

**PR1** Sun, 16:25-16:45 *Max Kade Auditorium*

NMR and IDPs  
Jane Dyson\*, Peter Wright  
*Scripps Research*

Today we recognize the central role that intrinsically disordered proteins (IDPs) play in cellular processes. This was not always the case. To understand why the IDP concept had not surfaced previously, we need to take ourselves back to the scientific mindset before 1990. The human genome project was not on anybody’s radar – very few if any genes had been sequenced. Cloning and expression of labeled proteins was in its infancy. Computing power was pitiful compared to today – the capacity of our institute-wide mainframe was less than that of a smartphone today. For protein NMR, the dominant technique was all-proton, and 2D was only introduced in 1977. Heteronuclear spectra of uniformly-labeled proteins were introduced in 1989. Our lab was focused on the study of peptides and small proteins, which seemed somehow qualitatively different – peptides were invariably disordered, sometimes with a propensity detectable by NMR for conformational preferences for local structure, whereas proteins were ordered in three dimensions, with a single overall structure that could be reflected in an X-ray crystal structure or, excitingly, by NMR. During the early 1990s we became increasingly puzzled by a series of proteins whose sequences were derived from the earliest sequenced genes. The genetic information was unequivocal on the location of the functional regions of these proteins, but when we looked at them in the NMR, they behaved like peptides. These proteins were disordered, yet still fully functional. Many of them fold into ordered structures upon binding to a partner; many remain fully or partly disordered even in partner complexes. In asking ourselves why disorder might be preferred over order in some types of proteins, we realized that disorder confers important advantages to the efficient operation of cells. Recognition of IDPs would not have been possible without the unique insights provided by NMR.

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**PR2** Sun, 16:45-17:05 *Max Kade Auditorium*

**Relaxing with IDPs: NMR analysis of dynamics and molecular interactions**  
Peter Wright\*, Jane Dyson  
*Scripps Research*

Intrinsically disordered proteins (IDPs) are highly abundant in the human proteome and are strongly associated with numerous devastating diseases, including cancers, age-related neurodegenerative disorders, diabetes, cardiovascular and infectious diseases. IDPs mediate critical regulatory functions in the cell, including transcription, translation, the cell cycle, and numerous signal transduction events. The lack of stable globular structure confers numerous functional advantages on IDPs, allowing them to exert an exquisite level of control over cellular signaling processes, but poses a major challenge to which traditional structural biology approaches are poorly suited. Many regulatory IDPs contain multiple interaction motifs. The intermolecular interface between such IDPs and their targets is energetically heterogeneous and is characterized by both static and dynamic interactions that mediate crosstalk between signaling pathways and lead to unique allosteric switches. NMR has emerged as the primary tool for elucidation of the structural ensembles, dynamics, interactions, posttranslational modifications, and functional mechanisms of IDPs. Relaxation measurements are especially important for characterization of IDP complexes, providing novel insights into the dynamic processes that mediate binding, competition for a common target, and allostery. The applications of NMR to elucidate the role of IDPs in dynamic cellular signaling will be illustrated by reference to the mechanism of action of a unidirectional, hypersensitive allosteric switch that downregulates the hypoxic response by displacing the hypoxia inducible factor HIF-1A from the general transcriptional coactivators CBP (CREB binding protein) and p300.

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## PR3

Sun, 17:20-17:40 *Max Kade Auditorium*

### DEER on cells with Gd(III)

Daniella Goldfarb\*

*Weizmann Institute of Science*

Observing proteins structural changes at during function in-side the cell is a challenge yet to be met. In this context we have been developing in-cell 95 GHz DEER ( double- electron electron resonance) distance measurement using Gd(III) spin labels. The developments that enabled in cell DEER on proteins, including instrumental aspects, Gd(III) spectroscopic properties, the chemistry of the Gd(III) labels and measurement methodology, will be first presented. Next, recent results showing that the monomer- dimer equilibrium constant of a protein is different in vitro and in the cell are different. Similarly, the protein conformation may also be different in these two very different environments.

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## PR4

Sun, 17:40-18:00 *Max Kade Auditorium*

### Everyone needs a little help from one's friends:

### Synergy between NMR, cryo-EM and large-Scale MD Simulations

Angela M. Gronenborn\*

*University of Pittsburgh School of Medicine*

Those of us engaged in active experimental research careers rarely have the opportunity to step back from the lab bench or the computer to reflect on our own scientific practice. Long before descriptive terms such as “multidisciplinary” or “integrative” were fashionable, biological NMR (BioNMR), from its very beginnings, was multidisciplinary in and by itself. Integration of complementary data has a long history in BioNMR and by now everyone in the scientific community is well aware that single types of methodologies are insufficient to adequately describe complex biological structures. I will describe the benefits of integrating solution NMR, MAS solid state NMR, crystallography, cryoEM and large-scale MD simulation, which permitted us to derive a realistic all-atom model for the entire HIV-1 capsid.

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**PR5**Sun, 18:15-18:35 *Max Kade Auditorium***Structure and Dynamics of Membrane Proteins in a Native Environment**David Goricanec<sup>1</sup>, Andrea Steiner<sup>2</sup>, Kolio Raltchev<sup>2</sup>, Elisabeth Häusler<sup>2</sup>,  
Jonas Miehl<sup>1</sup>, Melina Daniilidis<sup>1</sup>, Kai Fredriksson<sup>1</sup>, Franz Hagn<sup>\*</sup><sup>1</sup>*Department of Chemistry, Technical University of Munich*, <sup>2</sup>*Helmholtz Zentrum München*, <sup>3</sup>*Department of Chemistry and Institute for Advanced Study, Technical University of Munich and Helmholtz Zentrum München*

Membrane proteins are important players in signal transduction and the exchange of metabolites in cells. Thus, this protein class is the target of around 60% of currently marketed drugs, emphasizing their essential biological role. Besides production issues, a major bottleneck encountered in the structural characterization of membrane proteins is identifying a suitable membrane mimetic that provides a native environment. Thus, we are actively developing the phospholipid nanodisc technology for solution-state NMR spectroscopy. This versatile and size-tunable membrane mimetic provides a planar lipid bilayer membrane and can be used to study structure, dynamics and function of integral as well as peripheral membrane proteins. In this talk, our recent advances in the field of nanodisc development will be discussed as well as studies on the structure and dynamics of membrane proteins in suitable membrane mimetics, covering G-protein coupled receptors (GPCRs) and their complexes with G-proteins, a  $\gamma$ -Secretase substrate, the mitochondrial membrane protein VDAC1 as well as its counterpart in chloroplasts, called OEP21. These examples will emphasize the importance of choosing a suitable membrane mimetic for a particular application and membrane protein system.

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**PR6**

Mon, 08:40-09:20 *Max Kade Auditorium*

## Adventures with Long-Lived States

Malcolm Levitt\*

*University of Southampton*

Long-lived states are particular configurations of nuclear spins which are well-protected against relaxation mechanisms. These configurations appear as particular terms in the spin density operator associated with unusually slow decay rate constants. A seminal example arises in systems of homonuclear spin-1/2 pairs in solution. Singlet order, meaning a population imbalance between the singlet state and the triplet states, is protected against relaxation by the homonuclear dipole-dipole coupling between the two spins, and often has a decay time constant which exceeds  $T_1$  by a large factor. In one case, a singlet order lifetime exceeding 1 hour has been observed for a pair of  $^{13}\text{C}$  nuclei, even though  $T_1$  is about 1 minute under the same conditions.

Our group, and our collaborators, have been enjoying numerous adventures in the world of long-lived states. I will report on some of the following topics:

- using group theory to count and derive long-lived states
- using long-lived states to pump up the nuclear magnetization
- long-lived coherences, including the observation of coherent oscillations proceeding for tens of minutes - even when the sample is removed from the magnet
- relaxation mechanisms for long-lived states, including scalar relaxation in deuterated molecules
- new molecular systems supporting long-lived states
- a new master equation for spin dynamics far from equilibrium
- hyperpolarized long-lived singlet order generated by parahydrogen reactions: geminal-PHIP and trans-PHIP
- spin-isomer conversion of molecules trapped inside fullerenes

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**PR7**Mon, 09:20-09:40 *Max Kade Auditorium*

**New Methods for Dynamic Nuclear Polarization in Insulating Solids:  
The Overhauser Effect and Time Domain Techniques**

Thach Can\*

*Massachusetts Institute of Technology*

Dynamic nuclear polarization (DNP) is now established as a powerful technique for improving the sensitivity of NMR signals by several orders of magnitude, enabling otherwise impossible experiments. Unfortunately, the enhancements obtained at high magnetic fields ( $> 9$  T) are only a small fraction of the theoretical limit due to the fact that current DNP mechanisms, including the cross effect and solid effect, utilize continuous wave (CW) microwave irradiation, and scale unfavorably with  $B_0$ . This has motivated us to develop new DNP methods that do not suffer from the same field dependences.

Our first attempt resulted in the observation of the Overhauser effect in insulating solids doped with 1,3-bisdiphenylene-2-phenylallyl (BDPA) or sulfonated-BDPA (SA-BDPA) radical. As opposed to all other CW DNP mechanisms, the enhancement of the OE in insulating solids scales favorably with  $B_0$ . This finding sheds a new light on the seemingly well-understood Overhauser effect.

Our second approach is to perform time domain or pulsed DNP, which differs fundamentally from CW DNP, and like CP and INEPT transfers, is in principle independent of  $B_0$ . In particular, we have investigated the performance of two related pulse sequences including the nuclear orientation via electron spin locking (NOVEL) and integrated solid effect (ISE) at  $B_0$  ranging from 0.35 T to 3.35 T. The NOVEL pulse sequence relies on a matching condition between the nuclear Larmor frequency and the electron Rabi frequency, resulting in a fast polarization transfer from electron to protons (hundreds of ns time scale). Finally, we implemented a new version of the integrated solid effect (ISE) by modulating the microwave frequency instead of sweeping the  $B_0$ . In comparison to NOVEL, ISE gives similar DNP enhancement even far below the NOVEL condition. Our study sets the foundation for further development of time domain DNP at high fields.

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**PR8**

Mon, 09:40-10:00 *Max Kade Auditorium*

## Endogenous DNP from Paramagnetic Dopants for Probing Functional Inorganic Materials

Adi Harchol, Tamar Wolf, Asya Svirinovsky-Arblei, Guy Reuveni, Yehuda Buganim, Brijith Thomas, Michal Leskes\*

*Department of Materials and Interfaces, Weizmann Institute of Science*

In recent years magic angle spinning - dynamic nuclear polarization (MAS-DNP) developed as an excellent approach for boosting the sensitivity of solid state NMR (ssNMR) spectroscopy, thereby enabling the characterization of challenging systems in biology and chemistry. Most commonly, MAS-DNP is based on the use of nitroxide biradicals as polarizing agents. In materials science, since the use of nitroxides often limits the signal enhancement to the materials' surface and subsurface layers, there is need for hyperpolarization approaches which will provide sensitivity in the bulk of micron sized particles. Furthermore, for many functional materials, e.g. materials used for energy storage and conversion, the use of exogenous polarization agents is limited due to chemical reactivity at the materials' interface.

Here I will discuss the utilization of paramagnetic metal ion dopants as endogenous DNP agents for sensitivity enhancement in inorganic solids. By introducing the dopants at low concentrations, we obtain NMR signal enhancements of more than two orders of magnitude, thereby enabling the detection of structurally revealing nuclei such as  $^{17}\text{O}$  at natural abundance ( $<0.04\%$ ). I will describe the conditions for achieving sensitivity enhancement from a range of paramagnetic dopants and discuss their suitability for probing local vs. remote environments of the dopant. Finally, I will address some of the challenges in implementing this approach in functional solids such as electrode materials for batteries.

The approach offers an alternative route for efficiently detecting reactive surface species and opens the way for structural studies based on high sensitivity NMR of challenging nuclei in the bulk of inorganic solids.

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PR9

Wed, 13:30-13:40 Max Kade Auditorium

### Specific Cross-Relaxation Enhancement by Active Motions under Dynamic Nuclear Polarization: Technique & Applications

Victoria Aladin, Björn Corzilius<sup>2</sup>, Marc Vogel, Jiafei Mao, Clemens Glaubitz<sup>3</sup>, Beatrix Suess<sup>1</sup>

<sup>2</sup>Goethe University of Frankfurt am Main, <sup>3</sup>Institute of Biophysical Chemistry, Goethe University of Frankfurt am Main

Specific Cross-Relaxation Enhancement by Active Motions under DNP (SCREAM-DNP)[1] is a method which relies on direct-polarization transfer in solid-state DNP at typical DNP temperatures. The mechanism is based on cross-relaxation between  $^1\text{H}$  and  $^{13}\text{C}$  and is generated by the internal dynamics of methyl groups resulting in negative enhancement and inverted  $^{13}\text{C}$  signal in a direct DNP experiment[2]. Furthermore, this effect can be suppressed by  $^1\text{H}$  saturation. Through mathematical subtraction we can exclusively observe magnetization which was generated by cross relaxation. Therefore, the use of methyl groups as specific promotor for polarization transfer opens new applications in DNP. In this work, we show the application of SCREAM-DNP on a tetracycline-binding aptamer. Here,  $\text{CH}_3$  groups were introduced into the biomolecular complex by non-covalent complex formation between the aptamer and its highly specific ligand which carries three  $\text{CH}_3$ -groups. We use tetracycline as a source of cross-relaxation enhancement for binding studies. Moreover, we can influence the reorientation dynamics of methyl groups to a significant degree by changing the temperature, resulting in an increase of cross-relaxation efficiency. For utilization of this effect in proteins we also systematically investigated all natural methyl-bearing amino acids[3], where we illuminate the differences in methyl-dynamics in the context of sample temperature and sterical hindrance of the methyl group. Beyond that, we utilized SCREAM-DNP in green absorbing proteorhodopsin, where methyl-carrying retinal is used as a source of cross-relaxation enhancement. Here we could observe changes in the dynamics of the retinal  $\text{CH}_3$ -group through the all-trans to 13-cis isomerization as well as distance dependence of spin-diffusion following the SCREAM-DNP transfer. In conclusion, SCREAM-DNP is a promising method for different applications especially in site-specific DNP-studies.

**References:** [1] Aladin et al., Angewandte Chemie, 2019, 58,4863. [2] Daube et al., J. Am. Chem. Soc. 2016, 138, 16572. [3] Aladin and Corzilius, Solid State Nucl. Magn. Reson, 2019, 99,27.

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**PR10**Wed, 13:40-13:50 *Max Kade Auditorium*

**Insight into small molecule binding to the neonatal Fc receptor by  
X-ray crystallography and 100 kHz magic-angle-spinning NMR**

Daniel Friedrich<sup>\*,1</sup>, Alex Macpherson<sup>2</sup>, Susanne Smith-Penzel<sup>3</sup>, Nicolas Basse<sup>4</sup>, Fabien Lecomte<sup>2</sup>, Hervé Deboves<sup>5</sup>, Richard D. Taylor<sup>2</sup>, Tim Norman<sup>2</sup>, John Porter<sup>6</sup>, Lorna C. Waters<sup>7</sup>, Marta Westwood<sup>2</sup>, Ben Cossins<sup>2</sup>, Katharine Cain<sup>8</sup>, James White<sup>2</sup>, Robert Griffin<sup>2</sup>, Christine Prosser<sup>2</sup>, Sebastian Kelm<sup>2</sup>, Amy H. Sullivan<sup>9</sup>, David Fox III<sup>9</sup>, Mark D. Carr<sup>7</sup>

<sup>1</sup>*Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany,*

<sup>2</sup>*UCB Celltech, Slough, United Kingdom,* <sup>3</sup>*Laboratory of Physical Chemistry, ETH Zürich, Switzerland,* <sup>4</sup>*Sanofi, Strasbourg, France,* <sup>5</sup>*Evotec, Milton, United Kingdom,* <sup>6</sup>*Midatech Pharma Plc, Milton, United Kingdom,* <sup>7</sup>*Institute of Structural and Chemical Biology, University of Leicester, United Kingdom,* <sup>8</sup>*Vertex, Milton, United Kingdom,* <sup>9</sup>*Beryllium Discovery, Bedford, Massachusetts, United States of America*

Aiming at the design of an allosteric modulator of the neonatal Fc receptor (FcRn)–Immunoglobulin G (IgG) interaction, we developed a new methodology including NMR fragment screening, X-ray crystallography, and magic-angle-spinning (MAS) NMR at 100 kHz after sedimentation, exploiting very fast spinning of the nondeuterated soluble 42 kDa receptor construct to obtain resolved proton-detected 2D and 3D NMR spectra. FcRn plays a crucial role in regulation of IgG and serum albumin catabolism. It is a clinically validated drug target for the treatment of autoimmune diseases caused by pathogenic antibodies via the inhibition of its interaction with IgG. We herein present the discovery of a small molecule that binds into a conserved cavity of the heterodimeric, extracellular domain composed of an  $\alpha$ -chain and  $\beta$ 2-microglobulin ( $\beta$ 2m) (FcRn<sub>ECD</sub>, 373 residues). X-ray crystallography was used alongside NMR at 100 kHz MAS with sedimented soluble protein to explore possibilities for refining the compound as an allosteric modulator. Proton-detected MAS NMR experiments on fully protonated [<sup>13</sup>C,<sup>15</sup>N]-labeled FcRn<sub>ECD</sub> yielded ligand-induced chemical-shift perturbations (CSPs) for residues in the binding pocket and allosteric changes close to the interface of the two receptor heterodimers present in the asymmetric unit as well as potentially in the albumin interaction site. X-ray structures with and without ligand suggest the need for an optimized compound to displace the  $\alpha$ -chain with respect to  $\beta$ 2m, both of which participate in the FcRn<sub>ECD</sub>–IgG interaction site. Our investigation establishes a method to characterize structurally small molecule binding to nondeuterated large proteins by NMR, even in their glycosylated form, which may prove highly valuable for structure-based drug discovery campaigns.

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**PR11** Wed, 13:50-14:00 *Max Kade Auditorium*

**Time evolution of coupled spin systems in a generalized Wigner representation**

Bálint Koczor<sup>\*,1</sup>, Robert Zeier<sup>1,3</sup>, Steffen Glaser<sup>1</sup>

<sup>1</sup>*Technische Universität München*, <sup>2</sup>*University of Oxford (current)*,

<sup>3</sup>*Forschungszentrum Jülich GmbH (current)*

So-called phase-space representations such as Wigner functions, are a powerful tool for representing quantum states and characterizing their time evolution in the case of infinite-dimensional quantum systems and have been widely used in quantum optics and beyond. Continuous phase spaces have also been studied for the finite-dimensional quantum systems of individual spins. However, much less was known for coupled spin systems, and we present a complete theory of Wigner functions for this case. In particular, we provide a self-contained Wigner formalism for describing and predicting the time evolution of coupled spins which lends itself to visualizing the high-dimensional state space in a structured and intuitive way. We completely treat the case of an arbitrary number of coupled spins  $1/2$ , thereby establishing the equation of motion using Wigner functions. The explicit form of the time evolution is then calculated for up to three spins  $1/2$ . The underlying physical principles of our Wigner representations for coupled spin systems are illustrated for several NMR examples. This talk is based on *Annals of Physics* DOI 10.1016/j.aop.2018.11.020 (in press).

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**PR12**Wed, 14:00-14:10 *Max Kade Auditorium***Dynamic nuclear polarization of  $^{13}\text{C}$  in the liquid state over a 10 Tesla field range**Tomas Orlando<sup>\*,1</sup>, Riza Dervisoglu<sup>2</sup>, Marcel Levien<sup>1</sup>, Igor Tkach<sup>1</sup>,Thomas F. Prisner<sup>3</sup>, Loren Andreas<sup>2</sup>, Vasy Denysenkov<sup>3</sup>, Marina Bennati<sup>1</sup><sup>1</sup>*RG ESR Spectroscopy, Max Planck Institute for Biophysical Chemistry,*<sup>2</sup>*Department of NMR Based Structural Biology, Max Planck Institute for Biophysical Chemistry,* <sup>3</sup>*Institute of Physical and Theoretical Chemistry and Center for Biomolecular Resonance, Goethe University Frankfurt*

DNP in liquids is driven by electron-nuclear cross-relaxation, known as Overhauser effect (O-DNP). When relaxation is dominated by scalar hyperfine interaction, the enhancements can reach three orders of magnitudes, as recently reported for  $^{13}\text{C}$ -DNP at 3.4 T [1].

Hereby we present a systematic study performed at different magnetic fields on model systems doped with nitroxide radical (TEMPONE) as polarizing agent [2].  $^{13}\text{C}$  signal enhancements on organic small molecules in liquids at room temperature were observed as high as 800 at 1.2 Tesla and 600 at 9.4 Tesla. An accurate determination of Overhauser parameters allowed us to disclose the primary role of the scalar hyperfine interaction to the  $^{13}\text{C}$  nuclei as mediated by molecular collisions in the sub-picoseconds timescale.

Experimental measurements performed at 1.2 T, 9.4 T, and 14 Tesla allowed us to complete the characterization of the polarization transfer efficiency over a broad frequency range and described it by the subtle combination of dipolar and scalar relaxation.

Furthermore, we recognized that a proper choice of polarizing agent/target system is essential to optimize the efficiency of scalar O-DNP. Indeed, fullerene-nitroxide derivatives are superior to TEMPONE radical as polarizing agent at low fields, while halogens atoms (Cl, Br) bound to the target C nucleus seems to favor the scalar interaction.

The observation of sizable DNP of  $^{13}\text{CH}_2$  and  $^{13}\text{CH}_3$  groups in organic molecules at 9.4 T preserving NMR resolution opens perspectives for a broader application of this method as a tool to address  $^{13}\text{C}$ -NMR sensitivity issues at high fields.

**References:** [1] Liu G., Levien M., Karschin N., Parigi G., Luchinat C., and Bennati M. *Nat. Chem.* 9, 676-680 (2017). [2] Orlando T., Dervisoglu R., Levien M., Tkach I., Prisner T.F., Andreas L.B., Denysenkov V., Bennati M. *Angew. Chem. Int. Ed.* 58, 1402-1406 (2019).

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**PL21**

Mon, 17:40-18:30 *Max Kade Auditorium*

## Summarizing the static DNP mechanisms

Shimon Vega\*

*Weizmann Institute of Science*

During the last couple of years, we have been trying to understand the various stages of the DNP process leading to nuclear signal enhancements in static solid solutions, containing free organic radicals. Despite the fact that almost everything is known already, we have made an effort to clarify the basic spin dynamics resulting in these enhancements. For these studies, we performed experiments on the electrons and the nuclei in a variety of amorphous solids. In particular, the combination of EPR and ELDOR spectroscopy together with NMR measurements on the same sample, has led us to formulate computational models for explaining the line shapes of EPR and DNP spectra during microwave (MW) irradiation.

In this presentation, our findings in static samples will be summarized, by comparing simulated spectra of small model spin system with experimentally obtained spectra. For our computations, we use a cross relaxation mechanism to describe electron depolarization and rely on the indirect Cross Effect (iCE) for deriving DNP spectra from EPR spectra. The interaction regimes for the appearance of the Thermal Mixing (TM) phenomena will of course be discussed as well. For the analysis of experimental results, we show how we derive the change of the EPR lineshapes following microwave (MW) irradiation from ELDOR spectra, using the electron spectral diffusion (eSD) and TM models, and again rely on iCE to interpret experimental DNP spectra. Experimental results corresponding to the TM process will also be discussed and the dependence of the enhancements as a function of MW power, MW modulation and radical concentration will be verified.

Note: All static DNP studies have been performed in full collaboration with Daniella Goldfarb and Akiva Feintuch, and our post-doctoral and graduate students.

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### **Magnetic resonance for Cellular Structural Biology: from protein structures to functional processes in a cellular context**

Lucia Banci\*

*CERM and Dept. of Chemistry, University of Florence*

Magnetic resonance spectroscopies can provide unique contributions to describe cellular processes. Magnetic resonance is indeed suitable not only for characterizing the structural and dynamical properties of biomolecules but, even more importantly, for describing transient interactions and functional events with atomic resolution possibly in a cellular context. This requires the development of suitable methodologies capable of addressing multiple, specific, and sometimes non conventional aspects for describing functional processes in cells.

I will present some examples on how NMR, also integrated with other techniques, can contribute to advance the knowledge on functional cellular processes. They often involve transient interactions suitably studied by NMR, which can also characterize processes in living cells with atomic resolution. Transient interactions occur in metal transfer processes[1]. The presence of paramagnetic centers, such as iron-sulfur clusters, dramatically affects the NMR spectra, requiring the development of tailored experiments and the integration with multiple techniques, with EPR being essential for these systems. The power of NMR in describing cellular pathways will be presented for processes responsible for copper trafficking and for the biogenesis of iron-sulfur proteins. New major advancements in in-cell NMR[2,3] and in the characterization of highly paramagnetic systems[4] will be also discussed within an integrated approach where, from single structures to protein complexes, the processes are described in their cellular context within a molecular perspective.

**References:** [1] Banci L, et al. Affinity gradients drive copper to cellular destinations. *Nature* 465: 645-648, 2010. [2] Banci, L., et al. Atomic-resolution monitoring of protein maturation in live human cells. *Nat.Chem.Biol.* 9, 297-299, 2013. [3] Luchinat E, Banci L. In-Cell NMR in Human Cells: Direct Protein Expression Allows Structural Studies of Protein Folding and Maturation. *Acc Chem Res.* 51, 1550-1557, 2018. [4] Banci L, et al. The NMR contribution to protein-protein networking in Fe-S protein maturation. *J Biol Inorg Chem.* 23, 665-685, 2018.

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**PL23**

Tue, 09:20-10:00 *Max Kade Auditorium*

## Diffusion and electrophoretic NMR to characterize ion transport in electrolytes

Florian Schmidt, Gouverneur Martin, Mark Rosenwinkel,  
Marc Brinkkötter, Pinchas Nürnberg, Monika Schönhoff\*  
*Institute of Physical Chemistry, University of Muenster*

For application of electrolyte materials in energy storage devices their transport properties are essential. Multinuclear (e.g.  $^1\text{H}$ ,  $^7\text{Li}$ ,  $^{19}\text{F}$ ) Pulsed-Field-Gradient (PFG)-NMR diffusion has become a widely used method in this field. However, to identify the conductivity contribution of specific ion species remains a challenge, since the electrophoretic mobility  $\mu$  has to be known.

Electrophoretic NMR (eNMR) allows to directly measure the electrophoretic mobility of ions with NMR-active nuclei. During a PFG-NMR experiment an electric voltage is applied and the ion mobility is obtained from its drift velocity in the electric field. Provided that challenges arising from high conductivities and subsequent resistive heating can be overcome, even concentrated electrolyte systems can be investigated.

The lecture reviews our multinuclear eNMR studies on electrolytes for Li battery applications, e.g. Li salt-in-Ionic Liquid systems. Surprisingly, a negative mobility of  $\text{Li}^+$  may occur, implying a drift direction opposite to the expectation for a cation. This was attributed to a vehicular transport mechanism of Li in net negatively charged anion clusters and has strong implications for battery operation. In some systems a transition from a vehicular to a structural transport mechanism can be achieved by compositional variation.

We further report on various liquid electrolyte systems with organic additives and glyme-based solvate ionic liquids. Here, in addition to ion drift velocities, even a drift of uncharged molecular components in the electric field can be identified by  $^1\text{H}$  eNMR. Thus, conclusions on their coordination to  $\text{Li}^+$  ions are possible, shedding light on the mechanisms governing the Li transport.

In summary, electrophoretic NMR elucidates transport mechanisms on a molecular level, and provides unique information; in particular, where correlated motion of different ion species is involved.

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**PL24** Tue, 17:40-18:20 *Max Kade Auditorium*

**Transporter Conformational Dynamics from Spin Labeling EPR Spectroscopy**  
Hassane S. Mchaourab\*  
*Vanderbilt University*

Understanding the mechanisms of membrane proteins entails complementing static structures with the conformational changes in the structure. Recent advances in Double Electron-Electron Resonance (DEER) spectroscopy along with computational methods to generate restrained models of proteins are enabling unprecedented insights into the conformational dynamics of active transporters. My laboratory use EPR methods to define conformational equilibria that mediate the transport cycle of ion-coupled symporters and antiporters as well as ABC transporters. I will describe comparative analysis of ATP-binding cassette (ABC) exporters that reveals the role of dynamics in their transport cycles that resolve long standing question in the field . Together, these studies are illuminating the mechanistic commonalities and differences in the family.

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**PL25**

Wed, 08:40-09:20 *Max Kade Auditorium*

## Recent Developments of DNP Enhanced Solid-State NMR Spectroscopy at High Magnetic Field and Fast MAS

Anne Lesage\*  
*University of Lyon*

Dynamic Nuclear Polarization (DNP) has recently evolved into a cornerstone technology to overcome the sensitivity limitations of solid-state NMR. This technique, originally developed for low magnetic fields, has been shown to be applicable at high fields, opening new avenues in materials and life sciences. In this presentation we will review some recent advances from high magnetic field (18.8 T) and fast Magic Angle Spinning (MAS) (~ 40 kHz) DNP NMR. In particular, we will present our efforts towards the development of polarizing agents tailored for efficient DNP at high fields and fast MAS. Applications to the characterization of challenging catalytic surfaces and of biological assemblies will also be presented.

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**PL26** Wed, 09:20-10:00 *Max Kade Auditorium*

**Boosting clinical diffusion MRI with principles from solid-state and Laplace NMR**  
Daniel Topgaard\*  
*Lund University*

Diffusion MRI is today used in clinical routine for detecting stroke and grading prostate tumors, as well as in clinical research studies of for instance neurological diseases and normal brain development. The overwhelming majority of the diffusion MRI measurements are performed with motion encoding by the most basic form of the pulsed-gradient spin echo sequence from the mid-60s, which is sensitive to local diffusivities, restrictions, anisotropy, flow, and exchange. While it may be convenient to have a single experiment to detect a wide range of different diffusion properties, the lack of selectivity becomes a nuisance when attempting to assign the experimental observations for a complex, heterogeneous, and anisotropic material like the living brain to a specific diffusion mechanism. This lecture will give an overview of our recent work in redesigning diffusion MRI using principles that are well known in multidimensional solid-state NMR spectroscopy and low-field NMR of porous materials. The key features of this new “multidimensional diffusion MRI” approach are gradient waveforms targeting specific motion mechanisms and multidimensional acquisition and analysis protocols wherein the different mechanisms are separated and correlated. The gradient waveforms are often borrowed from classical sample reorientation techniques such as magic-angle spinning, variable-angle spinning, and double rotation. Data inversion is performed with algorithms from multidimensional Laplace NMR, in particular the more sophisticated Monte Carlo inversion generating ensembles of plausible distributions and estimates of the uncertainties of the obtained distributions and scalar parameters. Clinical application examples include studies of microstructure in meningioma and glioma brain tumors as well as white matter degeneration in multiple sclerosis.

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## PL27 Wed, 14:00-14:40 Max Kade Auditorium

### Stronger Together: Bacterial Weaving of Functional Amyloid and Polysaccharide Composites to Assemble Multicellular Biofilm Communities

Lynette Cegelski\*  
Stanford University

Biofilms are implicated in serious infectious diseases and have emerged as a target for anti-infectives. Our research program is inspired by the challenge and importance of elucidating chemical structure and function in complex biological systems and we strive to transform our discoveries into new therapeutic strategies. We have introduced new approaches integrating solid-state NMR with microscopy and biochemical and biophysical tools to reveal how amyloid-associated biofilm assembly and architecture in *E. coli* is influenced by chemical and molecular composition. Solid-state NMR is serving as a powerful discovery tool in these efforts and we recently reported the unprecedented discovery of a naturally produced chemically modified cellulose produced by *E. coli* and *Salmonella* strains. Solid-state NMR of the intact cellulosic material enabled the elucidation of the zwitterionic phosphoethanolamine modification and we identified the genetic and molecular basis for its assembly. I will report on our efforts to understand and describe the macromolecular assembly of the amyloid-polysaccharide composites in *E. coli* and their relation to biofilm function.

## PL28 Thu, 08:40-09:20 Max Kade Auditorium

### Optically-pumped dynamic nuclear polarization under ambient conditions via nitrogen-vacancy centers in diamond

Carlos Meriles\*  
CUNY - City College of New York

A broad effort is underway to improve the sensitivity of nuclear magnetic resonance through the use of dynamic nuclear polarization (DNP). Nitrogen-vacancy (NV) centers in diamond offer an appealing platform because these paramagnetic defects show efficient optical pumping at room temperature. This presentation focuses on the spin dynamics of NVs coupled to substitutional nitrogen (the so called P1 center) as a platform for DNP, with emphasis on recent schemes designed for powder geometries and optimal polarization transfer across the diamond surface. I will also discuss new phenomenology under NV-P1 cross-relaxation conditions revealing record fast nuclear spin diffusion constants as well as quick, homogeneous thermalization between bulk and strongly hyperfine-coupled nuclei. These observations highlight the need for DNP descriptions beyond classical models based on spin diffusion barriers.

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### High-resolution NMR spectroscopy applied for field inhomogeneity and spectral congestion

Zhong Chen\*

*Xiamen University*

NMR spectroscopy presents a non-invasive detection technique for molecular structure elucidation and dynamic effect analyses. In general NMR applications, spectral resolution is the key index determining the availability of resulting spectra. Due to limited chemical shift ranges and appended J coupling splittings, conventional 1H NMR spectra are subject to spectral congestions in complex samples. In addition, there exist adverse experimental conditions in which magnetic fields suffer from spatial or temporal inhomogeneity, constituting the second factor degrading spectral resolution in 1H NMR applications. Therefore, an NMR method available for high-resolution NMR measurements under the condition of inhomogeneous fields and complex samples is greatly demanded.

In our previous studies, a series of NMR methods based on intermolecular multiple-quantum coherence (iMQC)[1] is proposed for high-resolution applications in inhomogeneous magnetic field conditions. And other pure-shift based methods are also designed to eliminate J coupling splittings and further enhance spectral resolution.[3] For example, a high-resolution NMR method, named UPSIF, has been proposed to extract high-resolution 1D pure shift or 2D J-resolved spectra, suitable for direct analyses on biological samples. The proposed method is designed based on the combination of constant-time module and iMQC scheme. The constant-time module constitutes a direct decoupling manner for removing J couplings and extracting pure chemical shifts. The iMQC scheme is proved to be immune to field inhomogeneity. The performance of the proposed high-resolution method is demonstrated by experiments on biological samples with intrinsic field inhomogeneities and in situ electrochemical detection under externally adverse field conditions. Our proposed methods can be applied for high-resolution measurements under the condition of field inhomogeneity and spectral congestion.

**References:** [2] Z. Chen, S.H. Cai, Y.Q. Huang, Y.L. Lin, *Prog. Nucl. Magn. Reson. Spectrosc.*, 90-91, 1-31, 2015. [3] K. Zangger, H. Sterk, *J. Magn. Reson.*, 124, 486-489, 1997.

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**PL30**

Thu, 17:20-18:00 *Max Kade Auditorium*

**Pulsed magnetic resonance with a free-electron laser**

Mark Sherwin<sup>\*1</sup>, C. Blake Wilson<sup>1</sup>, Marzieh Kavand<sup>1</sup>, Xiaoling Wang<sup>1</sup>,

Jessica Clayton<sup>1</sup>, Nikolay Agladze<sup>1</sup>, Steffen Glaser<sup>2</sup>, Song-I Han<sup>3</sup>

<sup>1</sup>*University of California at Santa Barbara*, <sup>2</sup>*Department of Chemistry*,

*Technical University of Munich*, <sup>3</sup>*Department of Chemistry*,

*University of California at Santa Barbara*

In the highest-field NMR magnets (currently 23.5 T, 1 GHz proton NMR frequency), the Larmor precession frequency for spin-1/2 electrons is 660 GHz. The recently-demonstrated 32 T superconducting magnet at MagLab in Tallahassee pushes the Larmor frequency to nearly 900 GHz. However, at the present time, it is difficult to generate a programmable sequence of phase-coherent narrow-band pulses with kW peak powers above 100 GHz (3.5 T), precluding the rapid coherent manipulation of electron spins that is required for high-power pulsed EPR, pulsed electron-nuclear double resonance (ENDOR), and pulsed dynamic nuclear polarization (DNP)-enhanced NMR in modern NMR spectrometers. The UC Santa Barbara Free-Electron Lasers (FELs), which generate high-power quasi-continuous-wave (cw) pulses between 0.24 and 4.5 THz, are now being used to drive a pulsed EPR spectrometer at 8.6 T (240 GHz). This talk will include a discussion of methods we have developed for converting the FEL output into a sequence of one or two pulses with durations as short as a few ns, resonator-free  $\pi/2$  times below 10 ns, and, recently, multi-step phase-cycling. These pulse sequences, together with a home-built EPR spectrometer, have enabled measurements including Rabi oscillations, longitudinal and transverse relaxation times, and “instantaneous spectral diffusion” in systems including Nitrogen impurities (P1 centers) in diamond, and stable free radicals in both solid and solution phases. Current efforts to implement FEL-powered pulsed DNP and to generate more complex pulse sequences at 240 GHz will be described. Finally, I will discuss the outlook and scientific opportunities for FEL-powered EPR, DNP, and ENDOR at fields up to 30.5 T (1.3 GHz proton-NMR frequency, 854 GHz electron Larmor frequency). This work is supported by the NSF under grants DMR-1626681 and MCB-1617025.

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**PL31**

Fri, 08:40-09:20 *Max Kade Auditorium*

## NMR of Aromatic Side Chains in Large Proteins

Haribabu Arthanari<sup>1</sup>, Andras Boeszoermenyi<sup>2</sup>, Abhinav Dubey<sup>2</sup>,

Koh Takeuchi<sup>3</sup>, Ilya Kuprov<sup>4</sup>, Gerhard Wagner<sup>\*,5</sup>

<sup>1</sup>*Dana-Farber Cancer Institute*, <sup>2</sup>*Dana Farber Cancer Institute*, <sup>3</sup>*AIST*,

<sup>4</sup>*U. Southampton*, <sup>5</sup>*Harvard Medical School*

Current NMR methods for studying proteins are primarily focused on backbone resonances and on methyl bearing side chains. In contrast, NMR of aromatic side chains has been less pursued although these moieties form a large portion of the hydrophobic protein cores. This is in part due to the complexity of aromatic side-chain spectra, which appear in a narrow and crowded spectral region. <sup>13</sup>C-dispersion of spectra reduces the complexity but the large carbon-carbon and carbon-proton couplings make spectra difficult to analyze. The TROSY effect of aromatic carbons has been realized early on as an approach to make aromatic spin systems more accessible, and alternate <sup>13</sup>C labeling with judiciously chosen pyruvate precursors can render rather well resolved aromatic spectra that can yield NOE contacts with and between aromatic side chains in larger proteins. With the ability to introduce <sup>19</sup>F-labeled aromatic residues into proteins, we explored whether we could utilize the large CSA of fluorine to create a <sup>19</sup>F-<sup>13</sup>C TROSY effect for more efficient detection of aromatic signals in large proteins. Indeed, simulation of the relaxation properties using the Spinach program indicated that <sup>13</sup>C detected FC TROSY experiments can yield very sharp signals but need specific labeling strategies. Similar advances can also be made with <sup>19</sup>F-<sup>13</sup>C labeled nucleotide bases, some of which promise to yield very sharp TROSY signals that are expected to deteriorate little with larger systems. NMR access to mobility and interactions of aromatic side chains is particularly interesting for elucidating mechanisms of signal transduction of membrane protein receptors where binding of agonists or antagonists may cause small structural and dynamic changes to define signaling.

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**PL32** Fri, 09:20-10:00 *Max Kade Auditorium*

**Manipulation of Spin Dynamics for Extraction of Spectral Parameters,  
Ultra High Resolution and Sensitivity Enhancement: Application to small molecules**

Nagaraja rao Suryaprakash\*  
*Indian Institute of Science*

The smaller chemical shift dispersion and the pairwise interaction among all the abundant nuclear spins of the molecule renders <sup>1</sup>H NMR spectrum highly complex, severely hindering the straightforward analysis and the accurate determination of homo- and hetero- nuclear scalar couplings. The inherent insensitivity of NMR technique poses additional challenge. We have manipulated the spin dynamics and designed number of two dimensional techniques to partially address these problems. The developed NMR methodologies lead to the extraction of spectral parameters, resulted in ultra high resolution and enhanced sensitivity of <sup>1</sup>H detection. My talk will be focused on the application of some of our experimental methods to derive specific information on the small molecules. The special emphasis will be on our novel techniques, QG-SERF, Clean-G-SERF and PS-Clean-G-SERF. The QG-SERF permitted the rapid determination of spectral information, while the other two techniques completely eradicates the axial peaks and suppress the evolution of unwanted couplings while retaining only the couplings to the selectively excited proton thereby facilitating the accurate determination of indirect spin-spin couplings even from a complex proton NMR spectrum in an orchestrated manner.

**PL33** Fri, 13:40-14:20 *Max Kade Auditorium*

**Nanoscale magnetic spin resonance using the nitrogen vacancy centre in diamond**

Lloyd C. L. Hollenberg\*  
*School of Physics, University of Melbourne, Australia*

The nitrogen-vacancy (NV) centre in diamond is an optically addressable single spin-1 electronic system with relatively long coherence persisting at room temperature, making it ideal for a range of nanoscale quantum sensing applications. The NV system is sensitive to local magnetic and electric fields, as well as material properties such as strain and temperature. An exciting direction is the development of the NV centre as a quantum probe for nanoscopic magnetic resonance applications. In this talk, we will briefly review the NV centre and some of the seminal sensing and imaging demonstrations, and focus on new results based on controlled cross-relaxation between the NV quantum probe and target electron and nuclear spin systems. Experimental demonstrations of both electron and nuclear spin resonance using these techniques will be described, including applications in chemical detection, bio-magnetic characterisation, and hyperpolarisation of nuclear spins external to the diamond substrate. Finally, we will look at ideas for using such controllable quantum spin probes in bio-molecular structure determination.

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**PL34**

Fri, 14:20-15:00 *Max Kade Auditorium*

## Dynamic Complexes and Complex Dynamics - NMR Studies of Large Scale Protein Motions

Martin Blackledge\*

*Protein Dynamics and Flexibility by NMR*

Proteins are inherently dynamic, exhibiting conformational freedom on many timescales,[1] implicating structural rearrangements that play a major role in molecular interaction, thermodynamic stability and biological function. Intrinsically disordered proteins (IDPs) represent extreme examples where flexibility defines molecular function. IDPs exhibit highly heterogeneous local and long-range structural and dynamic propensities, sampling a much flatter energy landscape than their folded counterparts, allowing inter-conversion between a quasi-continuum of accessible conformations.[2] We are combining multifield NMR relaxation measurements and ensemble MD approaches to develop a unified description of the dynamics of IDPs as a function of environmental conditions.[3-5]

In spite of the ubiquitous presence of IDPs throughout biology, the molecular mechanisms regulating their interactions with physiological partners remain poorly understood. We use NMR spectroscopy to map these complex molecular recognition trajectories at atomic resolution, from the highly dynamic free-state equilibrium to the bound state ensemble. Examples include the replication machinery of Measles virus, where the highly (>70%) disordered phosphoprotein initiates transcription and replication exploiting weak interactions with ordered and disordered domains of the nucleoprotein,[6,7] the nuclear pore, where weak interactions between the nuclear transporter and highly flexible chains containing multiple ultra-short recognition motifs, facilitate fast passage into the nucleus.[8] and large-scale domain dynamics in Influenza H5N1 polymerase are essential for import into the nucleus of the infected cell.[9]

**References:** [1]. Lewandowski et al *Science* 348, 578 (2015). [2]. Jensen et al *Chem Rev* 114, 6632 (2014). [3]. Abyzov et al *J.A.C.S.* 138, 6240 (2016). [4]. Salvi et al *Angew Chem Int Ed.* 56, 14020 (2017). [5]. Salvi et al *Science Advances* In Press (2019). [6]. Schneider et al *J.A.C.S.* 137,1220 (2015). [7]. Milles et al *Science Advances* 163, 734 (2018). [8]. Milles et al *Cell* 112, 3409 (2015). [9]. Delaforge et al *J.A.C.S.* 137 (2015).

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**PS101**

Mon, 10:30-11:05

**Advances and Adventures with Compact NMR**

Bernhard Blümich\*

*RWTH Aachen University*

My adventures with NMR started in 1973 at the Technical University in Berlin with a just-for-fun project on a 90 MHz ‘high-field’ Fourier NMR spectrometer [1]. Fun and curiosity has been my main driver since to explore different measurement NMR methodologies along with novel applications. Building on experience with spectroscopy of liquids and solids in homogeneous fields, insights into imaging and the Hahn echo promised new challenges and discoveries with NMR in inhomogeneous fields [2,3]. Such fields can be produced with smaller and cheaper magnets than homogeneous magnetic fields and triggered my quest for the simplest NMR experiment, which lead to stray-field NMR and the NMR-MOUSE. Thanks to my good fortune of working together with brilliant students, postdocs, and colleagues, the NMR-MOUSE evolved into something useful and led to compact magnets with homogeneous fields suitable for NMR spectroscopy. The NMR-MOUSE and benchtop NMR-spectrometers from 40 MHz to 80 MHz are now in the Magritek portfolio, while my group explores the new opportunities provided by their commercial availability [4,5]. This lecture reminisces some of my steps in the world of NMR and reports recent adventures with compact NMR for relaxometry in museums and outdoors as well as for chemical analysis by spectroscopy on the tabletop.

**References:** [1] D. Ziessow, B. Blümich, Hadamard-NMR-Spektroskopie, Ber. Bunsenges. Phys. Chem. 78 (1974) 1168-1179. [2] B. Blümich, NMR Imaging of Materials, Clarendon Press, Oxford, 2000. [3] B. Blümich, Essential NMR, 2nd ed., Springer Nature, Cham, 2019 [4]. B. Blümich, K. Singh, Angew. Chem. Int. Ed. 57 (2018) 6996 – 7010 [5] B. Blümich, S. Haber-Pohlmeier, W. Zia, Compact NMR, de Gruyter, Berlin, 2014.

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**PS102**

Mon, 11:05-11:30

## Understanding novel spin physics to make clinical-scale hyperpolarization simple, fast and cheap

Warren Warren\*

*Duke University*

SABRE, a method pioneered about a decade ago, uses parahydrogen and reversible exchange in solution to hyperpolarize organic molecules. In recent years this method has been adapted to polarize heteroatoms such as  $^{15}\text{N}$ , rapidly (in seconds) on several hundred different molecules, with an apparatus that can be built for about 1% of the cost a DNP system. Two different strategies have been successful: direct transfer of order from parahydrogen at about 0.5 microtesla (where the resonance frequency difference between nitrogen and hydrogen is about the same as a J coupling) and very weak irradiation of the nitrogen resonance at high field (irradiation strength comparable to a J coupling). Both of these are unusual regimes for magnetic resonance, and provide unique opportunities for exploring spin physics in underexplored domains. This is important because, while progress in a short time has been phenomenal, the fundamental limitation of this technique today is scalability; these methods typically work well at low (mM) concentrations, and not that well in water. New pulse sequences we have developed, coupled with a new quantum Monte Carlo simulation approach, have drastically improved our understanding of the spin dynamics in these complex systems. They have also led to significant signal enhancements, new imaging agents (such as para-nitrogen gas and a variety of injectable compounds) and new strategies for clinical-scale hyperpolarization.

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**PS103**

Mon, 11:30-11:55

**Compact NMR for Metabolic Health Screening and Diabetes Prevention**

David Cistola

The world-wide diabetes pandemic has heightened the need for early screening and prevention. Type 2 diabetes develops slowly and insidiously, and the early stages are often undetected. Here we describe how NMR relaxometry using small table-top devices can be used for the early detection of insulin resistance and metabolic syndrome. These conditions affect nearly one half of US adults and confer a two-to-five-fold increased risk for developing type 2 diabetes.

Water  $T_2$ , measured using a small volume of unmodified human plasma, serum or whole blood, is exquisitely sensitive to early metabolic dysregulation. In a discovery cohort of asymptomatic non-diabetic human subjects, plasma and serum water  $T_2$  showed strong bivariate correlations with markers of insulin, glucose and lipid metabolism, as well as markers of the pro-inflammatory, pro-coagulation state. After correcting for confounding variables using multiple regression, low water  $T_2$  values were independently and additively associated with hyperinsulinemia, subclinical inflammation and dyslipidemia, even in subjects with normal glucose levels.

A fingerstick drop of settled whole blood yields two  $T_2$  values: one for the plasma supernatant ( $T_{2s}$ ) and another for the cell pellet ( $T_{2p}$ ). The  $T_{2s}$  value revealed a sixth-power dependence on hematocrit. This sixth-power relaxation enhancement results from a susceptibility gradient from the paramagnetic pellet into the diamagnetic supernatant. Paradoxically, the cell pellet  $T_{2p}$  correlated with metabolic markers like those observed with isolated plasma. Here,  $T_{2p}$  is sensing modifications in red blood cells resulting from changes in whole body metabolism.

In addition to isolated blood samples, NMR relaxometry measurements can be made non-invasively in living tissues. We will discuss early results obtained using a prototype compact NMR device designed for measuring  $T_2$  in the distal segment of the human finger. Compact NMR strategies for monitoring metabolic health are sufficiently practical for translation into point-of-care clinics and community settings.

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**PS104**

Mon, 11:55-12:30

## A MOUSE for Heritage: In Pursuit of Art and Culture

Christian Rehorn<sup>1</sup>, Lachlan Pham<sup>2</sup>, Bernhard Bluemich<sup>1</sup>, Maria Baías<sup>\*,2</sup>

<sup>1</sup>RWTH-Aachen University, <sup>2</sup>New York University Abu Dhabi

Ancient mummies and bones, paintings and violins – what do they have in common? First, they are all highly relevant to cultural heritage, and second, we can use mobile NMR to learn specific details about each of them.

Mobile NMR is a non-destructive technique that uses single-sided mobile NMR sensors capable of recording NMR signals from samples that are exterior to the magnet. The two main advantages of this method – its portability and non-invasiveness – fulfill the condition for analysis of precious objects that need to be kept safely in museums or archaeological sites and preserved during experiments. This makes mobile NMR an essential tool for studying objects and sites of high interest to the field of cultural heritage.[1,2]

My talk will focus on the applications of the Profile NMR-MOUSE (MOBILE Universal Surface Explorer) sensor to cultural heritage research. I will present how the NMR-MOUSE can be employed for the characterization of various objects of cultural heritage relevance, ranging from ancient mummies and bones to more recent artifacts, such as older and newer violins as well as modern paintings. I will illustrate how this method can offer information related to the state of conservation of mummies [3], reveal insights into building a master violin and help identifying forgeries in the world of modern paintings.

**References:** [1] M. Baías, Mobile NMR: An Essential Tool for Protecting our Cultural Heritage – Magn. Reson. Chem. 55 (2017) 33-37. [2] M. Baías, B. Bluemich, Nondestructive Testing of Objects from Cultural Heritage with NMR – Modern Magnetic Resonance (2017) 1-13, Springer International Publishing, Ed. Graham A. Webb. [3] F. Ruehli, T. Boeni, J. Perlo, F. Casanova, M. Baías, E. Egarter, B. Bluemich, Noninvasive spatial tissue discrimination in ancient mummies and bones by in situ portable nuclear magnetic resonance, Journal of Cultural Heritage 8 (2007) 257-263.

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**PS105**

Mon, 12:30-13:00

**Wide field range studies of nuclear magnetic relaxation using  
optically pumped magnetometers**Sven Bodenstedt\*, Michael C.D. Tayler, Morgan Mitchell*ICFO*

Recently, spin-exchange relaxation free (SERF) alkali-vapor magnetometers have been applied as detectors of nuclear magnetic resonance (NMR) in the zero to ultralow field (ZULF) regime [1]. In ZULF the reduction of spectral line broadening due to field gradients as well as the possible existence of long-lived coherences [2] may lead to spectra with high resolution. These can provide new chemical and physics insight into the sample, beyond the capability of existing analytical techniques. In recent work the technique has been used to provide chemical-specific insight into liquid mixtures after being imbibing into porous catalytic materials [3].

In this presentation, we discuss new methodology that extends the scope of ZULF NMR to study multi-phase materials, including liquids in porous catalytic materials and metals. One aim is to measure  $^1\text{H}$  NMR relaxation rates  $T_1$  and  $T_2$  at magnetic fields between a few nanotesla and several hundred microtesla, to interrogate slow dynamics associated with surface-site diffusion. These methods are applicable even to materials that cannot be studied with conventional magnetic resonance, including highly paramagnetic, disordered materials.

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Disordered Protein Complexes

Birthe B. Kragelund\*  
*University of Copenhagen*

Intrinsically disordered proteins (IDPs) (or –regions (IDRs)) are functional while existing in broad ensembles of near iso-energetic conformations. Despite their lack of structure, IDPs are involved in molecular communication forming associations ranging from binary, discrete complexes to large multicomponent assemblies. Similar to globular proteins their complexes serve structural, functional and regulatory roles, but due to their dynamic nature, they expand the types of association possible, enabling functional regulations by very different mechanisms. The fast dynamics characteristic of IDPs may persist in their complexes to various degrees. We have been exploring the role of disorder in cellular control processes including pH homeostasis, cytokine signalling, transcriptional regulation, and DNA metabolism, combining NMR with other biophysical methods as SAXS, neutron diffraction, single-molecule FRET and cell biology [1–4]. In one end, we observe folding-upon-binding forming nearly globular-like complexes with little disorder while at the other end, disorder may persist and results in complexes where both binding partners stay disordered in high-affinity binding [3]. Still, the kinetics combined with higher order complex formation allows regulation on biologically relevant timescales. Between these extremes, a continuum of dynamic complexes is possible. The characterisation and functional decoding of dynamic complexes challenges the methodological toolbox, but NMR continues to be a critical contributor in the understanding of disorder dependent biology.

**References:** [1] Bugge, K., et al (2016) A combined computational and structural model of the full-length human prolactin receptor. *Nat Comm* 7, 11578. [2] Bugge, K., et al. (2018) Structure of Radical-Induced Cell Death1 Hub Domain Reveals a Common  $\alpha\alpha$ -Scaffold for Disorder in Transcriptional Networks. *Structure* 26, 734–746. [3] Borgia, A., et al. (2018) Extreme disorder in an ultrahigh-affinity protein complex. *Nature*, 555, 61–66. [4] Hendus-Altenburger, R., et al (2016) The human Na(+)/H(+) exchanger 1 is a membrane scaffold protein for extracellular signal-regulated kinase 2. *BMC Biol*, 14, 31.

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**PS107**

Mon, 11:05-11:30

**Cross-correlated relaxation for studying intrinsically disordered proteins**Anna Zawadzka-Kazimierczuk<sup>\*,1</sup>, Clemens Kauffmann<sup>2</sup>,Krzysztof Kazimierczuk<sup>1</sup>, Thomas C. Schwarz<sup>2</sup>, Robert Konrat<sup>2</sup><sup>1</sup>University of Warsaw, <sup>2</sup>University of Vienna

Under physiological conditions intrinsically disordered proteins (IDPs) lack a rigid three-dimensional structure; they can be rather described as a large ensemble of possible structures, that are adopted only transiently [1]. Nonetheless, in organisms they play a variety of roles, e.g. related to signaling and regulation. Interestingly, their flexibility is often crucial for fulfilling these functions. The fact that IDPs are very common, especially in eukaryotic organisms, and their relations to many human diseases, makes them an important object to study.

Due to their relation to backbone dihedral angles, cross-correlated relaxation (CCR) rates are a valuable source of information on protein structure [2]. Such measurements are typically included in three-dimensional (3D) NMR experiments. CCR rates can be also studied for IDPs, where they report on the residual structure. Here, we present the new 4D experiment for the measurement of HNH $\alpha$  dipeptide-dipole – C' chemical shift anisotropy CCR rate. This rate offers interesting structural information.

The high dimensionality of the proposed experiment provides the resolution that enables efficient studies of IDPs. The inherent flexibility of IDPs causes their chemical shift range to be significantly narrower than in the case of folded proteins. In 3D spectra of an IDP the level of peak overlap can be substantial, limiting the amount of available information. To overcome this problem, the CCR rates measurements can be implemented into higher-dimensional experiments, where the peaks are distributed over a wider spectral space, thus decreasing the number of overlapping peaks [3].

To enable acquisition of high-dimensional data, non-uniform sampling was employed, and for processing a compressed sensing iterative soft thresholding algorithm [4] was used.

**References:** [1] *Biochimica et Biophysica Acta*, 1804(6): 1231-1246, 2010. [2] *Methods in enzymology* 338: 35-81, 2002. [3] *Angewandte Chemie – International Edition* 52: 4604-4606, 2013. [4] *Angewandte Chemie – International Edition* 50: 5556-5559, 2011.

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Mon, 11:30-11:55

NMR insight into transient structures and interactions within  
the RNA polymerase of bronchiolitis virus

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Virologie et Immunologie Moléculaires

The RNA polymerase (RNAPol) of respiratory syncytial virus (RSV), the main agent responsible for bronchiolitis infections, is a viral RNA synthesis machinery composed of at least of 2 proteins, a 250 kDa catalytic subunit and a 4x27 kDa phosphoprotein co-factor (RSV P). RNAPol associates with the viral nucleocapsid, a ribonucleic complex containing the genomic viral RNA matrix, as well as with several other proteins, either viral co-factors or cellular enzymes and signaling proteins. Low affinity towards its partners ensures processivity of the polymerase. Notably many proteins, involved in the RSV RNAPol and its complexes display low structural complexity regions, RSV P in particular, which is a linchpin of these complexes. We designed a combination of biochemical and NMR approaches to gain structural and dynamic insight into this protein, which is arranged as a tetramer, but otherwise lacks a defined 3-dimensional structure. NMR revealed structural features ranging from fully disordered regions to ordered regions in the form of isolated helices. This is an advantage for a protein that acts as a platform to recruit specific protein partners either to the RNAPol or to replication sites in infected cells. RSV P contains transient secondary structures, which provide plasticity of P and act as contact regions that lead to compaction of the protein or are recognized by protein partners. They may be stabilized in protein complexes. We identified at least four regions that correspond to different partners and complexes. One of these is the RSV M2-1 protein that keeps the RNAPol in transcription mode. We also characterized the PP1 phosphatase binding site on P, in the vicinity of that of its substrate, M2-1. Finally, we used NMR for probing protein-protein interaction inhibitors targeting the binding of the nucleocapsid to P, which could pave the way for new antiviral therapeutics.

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**PS109**

Mon, 11:55-12:30

**The role of proline residues in intrinsically disordered proteins**

Roberta Pierattelli\*

*CERM, University of Florence*

In recent years many examples of intrinsically disordered proteins (IDPs) appeared in the literature showing how their structural plasticity and intrinsic flexibility enable them to play key roles in many regulatory processes. Their mis-function has also been related to several diseases. The general properties of IDPs cannot be captured in ordered crystals, preventing them to be suitable targets for crystallographic studies. Thus, nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in their investigation, being the only method that allows a high resolution description of their structural and dynamic features in solution.

IDPs are often enriched in the so-called disorder promoting amino acids, among which proline residues have a particular prominence. Proline residues have peculiar physicochemical properties and generally they are not mapped with NMR experiments based on amide-proton detection. Notwithstanding, their role in globular proteins has been studied in detail since decades revealing interesting functional roles. On the contrary, despite their high abundance and presence in many peculiar motifs characteristic of IDPs, the role of proline residues in protein disorder has not yet been addressed in detail.

Examples of proline-rich IDPs will give us the opportunity to discuss the different roles played by proline residues depending on their sequence context.

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**PS110** Mon, 12:30-13:00

**The NMR structure of a gp41 cytoplasmic tail fragment reveals the structural basis of the transmembrane coupling of the HIV-1 envelope glycoprotein**  
Alessandro Piaì\*, Qingshan Fu, Wen Chen, James J. Chou  
*Harvard Medical School*

The antigenic structure of the HIV-1 envelope spike (Env) is a major consideration for vaccine design to induce effective immune responses. Recent studies suggest that the cytoplasmic tail (CT) of Env influences the antigenic properties of its ectodomain on the opposite side of membrane, but the structural basis of this conformational coupling is still unknown. Using nuclear magnetic resonance (NMR) spectroscopy, we determined the structure in near-lipid bilayer environment of an Env fragment encompassing the transmembrane domain (TMD) and a large portion of the CT, containing the Kennedy sequence (KS) and the lentivirus lytic peptide 2 (LLP2). Structure calculation faced the challenge of constraining the amphipathic CT at the water-lipid interface, which was overcome by implementing plane restraints obtained from NMR-based membrane partition analysis of the protein in bicelles. The structure revealed a molecular architecture never observed before, in which the CT folds into amphipathic helices attaching to the membrane and wraps around the C-terminal end of the TMD, thereby forming a support baseplate for the rest of Env. Biochemical data indicated that the CT-TMD interaction is important for the CT folding and trimerization, which help stabilizing the native conformation of the Env. Functional data from pseudovirus-based neutralization assays confirmed that loosening or disruption of the CT-TMD interaction can indeed affect the antibody binding to the Env ectodomain at the other side of the membrane, as weakening of the CT baseplate can destabilize the conformation of the TMD, which in turn affects that of the membrane-proximal external region (MPER) and the rest of the Env ectodomain, shifting it toward an open conformation. These results contribute to explaining why the CT has a profound effect on the antigenic structure of the ectodomain and can guide HIV-1 immunogen design.

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**PS111**

Mon, 10:30-11:05

**Deuterium Metabolic Imaging (DMI),  
a novel MR-based method for in vivo mapping of metabolism**

Robin de Graaf\*

*Yale University*

Deuterium metabolic imaging (DMI) is a novel 3D method to image metabolism of deuterium-labeled substrates in healthy or diseased human brain. DMI provides a powerful tool to reveal altered metabolism and provides completely novel information compared to standard MRI. Examples of aberrant metabolism in aggressive tumors demonstrate the unique metabolic dimension that DMI adds to the standard MRI arsenal. A range of biologically relevant, affordable deuterated substrates are available to probe multiple metabolic pathways. The robustness of DMI together with the high sensitivity, ease-of-use and affordability makes this novel technique highly relevant for 3D mapping of human brain metabolism.

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**PS112**

Mon, 11:05-11:30

## Quantitative Heterogeneity MRS (qhMRS) - A New Type of Line Shape Analysis Applicable to NMR Resonances Sensitive to Suitable Physicochemical Parameters

Norbert W. Lutz\*, Monique Bernard

*CRMBM, Aix-Marseille University*

NMR line shapes have long been analyzed to study coupling constants and multiplicities, molecular structure and mobility, chemical exchange and other molecular properties. Line shape narrowing by complex shim procedures, sample spinning and/or other techniques has been extremely important for optimal suppression of line shape contributions not related to molecular properties. In biomedical *in vivo* NMR spectroscopy, relatively broad resonances were considered an inevitable annoyance reducing spectral resolution. Indeed, line widths in spectra from biological objects are affected by susceptibility gradients (as a result of tissue structures) that cannot be canceled by shim gradients.

In addition, line shapes of **suitable** resonances can be characteristically broadened due to specific **physicochemical parameters** varying across a measured volume of tissue (or other heterogeneous material). A case in point is the shape of the inorganic phosphate ( $P_i$ )  $^{31}P$  resonance as its chemical shift is a function of intracellular pH ( $pH_i$ ) [1,2]; or the H2  $^1H$  resonance of exogenous imidazole ethoxycarbonylpropionic acid (IEPA) whose chemical shift varies with extracellular pH ( $pH_e$ ) [3]. Here, **line shapes** are not only broadened, but actually **encode information** on the **statistical distribution** of parameter values ( $pH_i$  or  $pH_e$ ) within the measured pH-heterogeneous sample. We suggest to decode this information by statistical line shape analysis ('quantitative heterogeneity MRS', qhMRS), and present here the **experimental proof of principle** of our approach, applied to judiciously designed IEPA solutions (phantoms).

This was accomplished by calculating  $\geq 8$  **quantitative descriptors** (in addition to the curve maximum) characterizing each statistical distribution of pH values within a given volume or voxel. Based on the H2-IEPA resonance, our qhMRS technique enables integration of **statistical pH heterogeneity analysis** into  $^1H$  MRSI protocols.

**References:** [1] Lutz NW et al., Cancer Res 2013;73:4616. [2] Graham RA et al., Am J Physiol 1994;266:R638. [3] van Sluis R et al., Magn Reson Med 1999;41:743.

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**PS113**

Mon, 11:30-11:55

**In-vivo NMR and MRI study of Superparamagnetic Iron  
Oxide Nanoparticles (SPIONs) on Daphnia Magna**

Bing Wu<sup>\*1</sup>, Jenne Amy<sup>1</sup>, Ronald Soong<sup>1</sup>, Dieter Gross<sup>2</sup>, Oliver Gruschke<sup>2</sup>, Volker Nieman<sup>2</sup>,  
Klaus Zick<sup>2</sup>, Patricia Monks<sup>3</sup>, Cara Moloney<sup>3</sup>, Dermot Brougham<sup>3</sup>, Andre Simpson<sup>1</sup>

<sup>1</sup>University of Toronto, <sup>2</sup>Bruker BioSpin GmbH, <sup>3</sup>University College Dublin

Superparamagnetic Iron Oxide Nanoparticles (SPIONs) are used extensively in multiple biomedical applications such as hyperthermia, MRI enhancing reagents and drug delivery. However, despite their wide spread application, their environmental impacts, especially on the aquatic environment are not well understood. *Daphnia magna* (water fleas) are key-stone species ubiquitous in freshwater ecosystems and are one of the most common species used in toxicity testing. This research combines magnetic resonance imaging, relaxometry and metabolomics (both in-vivo and ex-vivo) to build a holistic understanding of SPION toxicity. SPIONs with three different core sizes (8 nm, 10 nm, 12 nm), and three different ligand sizes (1 KD, 5 KD, 10 KD) were exposed to *Daphnia* over 24h. The impacts and potential compartmentalization was firstly evaluated by T<sub>2</sub> weighted MRI. Secondly, T<sub>1</sub> and T<sub>2</sub> weighted 2D NMR analyses provided complementary information on the types of biomolecules that interact with the SPIONs inside the organisms. Finally, 2D in-vivo metabolomics provides insight into how the organisms themselves respond on exposure which helps explain the toxic-mode-of-action of the nanoparticles. To our knowledge this is the first time MRI, relaxometry and metabolomics have been combined to provide a comprehensive overview of toxicity inside whole organisms.

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**PS114** Mon, 11:55-12:30

**Imaging Human Brain Metabolism Exploiting Ultra-High Field MRI**  
Anke Henning\*  
*University of Texas Southwestern Medical Center*

In vivo magnetic resonance spectroscopy has evolved during the last 25 years in terms of localization quality and spatial resolution, acquisition speed, artifact suppression, number of detectable metabolites and quantification precision and has benefited from the significant increase of magnetic field strength that recently became available for in vivo investigations. Today it allows for non-invasive and non-ionizing determination of tissue concentrations and metabolic turn-over rates of more than 20 metabolites and compounds with high spatial resolution in the human brain and has established as an important tool for neurophysiological research. This presentation summarizes our recent work using a human 9.4T whole-body MRI scanner. Advantages and technical challenges of ultra-high field human MRI as well as related hardware (RF coils, B0 shimming), data acquisition (RF pulses, Sequences) and data reconstruction approaches are discussed. The high signal-to-noise ratio (SNR) and the spectral resolution at 9.4T in combination with optimized 1H MRSI acquisition and image reconstruction techniques allows for mapping the spatial distribution of a total of 12 metabolites including neurotransmitters, second messengers and antioxidants in the living human brain. Other measurable substances are involved in energy and membrane metabolism. Visualization of concentration differences between gray and white matter and identification of gyri in metabolic MR brain images becomes possible for the first time. In vivo detection of amino acids in vivo is demonstrated and histograms of the amino acid chemical shift distributions extracted from the protein NMR database are used as a fitting model to quantify them. The SNR and spectral separation at UHF also allows for regional functional metabolic readouts and reveal a modulation of energy metabolites and neurotransmitter concentrations. 31P spectroscopy and spectroscopic imaging of the human brain allow mapping of the spatial distribution of energy metabolites such as ATP and NADH. Finally potential neuroscientific and clinical applications are identified.

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**PS115**

Mon, 12:30-13:00

**MRI at 2.15 MHz in a large-bore Halbach Array**

Thomas O'Reilly, Wouter Teeuwisse, Kirsten Koolstra, Andrew Webb\*  
*C.J. Gorter Center for High Field MRI, Leiden University Medical Center*

**Introduction:** Modern clinical MRI systems are able to offer sub-millimetre imaging of the human body. However, the high up-front and maintenance cost of these systems means that much of the world lacks access to this technology. For clinical conditions such as hydrocephalus where very high resolution images are not required, low-field MRI systems can offer a low-cost approach towards providing clinically useful MR images in low-resource settings. In this work we designed and constructed a large-bore, low-cost, low-field, Halbach-based MRI scanner intended for neuroimaging in young children.

**Methods:** The Halbach array is constructed from 23 layers of 12 mm cuboid N48 neodymium magnets, each layer consisting of two rings of magnets with a total magnet length of 50.6 cm with a bore size of 27 cm. The homogeneity of the magnet is improved by tapering the diameter of the Halbach array at the ends. Shimming was performed by placing additional 3 mm cuboid magnets inside the bore of the magnet. Gradient coils were constructed using 1.5 mm diameter copper wire wound on plastic cylindrical formers. A solenoidal RF coil was used for transmit/receive. Total hardware costs were less than 30 000 euros.

**Results & discussion:** The field strength was 50.45 mT with homogeneity 2500 ppm over a 20 cm diameter spherical volume (DSV). Two dimensional phantom images have been acquired with a resolution of 1x1x35 mm in 16 minutes using a spin echo sequence. Image distortion due to gradient non-linearity was corrected by including simulated gradient fields in a model-based reconstruction.

**Conclusion:** The homogeneity of a Halbach array can be improved by tapering the diameter of the magnet away from the center. Initial 2D images have been acquired demonstrating the potential for a low-cost MR system.

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**PS116** Mon, 10:30-11:05

New methods and theory for conformational dynamics of macromolecules  
Arthur Palmer\*  
*Columbia University*

Spin relaxation in NMR spectroscopy is a powerful approach for probing aspects of conformational dynamics in biological macromolecules. Methods for characterizing dynamics on picosecond-nanosecond and on microsecond-millisecond time scales, emphasizing the information content provided by multiple static magnetic fields, are illustrated by applications to the enzyme ribonuclease H and the yeast transcription factor GCN4. Theoretical approaches generalize two-state descriptions of the  $R_1\rho$ , Carr-Purcell-Meiboom-Gill, and CEST experiments to N states with arbitrary kinetic topologies, facilitating applications to complex biological phenomena.

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**PS117**

Mon, 11:05-11:30

**Localization of ligands in human carbonic anhydrase by  
19F PCS NMR and new lanthanide chelating tags**

Daniel Häussinger<sup>\*,1</sup>, Daniel Joss<sup>2</sup>, Raphael Vogel

<sup>1</sup>University of Basel, <sup>2</sup>Universität Basel

Pseudocontact shift (PCS) NMR is a powerful tool to gain long-range structural information on protein-protein and protein-ligand interactions. We have shown the localization of three different fluorine containing high affinity ligands within the 30 kDa enzyme human carbonic anhydrase using only <sup>19</sup>F PCS NMR [1]. The distances between the <sup>19</sup>F atoms and the lanthanide cations ranged between 22 and 38 Å, generating <sup>19</sup>F PCS from 0.409 to 0.078 ppm. Up to five different PCS tensors were analysed to extract the fluorine positions with an accuracy of up to 0.8 Å. A careful investigation on the influence of the number and orientation of the tensors for the different ligands on the precision of the localization revealed new insights into the potentials and limits of PCS NMR, especially for ligand screening.

We would like to report in addition on several new lanthanide chelating tags (LCT), based on sterically overcrowded tetra-methyl- or tetra-isopropyl-cyclen scaffolds.

Combination with bulky side-arms and a highly reactive, reduction stable pyridine-thiazolo linker results in versatile LCT. All new tags were benchmarked on ubiquitin and also on human carbonic anhydrase and demonstrated very large PCS and RDC. In order to explore the theoretical PCS limit for an LCT with ideal rigidity relative to the protein, we determined for the first time the intrinsic anisotropy parameters of strongly paramagnetic LCT on the free tags. To assign the proton NMR spectra that display a proton chemical shift range of up to 1500 ppm and a T2 relaxation time of less than 50 µs, various isotope labelling schemes had to be used, as 2D NMR spectra are not feasible. Besides unusually large susceptibility tensors, we also unravelled Fermi contact shifts in the proton spectra that are in contradiction to current theoretical models.

**References:** [1] K. Zimmermann et al., Chem. Sci. **2019**, DOI: 10.1039/c8sc05683h.

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**PS118**

Mon, 11:30-11:55

## Accurate Measurement of Transverse Relaxation Rates in Systems with Coupled Protons

Peter Kiraly\*, Mathias Nilsson, Gareth Morris  
*University of Manchester*

Measuring transverse relaxation rates provides insight into the dynamics of molecules. For example, measurements of relaxation dispersion allow the study of invisible conformations of proteins. Such applications are currently restricted to sparse spin systems in which homonuclear couplings can be neglected, such as 15NH or selectively labelled 13CHD2 groups in proteins. The accurate measurement of transverse relaxation rates is also essential if quantitative results are to be obtained from multipulse NMR experiments where there are T2 differences among the spins observed.

The common experiments for measuring T2 in coupled spin systems, CPMG [1] and PROJECT [2], can suppress the signal modulations caused by homonuclear J couplings. However in both cases the relaxation measured is not specific to a given signal, but contains contributions from all the spins in a coupled system because of the sharing of coherence. Measured T2 values therefore differ from the true T2s. We propose a new approach, Active Spin Refocusing (ASR) T2 measurement, which allows broadband measurement of 'true' transverse relaxation rates even in coupled spin systems. It uses a single spin echo, with the refocusing element flanked by variable delays instead of trains of pulses so that the measured signal decay reflects the true loss of transverse magnetisation during free precession. J modulation is suppressed by using an active spin refocusing element of the sort now commonly used in pure shift NMR methods such as that of Zangger and Sterk [3]. This allows true T2 measurements on all signals in a spectrum in a single experiment, albeit at the price of a reduction in sensitivity.

**References:** [1] S. Meiboom and D. Gill, Rev. Sci. Instr., 1958, 29, 688-691. [2] J.A. Aguilar et al, Chem. Commun., 2012, 48, 811-813. [3] K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.

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**PS119**

Mon, 11:55-12:30

## Conformation Changes in Proteins Made Visible by Lanthanide Tags

Gottfried Otting\*

*Australian National University*

The coordinates of a nuclear spin relative to a lanthanide ion can be determined with high accuracy in a system, where a paramagnetic lanthanide tag is attached to a protein of known three-dimensional structure. First, pseudocontact shifts (PCS) of the protein are measured by NMR to determine the coordinate frame of the magnetic susceptibility anisotropy (DeltaChi) tensor associated with the lanthanide tag. Next, the PCS of the nuclear spin of interest is used positions it relative to the DeltaChi tensor. With lanthanide tags at three different sites, the position of the nuclear spin can be restricted in a way analogous to the global positioning system (GPS) of a mobile phone. Examples of this approach are shown for changes in amino acid side chain position triggered by metal binding and in response to ligand binding.

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**PS120** Mon, 12:30-13:00

**De-correlating kinetic and relaxation parameters in exchange saturation transfer NMR**  
Alberto Ceccon, G. Marius Clore, Vitali Tugarinov\*  
*National Institutes of Health*

The interaction of the N-terminal domain of huntingtin exon-1 with membrane surfaces promotes poly-glutamine mediated aggregation, and is thought to play a role in the etiology of Huntington's disease. We investigated the kinetics of binding of two huntingtin peptides, comprising the 16-residue N-terminal amphiphilic domain alone (httNT) and with a seven residue poly-glutamine C-terminal tract (httNTQ7), to small unilamellar lipid vesicles (SUV), ~31 nm in diameter and ~4.3 MDa in molecular weight, using solution NMR experiments designed to probe interactions of NMR visible states with sparsely-populated, invisible or 'dark', high molecular weight species. Specifically, we make use of Dark state Exchange Saturation Transfer (DEST) and lifetime line broadening (dR2) supplemented with the measurements of the maximal value of the contribution of fast-relaxing magnetization component to the total NMR signal, Cfast\_max. In the exchange regime where the transverse spin relaxation rates in the bound state are smaller than the strength of the DEST saturation radio-frequency field, the combination of DEST and dR2 data is not sufficient to unambiguously determine the population of the bound state (pB) and its transverse relaxation rates at the same time. We show that these exchange and relaxation parameters can be de-correlated by the measurement of Cfast\_max which is directly proportional to pB. When integrated into the analysis of DEST/dR2 data, Cfast\_max provides an indispensable source of information for quantitative studies of exchange involving high-molecular-weight dark states. While the population of the species bound to the SUV surface is substantial, on the order of 7-8%, the exchange between the free peptides and the SUV-bound states is slow on the relaxation time-scale (kex~200 1/s). The C-terminal regions of the peptides remain flexible even in the SUV-bound form due to transient detachment from the lipid surface that occurs on a time-scale several-fold faster than the binding proper.

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**PS121**

Mon, 10:30-11:05

## Pulsed EPR and ENDOR with Photonic Bandgap Superconducting Microresonators

Anthony Sigillito<sup>1</sup>, Alexei Tyryshkin<sup>1</sup>, Schenkel Thomas<sup>2</sup>, Andrew Houck<sup>1</sup>, Stephen Lyon <sup>\*,1</sup>

<sup>1</sup>Princeton University, <sup>2</sup>Lawrence Berkeley National Lab

Superconducting microresonators are powerful tools for measuring electron paramagnetic resonance in very small sample volumes. By keeping the thickness of the superconductor below a penetration depth, and aligning the DC magnetic field in the plane of the superconductor, high fields (much larger than the critical field) are possible. With transmission-line geometry resonators (typically coplanar waveguide structures) the mode volume can be a few microns in two dimensions, while of order a wavelength in the third dimension. By utilizing unique properties of superconductors, such as large kinetic inductance, the length of the resonators can be substantially reduced, and planar lumped-element structures can be smaller still. Here we will discuss transmission-line structures which employ ‘mirrors’ consisting of a periodically modulated impedance transmission line. Coplanar waveguide based structures of this variety have a continuous center conductor, allowing DC and low-frequency driving signals, as well as the microwaves for the EPR. A DC current can be used to electrically tune a resonator, using kinetic inductance, though here we will discuss ENDOR experiments in which the RF current is driven through the center conductor. We demonstrate this ENDOR microresonator using phosphorus and arsenic donors in isotopically enriched silicon. Surprisingly, the nuclear spin transitions can also be driven by a resonant electric field (no current) applied to the center pin. For Si:P the effect appears to be mediated by the hyperfine interaction between the donor electron and the nuclear spin. In the case of Si:As, however, the nuclear spins are being driven directly through the nuclear quadrupole interaction. These appear to be the first observations of quadrupolar nuclear electric resonance in a nonpolar crystal.

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**PS122**

Mon, 11:05-11:30

**Chemical Exchange Processes Studied by 95 GHz 2D-ELDOR**Boris Dzikovski<sup>\*,1</sup>, Valery Khramtsov<sup>2</sup>, Curt Dunnam<sup>1</sup>,  
Siddarth Chandrasekaran<sup>1</sup>, Jack Freed<sup>1</sup><sup>1</sup>Cornell University, <sup>2</sup>West Virginia University

Exchange processes which include conformational change, protonation/deprotonation, binding equilibria etc. are routinely studied by various 2D NMR techniques, e.g. EXSY, ZZ-exchange, CEST. In these techniques the information about exchange of nuclei between environments with different NMR parameters is obtained from the cross-peak development. Cross-peaks due to chemical exchange have been previously seen in EPR, but for most common EPR probes their observation and analysis at low EPR frequencies is difficult because the exchanging states are poorly resolved and their separation is comparable or less than their individual linewidths. With 2D ELDOR spectroscopy at 95GHz we benefited from the increased g-factor resolution to study chemical and physical exchange for protonation/deprotonation and partition equilibria of nitroxide radicals. The protonation/deprotonation process was studied for a pH sensitive imidazoline spin label, with both the relative ratio of exchanging states and the exchange rate controlled by the composition and the concentration of the buffer solution respectively[1]. This allowed for reliable assignments of cross-peaks related to chemical exchange and for separating them from cross-peaks emerging from Heisenberg exchange and Electron-Nuclear Dipole (END) interactions. The exchange rate obtained from the cross-peaks is in good agreement with the changes in relaxation times of the exchanging states derived from the same 2D ELDOR experiment and other EPR experiments. For a totally different system of a nitroxide radical partitioning between polar and non-polar environments in microemulsions and multilamellar lipid vesicles we also demonstrated the cross-peak development owing to physical exchange between different phases and measured its rate. These experiments were carried out on ACERT's newly rebuilt 95 GHz 2D ELDOR spectrometer.

**References:** [1] Khramtsov V, Bobko A, Tseitlin M, Driesschaert B. Exchange Phenomena in the Electron Paramagnetic Resonance Spectra of the Nitroxyl and Trityl Radicals: Multifunctional Spectroscopy and Imaging of Local Chemical Microenvironment. *Anal Chem.* 2017 89(9):4758-4771.

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**PS123**

Mon, 11:30-11:55

**Tuning Spin Dynamics in Crystalline Tetracene**

Naitik A. Panjwani<sup>\*,1</sup>, Sam L. Bayliss<sup>1</sup>, Felix Kraffert<sup>1</sup>, Rui Wang<sup>2</sup>,  
Chunfeng Zhang<sup>3</sup>, Robert Bittl<sup>1</sup>, Jan Behrends<sup>1</sup>

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<sup>2</sup>*National Laboratory of Solid State Microstructures, School of Physics, Nanjing University, Nanjing 210093, China,*

<sup>3</sup>*National Laboratory of Solid State Microstructures, School of Physics, Nanjing University, Nanjing 210093, China; Synergetic Innovation Center in Quantum Information and Quantum Physics, University of Science and Technology of China, Hefei, Anhui 230026, China*

Tetracene is an archetypal material undergoing singlet fission—the generation of a pair of triplet excitons from one singlet exciton. Here, using time-resolved electron spin resonance, we show how the spin dynamics in tetracene crystals are influenced by temperature and morphology. Upon cooling from 300 to 200 K, we observe a switch between singlet fission and intersystem crossing generated triplets, manifesting as an inversion in transient spin polarization. We extract a spin dephasing time of approximately 40 ns for fission-generated triplets at room temperature, nearly 100 times shorter than the dephasing time that we measure for triplets localized on isolated tetracene molecules. These results highlight the importance of morphology and thermal activation in singlet fission systems. In addition, we present recent findings on single crystal tetracene studied using optically detected magnetic resonance, where additional features in the spectrum are observed.

We acknowledge support from DFG SPP-1601 (BI 464/10-2, BE 5126/1-2) and the Nanoscale project within the excellence initiative of the Freie Universität Berlin. The work at Nanjing University is supported by the National Natural Science Foundation of China (21873047). We thank L. R. Weiss for insightful discussions.

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**PS124**

Mon, 11:55-12:30

**Trityls vs nitroxides as spin labels**

Elena Bagryanskaya\*

*N.N.Vorozhtsov Institute of Organic Chemistry SB RAS*

Pulse dipolar EPR is widely used to study tertiary structure, dynamics and functional features of biomolecules. Nitroxides are commonly used spin labels. Trityl radicals or TAMs have appeared recently as an alternative source of spin labels using PDEPR[1]. In this presentation we compared functional properties of spin labels based on TAMs and nitroxides. PDEPR in combination with MD were used to investigate the conformational changes in DNA with AP site and in complex with AP endonuclease1 (APE1). For this sake, triaryl-methyl (TAM) based spin labels were attached to the 5'-ends of oligonucleotide duplex, and nitroxide spin labels were introduced into the APE1 site. In this way, we for the first time created the system that allowed to follow the conformational changes of the main APE1 substrate by EPR. The use of different (orthogonal) spin labels in the enzyme and in DNA substrate has crucial advantage and allows detailed investigation of local damages and conformational changes in AP-DNA alone, as well as in its complex with APE1. We use very hydrophilic OX063 with very-low toxicity and little tendency for aggregation as the basis for a spin label for human serum albumin (HSA). EPR spectra of HSA-OX063 have an intense, narrow line typical of TAM radicals in solution while HSA-FTAM showed extensive aggregation. In pulse EPR measurements, the measured  $T_m$  for HSA-OX063 is 6.3  $\mu$ s at 50 K, the longest yet obtained with trityl based spin-labels[3]. CW and PDEPR were used to study of the mechanism of penetration of TAM or nitroxide spin labeled intrinsically disordered protein into human cell.

This study was supported by Ministry of Science and Education of the RF(grant14.W03.31.0034).

**References:** [1] O.Krumkacheva, E.Bagryanskaya, Trityl radicals as spin labels, From the book: Electron Paramagnetic Resonance:v. 25, 2016,25. [2] O.Krumkacheva et al.,NAR, 2019, submitted. [3] V.Tormyshev,et al., Chem. Eur. Jour. 2019, submitted.

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**PS125**

Mon, 12:30-13:00

**Deep neural network analysis of DEER data**Steven Worswick<sup>1</sup>, James Spencer<sup>1</sup>, Gunnar Jeschke<sup>2</sup>, Ilya Kuprov<sup>\*,1</sup><sup>1</sup>University of Southampton, <sup>2</sup>ETH Zurich

It is demonstrated that deep neural networks (DNN) are a powerful alternative to Tikhonov regularisation methods for the interpretation of double electron-electron resonance (DEER) data. Networks trained using large databases of synthetic DEER traces with carefully modelled distortions and noise are found to process previously unseen experimental data with results comparable to, and occasionally better than, the state-of-the-art Tikhonov methods.

The current best practice for DEER processing is to use Tikhonov regularised deconvolution, a procedure that works well in simple spin- $\frac{1}{2}$  pairs, but becomes difficult for more complex systems [1]. Using DNNs trained on simulated data is attractive because training data can be generated to include all complications. Such neural networks can be made resilient to the presence of zero-field splittings, exchange, and out-of-pair inter-electron interactions.

DNN performance strongly depends on the quality of the training dataset. To ensure that the network can successfully process previously unseen datasets, the training database must be representative of the entire range of real experimental systems. The relevant functionality has recently become available in the Spinach library. There are also important factors around the network architecture, pre- and post-processing of data, and the training process.

In this communication we introduce DEERNet and describe how we have created the training database, tuned the network parameters, and handled data pre-processing to achieve excellent performance on real-life DEER data [2].

**References:** [1] G. Jeschke, Y. Polyhach, Phys. Chem. Chem. Phys., 2007, 9, 1895-1910. [2] S.G. Worswick, J.A. Spencer, G. Jeschke, I. Kuprov, Science Advances, 2018, 4 (8), eaat5218.

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**PS126** Mon, 16:15-16:50

**New Long-Distance SSNMR Techniques and  
Their Applications to Protein Structure Determination**  
Mei Hong\*  
*MIT*

In this talk I will first present our recent development of multiple <sup>19</sup>F-based solid-state NMR techniques that increase the distance reach of NMR to ~2 nm. <sup>19</sup>F-<sup>19</sup>F distances can be measured by both spin diffusion and dipolar recoupling techniques under fast MAS frequencies of 25 – 55 kHz at the medium-high magnetic field of 14.1 Tesla. Fast MAS suppresses the <sup>19</sup>F chemical shift anisotropy sidebands without compromising the dipolar coupling measurements. An empirically calibrated master curve has been obtained from <sup>19</sup>F-<sup>19</sup>F spin diffusion buildup rates to allow extraction of semi-quantitative distances. We also demonstrate <sup>13</sup>C-<sup>19</sup>F and <sup>1</sup>H-<sup>19</sup>F distance measurements under fast MAS. These long-distance <sup>19</sup>F SSNMR techniques are instrumental for determining the cholesterol-binding site of the influenza M2 protein, the oligomeric structure of the HIV fusion protein gp41, and the interhelical packing of the influenza B virus M2 protein. In the second part of the talk, I will describe the determination of a novel amyloid fibril structure formed by the anti-hypoglycemia pharmaceutical peptide, glucagon.

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**PS127**

Mon, 16:50-17:15

**Molecular mechanisms behind Remorin nanodomain formation**

Anthony Legrand<sup>1</sup>, Denis Martinez<sup>2</sup>, Julien Gronnier<sup>3</sup>, Melanie Berbon<sup>4</sup>,  
 Marion Decossas<sup>5</sup>, Paul Gouget<sup>3</sup>, Axelle Grelard<sup>2</sup>, Veronique Germain<sup>3</sup>,  
 Olivier Lambert<sup>6</sup>, Antoine Loquet<sup>2</sup>, Sebastien Mongrand<sup>3</sup>, Birgit Habenstein<sup>\*,2</sup>

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 5248 CNRS University of Bordeaux, <sup>3</sup>LBM UMR 5200 CNRS University of Bordeaux,

<sup>4</sup>CBMN / IECB UMR 5248 CNRS University of Bordeaux, <sup>5</sup>CBMN UMR 5248  
 CNRS University of Bordeaux, <sup>6</sup>CBMN UMR 5248 CNRS University of Bordeaux

Protein and lipid components in biological membranes act as a dynamic network of subtle molecular interactions segregating the membrane into particular highly dynamic regions called nanodomains. Nanodomains constitute functional platforms enriched in specific lipids (such as sterols and phosphoinositides) and proteins to perform their diverse activities. Remorins (REMs) are plant proteins and well-established nanodomain markers and can, as such, be considered as paradigm to provide a mechanistic description of membrane organisation into functional nanodomains. In a divide-and-conquer approach, we describe the impact of StREM1.3's C-terminal membrane anchor [1], its oligomerization domain [2] and the intrinsically disordered region on membrane structure and dynamics. Furthermore, we tackle the structural features of StREM1.3 and its domains when associated to nanodomain-mimicking membranes by solid-state NMR. We show that StREM1.3 drives nanodomain organisation by concerted lipid-protein and protein-protein interactions, highlighting the dedicated role of each domain. We reveal a delicate balance between hydrophobic and electrostatic effects leading up to the protein's characteristic affinity for negatively charged phospholipids.

**References:** [1] Gronnier J, et al.; Elife. 2017; 6. pii: e26404. [2] Martinez D, et al.; J Struct Biol. 2019; 206(1); 12-19.

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**PS128**

Mon, 17:15-17:40

## Solution NMR of nanodisc-embedded proteins: new molecular insights into protein-protein and protein-membrane interactions

Inbal Sher, Arwa Qasim, Netanel Mendelman, Adi Yahalom, Hadassa Shaked, Jordan Chill\*

*Department of Chemistry, Bar Ilan University*

Membrane-associated proteins (MAPs), such as channels, pumps and receptors, are notoriously difficult to study by structural methods because they require a stabilizing surrogate lipo-environment, often making sample preparation and data acquisition challenging. At the same time biological processes occurring at the cell membrane, particularly protein-protein and protein-membrane interactions, are deeply involved in homeostasis and disease; indeed, over 50% of approved pharmaceuticals target this class of cellular contacts. Thus, there is great motivation to reach a structural understanding of membrane protein biochemistry despite these objective challenges. Here we demonstrate successful applications of the lipoprotein nanodisc (LPN) technology, providing close-to-native membrane assemblies, to addressing structural questions in the membrane environment.

By incorporating the potassium channel KcsA in LPNs we could reliably identify the molecular basis of biological function in two regions of interest, the toxin-binding extracellular region and the putative pH-gating region in the cytoplasmic C-terminal domain. We used NMR to determine the structures of de novo and natural KcsA-blocking toxins, and, in combination with electrophysiology measurements, the basis for specific recognition between toxins and various channels. LPNs also enabled us to follow the tetramer-to-monomer transition in the channel's cytoplasmic domain by NMR and EPR in terms of pH-gating, a subject of controversy in previous studies, as well as coupling to other gates. Finally, we employed LPNs to investigate host membrane-targeting by the cytotoxic effector BteA, secreted by the pathogen *Bordetella pertussis* responsible for causing whooping cough. Chemical shift perturbation analysis of wildtype and mutant BteA, backed by additional biophysical methods, showed that this four-helix bundle domain binds to membranes in a phosphatidylinositol-dependent manner, and defined a membrane-targeting motif that differs from that of previously described effectors. We thus demonstrate the utility of NMR methods in conjunction with LPNs in elucidating the structural biology of membrane-associated proteins.

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**PS129**

Mon, 16:15-16:50

## Probing Ion Mobility Mechanisms in Solid Electrolytes using Solid-State NMR

Karen Johnston<sup>\*1</sup>, Tavleen Attari<sup>1</sup>, Theodosis Famprikis<sup>2</sup>,

James Dawson<sup>3</sup>, Christian Masquelier<sup>2</sup>, Saiful Islam<sup>3</sup>

<sup>1</sup>Durham University, <sup>2</sup>Universite de Picardie Jules Verne, <sup>3</sup>University of Bath

All-solid-state Li-ion batteries are attracting considerable attention as possible alternatives to conventional liquid electrolyte-based devices as they present a viable opportunity for increased energy density and safety. In recent years, a number of candidate materials have been explored as possible solid electrolytes, including garnets, Li-stuffed garnets, Li-rich anti-perovskites (LiRAPs), thio-LISICONs and complex spinels. LiRAPs, including  $\text{Li}_{3-x}\text{OH}_x\text{Cl}$ , have generated considerable interest based on their reported ionic conductivities (on the order of  $10^{-3} \text{ S cm}^{-1}$ ). [1],[2] However, until very recently, their lithium and proton transport capabilities as a function of composition were not fully understood. Hence, current research efforts have focused on the synthesis and structural characterisation of  $\text{Li}_{3-x}\text{OH}_x\text{Cl}$  using a combination of *ab initio* molecular dynamics and variable-temperature  $^1\text{H}$ ,  $^7\text{Li}$  and  $^{35}\text{Cl}$  solid-state NMR spectroscopy. Using this unique combination of techniques, it is possible to study the mobility of both the Li ions and protons. We will demonstrate that Li-ion transport is highly correlated with the proton and Li-ion vacancy concentrations. In particular, we will show that the Li ions are free to move throughout the structure, whilst the protons are restricted to solely rotation of the  $\text{OH}^-$  groups. Based on these findings, and the strong correlation between long-range Li-ion transport and  $\text{OH}^-$  rotation, we have proposed a new Li-ion hopping mechanism, which suggests that the Li-rich anti-perovskite system is an excellent candidate electrolyte for all-solid-state batteries. [3] However, to fully understand the mechanism for conduction, multiple, complementary characterisation techniques are needed.

**References:** [1] Y. Zhao and L. L. Daemen, *J. Am. Chem. Soc.*, 2012, **134**, 15042. [2] A. Emly, E. Kioupakis and A. Van der Ven, *Chem. Mater.*, 2013, **25**, 4663. [3] J. A. Dawson, T. S. Attari, H. Chen, S. P. Emge, K. E. Johnston and M. S. Islam, *Energy Environ. Sci.*, 2018, **10**, 2993.

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**PS130**

Mon, 16:50-17:15

**Structure and Dynamics of Defects in Metal-organic Frameworks studied by Solid-state NMR**

Yao Fu<sup>\*1</sup>, Alex Forse<sup>2</sup>, Jeffrey Reimer<sup>2</sup>, Xueqian Kong<sup>1</sup>

<sup>1</sup>Zhejiang University, <sup>2</sup>UC Berkeley

Metal-organic frameworks (MOFs) are porous crystalline materials with promising applications in molecular adsorption, separation, and catalysis. It has been discovered recently that structural defects introduced unintentionally or by design could have a significant impact on their properties. However, the exact chemical composition and structural evolution under different conditions at the defects are still under debate.

In the first part, we probed the existence of residual modulator: the commonly-used acetic acid, which controls the formation of defects in UIO-66. We discovered that acetate molecules coordinate to a single metal site monodentately and pair with water at the neighboring position. The acetates are highly flexible which undergo fast libration as well as a slow kinetic exchange with water through dynamic hydrogen bonds. The dynamic processes under variable temperatures and different hydration levels have been quantitatively analyzed by SUPER and CODEX experiments. The integration of SSNMR and computer simulations allow a precision probe into defective MOF structures with intrinsic dynamics and disorder.

In another related work, we studied Mg<sub>2</sub>(dobpdc) (dobpdc<sup>4-</sup> = 4,4'-dioxidobiphenyl-3,3'-dicarboxylate) that contains accessible coordinatively unsaturated metal sites. We investigated the defect chemistry of Mg<sub>2</sub>(dobpdc) when synthesized with 4-fluorosalicylic modulators. We illustrated that by varying the concentration of modulator, the linker vacancies can be tuned systematically and the concentration of the ligand substitution defects can be as high as ~35%. We uncovered the detailed structure of modulated Mg<sub>2</sub>(dobpdc) and the defects distribution by REDOR solid-state NMR experiments.

Y. Fu, Z. Kang, J. Yin, W. Cao, Y. Tu, Q. Wang, X. Kong *Nano Letters*, 2019, 19.1618-1624

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**PS131**

Mon, 17:15-17:40

**Solid-state and in situ NMR studies of flexible metal-organic frameworks**

Eike Brunner<sup>\*</sup>, Sebastian Ehrling, Stefan Kaskel, Silvia Paasch,  
 Marcus Rauche, Irena Senkovska  
 TU Dresden

Framework flexibility (elasticity), i.e., the ability of a metal-organic framework (MOF) to considerably change its structure as a function of relevant parameters like pressure, temperature, and type of adsorbed molecules is only observed for some special compounds. The MOF compound  $\text{Ni}_2(2,6\text{-ndc})_2(\text{dabco})$  [2,6-ndc: 2,6-naphthalenedicarboxylate, dabco: 1,4-diazabicyclo[2.2.2]octane, further denoted as DUT-8(Ni)] can be synthesized in flexible and non-flexible (rigid) form of equal chemical composition just by controlling the particle size [1]. The unit cell of the flexible form changes its volume by more than 100% during the reversible, adsorption-induced structure opening! Here, we comparatively study flexible and non-flexible DUT-8(Ni) in order to answer two questions: What are the structural differences between these two variants? And does framework flexibility influence adsorption selectivity from gas mixtures? To answer the first question, we selectively isotope-labeled promising framework positions with  $^{13}\text{C}$  and  $^2\text{H}$ . This allows to selectively detect carboxylate  $^{13}\text{C}$  atoms close by the Ni centers and to study the mobility of the organic linker molecules by  $^2\text{H}$  NMR spectroscopy. Extended solid-state NMR investigations encompassing  $^2\text{H}$  exchange spectroscopy (EXSY),  $^{13}\text{C}$ - $^1\text{H}$  heteronuclear correlation (HETCOR), and others revealed that the non-flexible form exhibits a higher fraction of defects and dynamically disordered linker molecules compared to the flexible variant. The second question is studied by quantitative high-pressure *in situ*  $^{13}\text{C}$  NMR spectroscopy of gas adsorption from mixtures containing  $^{13}\text{C}$ -enriched  $\text{CO}_2$  and  $\text{CH}_4$ . Flexible DUT-8(Ni) indeed exhibits a significantly higher selectivity for carbon dioxide adsorption from these mixtures than the rigid form [2]. That means, framework flexibility seems to influence adsorption selectivity.

**References:** [1] N. Kavoosi, V. Bon, I. Senkovska, S. Krause, C. Atzori, F. Bonino, J. Pallmann, S. Paasch, E. Brunner, S. Kaskel, *Dalton Trans.* **2017**, 46, 4685. [2] M. Sin, N. Kavoosi, M. Rauche, J. Pallmann, S. Paasch, I. Senkovska, S. Kaskel, E. Brunner, *Langmuir* **2019**, 35, 3162.

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**PS132**

Mon, 16:15-16:50

**Electron Spin Relaxation Mechanisms of Atomic Hydrogen Trapped in Silsesquioxane Cages: the Role of Isotope Substitution**George Mitrikas<sup>\*,1</sup>, Raanan Carmieli<sup>2</sup><sup>1</sup>*Institute of Nanoscience and Nanotechnology, NCSR Demokritos,*<sup>2</sup>*Department of Chemical Research Support, Weizmann Institute of Science*

Encapsulated atomic hydrogen in silsesquioxane cages is a promising candidate for applications in emerging technologies like spin-based quantum computing, magnetic field sensing, and atomic clock devices. Compared to endohedral fullerenes ( $N@C_{60}$  or  $P@C_{60}$ ), which are currently the most used molecular spin systems for demonstrating single-quantum gates and quantum memories, atomic hydrogen is more attractive due to its simpler electronic 1s state and the exceptionally large hyperfine coupling of about 1420 MHz. Detailed pulsed EPR studies of parameters relevant to quantum computing like electron spin-lattice ( $T_1$ ) and phase memory ( $T_M$ ) relaxation times are scarce and concern exclusively cages of the type  $Si_8O_{12}R_8$  with  $R=C_2H_5$  [1],  $R=C_3H_7$  (n-propyl) [2], and  $R=OSi(CH_3)_2H$  [3]. Recently, [4] we applied dynamical decoupling methods in order to suppress nuclear spin diffusion in  $H@h_{72}Q_8M_8$ , the derivative with  $R=OSi(CH_3)_3$ . Herein we examine for the first time the effect of deuterium isotopic substitution on the spin relaxation properties of  $H@h_{72}Q_8M_8$ , by applying pulsed electron paramagnetic resonance (EPR) methods on its deuterated analogues  $H@d_{72}Q_8M_8$  and  $D@d_{72}Q_8M_8$ . For the latter species we measure a phase memory time of 60  $\mu s$  at 180 K, the largest obtained so far for this family of molecular spins. We show that selective substitution of encapsulated or peripheral hydrogen atoms with deuterium reveals high-temperature relaxation mechanisms that were previously hidden by proton nuclear spin diffusion. Unusually short  $T_M$  values observed for all deuterated species even at liquid helium temperatures are discussed in terms of tunneling reorientation of methyl groups.

**References:** [1] Weiden et al, Appl. Magn. Reson. 2001, 21, 507–516. [2] Schoenfeld et al, Phys. Status Solidi B 2006, 243, 3008–3012. [3] G. Mitrikas, Phys. Chem. Chem. Phys., 2012, 14, 3782–3790. [4] G. Mitrikas et al, Phys. Chem. Chem. Phys., 2014, 16, 2378–2383.

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**PS133**

Mon, 16:50-17:15

**Coherent control of solid state nuclear spin nano-ensembles****Boris Naydenov**<sup>\*,1</sup>, Jochen Scheuer<sup>2</sup>, Thomas Unden<sup>2</sup>, Nikolas Tomek<sup>2</sup>,Paz London<sup>3</sup>, Ilai Schwartz<sup>4</sup>, Martin Plenio<sup>4</sup>, Fedor Jelezko<sup>2</sup>

<sup>1</sup>*Institute for Nanospectroscopy, Helmholtz Zentrum Berlin für Materialien und Energie, Kekulestr. 5, 12489 Berlin,* <sup>2</sup>*Institute of Quantum Optics, Ulm University, Albert Einstein Allee 11, 89081 Ulm, Germany,* <sup>3</sup>*Department of Physics, Technion, Israel Institute of Technology, Haifa 32000, Israel,* <sup>4</sup>*Institute of Theoretical Physics, Ulm University, Albert Einstein Allee 11, 89069 Ulm, Germany*

Nitrogen-vacancy color centers (NVs) in diamond can be measured at the single cite level even at room temperature, allowing to perform a variety of fundamental experiments.

Here the recent progress in controlling small nuclear spin ensembles at ambient conditions will be presented. A Dynamic Nuclear spin Polarization (DNP) method was developed to transfer the NV's high (> 92 %) electron spin polarization induced by short laser pulses to the surrounding <sup>13</sup>C carbon nuclear spins ( $I = 1/2$ ). Here the NV is repeatedly repolarized optically, thus providing an effectively infinite polarization reservoir. The polarisation of the nuclear spin ensemble was so high, that it lead to narrowing of the line width of a single NV electron spin transition by a factor of eight [1]. The same technique was used to polarize a macroscopic ensemble of <sup>13</sup>C spins, where we achieved an increase of the NMR signal by a factor of 45 [2]. A novel method for both polarization of the nuclear spin bath and its quantitative measurement will be demonstrated - Polarization Read Out by Polarization Inversion (PROPI) [3]. With this technique we are able to determine the exact number of spin quanta transferred from the NV's electron spin to the nuclear spin bath. Finally, NMR spectroscopy of few tens of nuclear spins is demonstrated by using radio frequency pulses for coherent control and PROPI for polarization and readout of the spin ensemble [4].

**References:** [1] P. London et al., Phys. Rev. Lett. 111, 067601 (2013). [2] J. Scheuer et al., New J. Phys. 18, 013040 (2016). [3] J. Scheuer et al., Phys. Rev. B 96, 174436 (2017). [4] T. Unden et al., npj Quantum Information 4, 39 (2018).

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**PS134**

Mon, 17:15-17:40

**Stable radicals tethered to pentacene studied using time resolved EPR and transient absorption spectroscopy**

Claudia E. Avalos<sup>\*1</sup>, Sabine Richert<sup>2</sup>, Etienne Socie<sup>1</sup>, Ganesan Karthikeyan<sup>3</sup>,  
 Gabriele Stevanato<sup>1</sup>, Dominik J. Kubicki<sup>1</sup>, Jacques -E. Moser<sup>1</sup>, Christiane R. Timmel<sup>2</sup>,  
 Moreno Lelli<sup>4</sup>, Aaron J. Rossini<sup>5</sup>, Olivier Ouari<sup>6</sup>, Lyndon Emsley<sup>1</sup>

<sup>1</sup>*Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne,*

<sup>2</sup>*Department of Chemistry, University of Oxford, Inorganic Chemistry Laboratory,*

<sup>3</sup>*Aix-Marseille Univ, CNRS, ICR, 13397 Marseille, France,* <sup>4</sup>*Department of Chemistry,*

*University of Florence, Center for Magnetic Resonance, Sesto Fiorentino (FI), Italy,*

<sup>5</sup>*Department of Chemistry, Iowa State University,* <sup>6</sup>*Aix-Marseille Université, CNRS*

The ability to generate well-defined states with large electron spin polarization is useful for applications in molecular spintronics, high-energy physics and magnetic resonance spectroscopy. Pentacene-radical derivatives can rapidly form triplet excited states through enhanced intersystem crossing and under the right conditions this can in turn lead to polarization of the tethered radical [1]. The magnitude of the spin polarization on the radical substituent depends on many factors: local magnetic and electric fields, molecular geometry, and spin-spin coupling [2-4]. In this work we present time resolved electron paramagnetic resonance (TREPR) and field swept echo detected electron paramagnetic resonance (FSEPR) measurements on three pentacene derivatives with trityl, BDPA or TEMPO substituents. We observe polarization transfer between the pentacene excited triplet and the TRITYL radical, but do not observe the same for the BDPA and TEMPO derivatives. We also investigate polarization transfer in the pentacene-TRITYL system in different glassy environments and observe distinct polarization transfer behavior depending on the solvent used. We explain the TREPR and FSEPR measurements by comparing the excited-state dynamics of the three pentacene derivatives from nanosecond and femtosecond transient absorption measurements. We observe a two order of magnitude difference in the timescale of triplet formation of the pentacene TRITYL system when compared to the pentacene with the BDPA and TEMPO substituents.

**References:** [1] Chernick, E. T.; Casillas, R.; Zirzmeier, J.; Gardner, D. M.; Gruber, M.; Kropp, H.; Meyer, K.; Wasielewski, M. R.; Guldi, D. M.; Tykwinski, R. R., *J Am Chem Soc* 2015, 137, 857-863. [2] Ito, A.; Shimizu, A.; Kishida, N.; Kawanaka, Y.; Kosumi, D.; Hashimoto, H.; Teki, Y., *Angew Chem Int Edit* 2014, 53, 6715-6719. [3] Jenks, W. S.; Turro, N. J., *J Am Chem Soc* 1990, 112, 9009-9011. [4] Ishii, K.; Takeuchi, S.; Kobayashi, N. *J Phys Chem A* 2001, 105, 6794-6799.

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**PS135**

Mon, 16:15-16:50

## Copper trafficking in eukaryotic systems:

### Current knowledge from experimental and computational efforts

Sharon Ruthstein\*

*Bar Ilan University*

Copper plays a vital role in fundamental cellular functions, and its concentration in the cell must be tightly regulated, as dysfunction of copper homeostasis is linked to severe neurological diseases and cancer. This talk provides a compendium of current knowledge regarding the mechanism of copper transfer from the blood system to the Golgi apparatus; this mechanism involves the blood carrier protein, human serum albumin (HSA), the copper transporter hCtr1, the metallochaperone Atox1, and the ATPases ATP7A/B. I will discuss key insights regarding the structural and functional properties of the hCtr1-Atox1-ATP7B cycle, obtained from diverse studies relying on Electron Paramagnetic Resonance (EPR) measurements, complementary biophysical methods, biochemical, and computational methods. Last, I will demonstrate how basic understanding of the function of these systems can assist us in designing new class of bi-markers and therapeutic agents.

**PS136**

Mon, 16:50-17:15

## Structural description of the target search process by a disordered transcription factor

Conor Kelly\*, Christina Redfield, Sarah Shammass

*University of Oxford*

Specific transcription factors must search for their target sites amongst a vast excess of non-specific DNA. They find their sites quickly using a combination of sliding, jumping, hopping and intersegmental transfer. DNA binding domains (DBDs) are usually thought of as structured, however this is not always the case. There are also 3 very large classes of transcription factors whose DBD are disordered in the absence of DNA – bZIPs, bHLH and AT hooks. I have been examining a prominent model protein, cyclic-AMP response element binding (CREB) protein, a member of the bZIP family, to determine what role protein disorder might play in the target search process. CREB binds to its target as a homodimer. We present structural data from NMR for both monomeric and dimeric CREB that describes its secondary structure propensity and dynamics when free in solution, sliding along the DNA and bound to its target site. We demonstrate that whilst searching for its target site the protein remains highly dynamic with limited helical content, and that the protein forms a dimer before binding to its target site. To our knowledge our results constitute the most complete structural description of the search process by a disordered transcription factor to date.

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**PS137**

Mon, 17:15-17:40

**A generalized approach for NMR studies of lipid–protein interactions based on sparse fluorination of acyl chains**Tammo Diercks<sup>\*,1</sup>, Francisco J. Blanco<sup>1</sup>, Alfredo De Biasio<sup>2</sup>,Alain Ibañez de Opacua<sup>3</sup>, Daniel Nietlispach<sup>4</sup>, Mark J. Bostock<sup>5</sup><sup>1</sup>CiC bioGUNE, <sup>2</sup>University of Leicester, <sup>3</sup>Max Planck Institute forBiophysical Chemistry, <sup>4</sup>University of Cambridge (UK), <sup>5</sup>University of Cambridge

High-resolution NMR studies on protein-lipid interactions are severely limited by poor <sup>1</sup>H signal dispersion in the lipids' acyl chains, where uniform <sup>13</sup>C enrichment cannot resolve all overlap problems and introduces no distinct molecular marker from a likewise <sup>13</sup>C enriched protein to separate inter- from intramolecular NOE signals. We present a new approach [1] that relies on *sparse fluorination of lipid acyl chains* and exploits fluorine both *indirectly*, as a *shift reagent* affecting nearby spins, and *directly*, as a *distinctive isotope* (<sup>19</sup>F) with superb NMR properties. The introduced fluorine atoms then solve the NMR resolution problem for acyl chains by (i) increasing their <sup>1</sup>H signal dispersion via local deshielding, (ii) enabling clean molecular distinction via <sup>19</sup>F filtering, and (iii) allowing further resolution enhancement via <sup>19</sup>F editing. While the number of H/F substitutions must be minimised to mitigate any biophysical impact, prevent complications from J<sub>FF</sub> coupling, and preserve a high <sup>1</sup>H<sub>lipid</sub> density to probe intermolecular contacts via <sup>1</sup>H<sub>lipid</sub>-<sup>1</sup>H<sub>protein</sub> NOE signals, a *minimal fluorination scheme* is defined by the reach of fluorine induced deshielding and J<sub>FF</sub> coupling. We, thus, designed di-(4-fluoro)heptanoylphosphocholine (4F-DHPC<sub>7</sub>) that forms stable micelles with similar size as DHPC<sub>7</sub>, but with fully dispersed high-resolution <sup>1</sup>H and <sup>19</sup>F spectra. Both DHPC<sub>7</sub> and 4F-DHPC<sub>7</sub> micelles readily stabilise the phototaxis receptor sensory rhodopsin II (pSRII) and outer membrane protein X (OmpX), where <sup>15</sup>N TROSY signals differ notably only for residues near the fluorine atoms in modelled 4F-DHPC<sub>7</sub> micelles. Thus, H/F substitution in lipid chains also causes localised *fluorine induced chemical shift perturbations* (CSP<sub>F</sub>) in solubilised proteins, indicating their lipid layer insertion similar to paramagnetic markers, but with minimal steric impact and finer distance resolution. Finally, a first <sup>19</sup>F filtered NOESY spectrum unambiguously brought out intermolecular contacts between 4F-DHPC<sub>7</sub> and a bihelical integrin fragment.

**References:** [1] De Biasio *et al*, *Chem.Comm.* (2018) 54, 7306-7309.

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**PS138**

Mon, 16:15-16:50

**Selective High-Resolution DNP-Enhanced NMR of Biomolecular Binding Sites**

Sabine Hediger

Locating binding sites in biomolecular assemblies and solving their structures is crucial to unravel functional aspects of the system and provide experimental data that can be used for structure-based drug design. This often still remains a challenge, both in terms of selectivity and sensitivity for X-ray crystallography, cryo-electron microscopy and NMR.

Dynamic Nuclear Polarization (DNP) has revolutionized the scope of many solid-state NMR experiments by enabling new sensitivity-limited experiments to be recorded. Its use for biomolecular systems is however often limited by the necessity to run experiments at cryogenic temperatures, which can induce line broadening and loss of resolution. We present here a new method, called Selective DNP (Sel-DNP) that provides specific selectivity with high spectral resolution for the binding region of biomolecules, allowing for the identification of the residues present in the binding site. This powerful site-directed approach relies on the combined use of localized paramagnetic relaxation enhancement, induced by a ligand-functionalized paramagnetic construct, and difference spectroscopy to recover high-resolution and high-sensitivity information from binding sites.

The Sel-DNP approach is demonstrated on the galactophilic lectin LecA, a 12.75 kDa protein. The identification of residue types present in the galactose-binding region occurs using spectral fingerprints obtained from a set of high-resolution multidimensional spectra with varying selectivity. Experimental and computational strategies are then combined to assign the identified residues types to the specific residues in the sequence of the protein. In particular, a hierarchical alignment procedure using a modified genetic algorithm will be presented, which is able to perform de novo assignment and to locate the binding site in the protein sequence on the sole basis of the residue-type list extracted from Sel-DNP spectra.

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**PS139** Mon, 16:50-17:15

**Interaction between Cell-Wall and Biosynthetic Enzymes  
Using a Combination of Liquid- and Solid-State NMR Approaches**  
Catherine Bougault, Cedric Laguri, Isabel Ayala, Waldemar Vollmer,  
Sabine Hediger, Paul Schanda, Jean-Pierre Simorre\*

The cell wall is essential for the survival of bacteria. It gives the bacterial cell its shape and protects it against osmotic pressure, while allowing cell growth and division.

The machinery involved in the synthesis of this envelop is crucial and is one of the main antibiotic target. Different proteins as transpeptidases, transpeptidase activators or hydrolases are recruited to maintain the morphogenesis of this polymer during the bacterial cell cycle. Based on few examples involved in the machinery of synthesis of the peptidoglycan, we will present a combination of liquid and solid-state NMR that can be a powerful tool to screen for cell-wall interacting proteins in vitro and on cell.

In particular, we have explored the possibilities to study the PG with ultra-fast (100 kHz) magic-angle spinning NMR. We show that highly resolved spectra can be obtained, and we have developed strategies to obtain site-specific resonance assignments and distance information. we have also in parallel investigated the potential of Dynamic Nuclear Polarization (DNP) to investigate cell surface directly in intact cells.

Altogether, NMR approaches developed here propose new routes to fill the gap between in vitro studies of isolated biomacromolecules and in vivo cell biology studies in order to investigate cell surfaces and decipher key biological processes involved.

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**PS140**

Mon, 17:15-17:40

**Unravelling Glycan-Lectin Interactions: from STD to Paramagnetic NMR**

Angeles Canales, Javier Cañada<sup>2</sup>, Beatriz Fernandez de Toro<sup>3</sup>,  
 James C Paulson<sup>4</sup>, Carlo Unverzagt<sup>5</sup>, Jesus Jimenez-Barbero<sup>\*,6</sup>  
<sup>2</sup>CIB-CSIC, <sup>3</sup>CIB CSIC, <sup>4</sup>TSRI, <sup>5</sup>University Bayreuth, <sup>6</sup>CIC bioGUNE

Molecular recognition by specific targets is at the heart of the life processes. In recent years, it has been shown that the interactions between proteins (lectins, enzymes, antibodies) and carbohydrates mediate a broad range of biological activities, from fertilization and tissue maturation, to pathological processes. The elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, unravelling the structural and conformational factors and the physicochemical features that rule the interactions of these molecules is of paramount interest. The key tool for studying at atomic resolution the recognition processes in which glycans are involved is NMR. Thus, we use NMR as key tool for analysing key molecular recognition processes in which glycans are involved at atomic resolution.[1-8] Although the inherent flexibility of N-glycans and the chemical equivalence of individual branches precludes their NMR characterization using standard NMR methods, using multi-antennary N-glycans conjugated to a lanthanide binding tag, we have been able to discriminate the NMR signals of bi and multiantennary glycans with unprecedented resolution. As recent example, key details of biantennary glycan recognition by influenza hemagglutinin will be shown, with special emphasis in the application of novel paramagnetic-NMR methods to evaluate the relative importance of polar (hydrogen bonding, electrostatic interactions) and non-polar (van der Waals, CH- $\pi$ ) forces in the recognition process.

**References:** [1] A. Gimeno, et al. ACS Chem Biol. 2017, 12, 1104. [2] L. Unione, et al., Chem Eur J. 2017, 23, 3957. [3] A. Canales, et al., Angew Chem Int Ed. 2017, 56, 14987. [4] B. Fernández de Toro, et al., Angew Chem Int Ed. 2018, 57, 15051. [5] T. Diercks et al., Chem Eur J. 2018, 24, 15761. [6] A. Ardá, J. Jiménez-Barbero, Chem Commun. 2018, 54, 4761.

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**PS141** Tue, 10:30-11:05

**NMR investigation of the activation mechanism of the guardian of the germ cell TAp63a**

Jakob Gebel<sup>1</sup>, Marcel Tuppi<sup>2</sup>, Apirat Chaikuad<sup>1</sup>, Katharina Hötte<sup>1</sup>,  
Laura Schulz<sup>3</sup>, Frank Löhr<sup>1</sup>, Niklas Gutfreund<sup>1</sup>, Ralf Lehnert<sup>4</sup>,  
Gerhard Hummer<sup>3</sup>, Ernst Stelzer<sup>1</sup>, Stefan Knapp<sup>1</sup>, Volker Dötsch<sup>\*,1</sup>

<sup>1</sup>Goethe University, <sup>2</sup>The Francis Crick Institute,

<sup>3</sup>Max Planck Institute for Biophysics, <sup>4</sup>Mathezentrum, Goethe University

Cell fate decisions like cell division or apoptosis require cells to translate signals into a final yes/no answer. Primary oocytes are a special type of cells that are arrested in prophase of meiosis I in which they last for up to 50 years in humans. The number of primary oocytes determines the reproductive capacity of females. Due to the importance and the long arrest time of these cells they have evolved a special type of genetic quality control not present in somatic cells. Regulating this control mechanism is of very high importance: Tight genetic quality control is necessary to maintain the genetic integrity of the entire species but a too stringent mechanism can deplete the whole primary oocyte pool leading to infertility. In female germs cells this genetic quality is monitored by the p53 homolog TAp63α. After DNA damage it gets activated by phosphorylation triggering the transition from a closed dimeric state to an open tetramer. We have used NMR spectroscopy to investigate how phosphorylation determines the critical threshold level for elimination of a primary oocyte. Through measuring single site phosphorylation kinetics in isolated peptides as well as in full-length protein we show that phosphorylation follows a biphasic behavior. We reveal the structural mechanism and show by quantitative simulation that the slow phase determines the threshold of DNA damage that is necessary to induce apoptosis.

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**PS142**

Tue, 11:05-11:30

**Flavivirus capsid assembly and dynamics: evidence of a structure-driven regulation of protein interaction with intracellular hydrophobic interfaces.**Gabriela Araujo<sup>1</sup>, Glaucé M. Barbosa<sup>1</sup>, Maria A. Morando<sup>2</sup>,  
Christine Cruz-Oliveira<sup>1</sup>, Andreia T. Da Poian<sup>1</sup>, Fabio C. L. Almeida<sup>\*,1</sup><sup>1</sup>*Federal University of Rio de Janeiro (UFRJ),*<sup>2</sup>*Centro de Desenvolvimento de Tecnologia em Saúde, FIOCRUZ*

Dengue (DENV) and Zika (ZIKV) are major arthropod-borne human viral disease, for which no specific treatment is available. They are a worldwide important health concern, which causes neurological disorders and hemorrhagic syndrome. Although the structure of ZIKV and DENV virion has been determined, information on the nucleocapsid is lacking. The most accepted hypothesis is of a disorganized nucleocapsid. Using NMR, we solved the structure and dynamics of full length ZIKV capsid protein (ZIKVC) and the dynamics of DENV capsid protein (DENV C). We showed that the addition of oligonucleotides can form an organized nucleocapsid-like particles (NC-like). The binding to intracellular hydrophobic interfaces, such as endoplasmic reticulum and/or lipid droplets is essential for virus replication. The hydrophobic cleft is the binding site, along with the intrinsically disordered region, and an open-close dynamic of the globular domain that are species-specific. For ZIKVC,  $\alpha$ -helix 1 is smaller and partially occludes protein hydrophobic cleft. Measurements of the dynamics of  $\alpha$ -helix 1, surface exposure and thermal susceptibility of each backbone amide hydrogen in protein structure revealed the occlusion of the hydrophobic cleft by  $\alpha 1/\alpha 1'$  and supported a  $\alpha$ -helix 1 position uncertainty. Based on the findings, we propose that the dynamics of flaviviruses structural elements responds for a structure-driven regulation of protein interaction with intracellular hydrophobic interfaces, which would impact in the switches necessary for nucleocapsid assembly. Subtle differences in the sequence of helix 1 impact on its size and orientation and on the degree of exposure of the hydrophobic cleft, suggesting that  $\alpha$ -helix 1 is a hotspot for evolutionary adaptation of flaviviruses' capsid proteins.

Acknowledgements: FAPERJ, CAPES, CNPq, INBEB-CNPq.

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**PS143** Tue, 11:30-11:55

**Progress of the structural characterization on a  
eukaryotic rhodopsin by solid-state NMR**  
Shenlin Wang\*  
*Peking University*

Magic-angle-spinning solid-state NMR (MAS-SSNMR) has emerged as a powerful technique of structural biology. It is particularly attractive for its unique capability of providing structure and dynamic for membrane proteins in lipid bilayers. In this presentation I will introduce our recent progress in structure and dynamic characterization of Leptosphaeria rhodopsin (LR).

LR was the first discovered eukaryotic light-driven proton pump, which uses light energy to transport protons across the cell membranes. LR shares the typical heptahelical topology and has a retinal covalently bound to the protein core. However, the structure and the detailed mechanisms of LR are still unknown. The proteins were prepared by *P. pastoris* expression system. To determine the structure of LR, the 2D and 3D MAS-SSNMR spectra were collected to achieve backbone and side-chain assignments. Sparsely <sup>13</sup>C labeled protocol for *P. pastoris* expression systems were developed to obtain long-range distances for structural illustrations. The LR forms homo-trimers in lipid environments. Paramagnetic relaxation enhancements were applied in characterization of the intermonomer interface.

The LR shares heptahelical transmembrane topology. The hydrogen-bonding networks formed by the proton of the protonated Schiff-base and critical Asp residues are the key elements for LR function. The chemical shift of Asp residues suggested the carboxyl sidechains of D139 and D266 are deprotonated, consistent with the common knowledge of microbial rhodopsin. On the other hand, the solid-state H/D exchange experiments suggested the rapid exchange between solvent water and the proton of the protonated Schiff-base. This indicated the participation of water molecule in the pathway of proton pump of LR.

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**PS144**

Tue, 11:55-12:30

**Insights into the Antifungal Activity of Amphotericin B from Solid-State NMR**

Chad Rienstra\*

*University of Illinois at Urbana-Champaign*

In this talk, I will describe ongoing efforts in my laboratory (in collaboration with Prof. Martin D. Burke at Illinois) to understand the mode of action of the gold standard antifungal drug amphotericin B (AmB). We have previously proposed a hypothesis that AmB acts as a sterol sponge, a high molecular weight assembly that cooperatively assembles and extracts ergosterol from the yeast plasma membrane. Binding of ergosterol is correlated with antifungal activity and binding of cholesterol with toxicity. Thus the sponge model predicts that analogs of AmB with greater binding specificity for ergosterol v. cholesterol will have an improved therapeutic index. The structural basis for this activity, however, remains incompletely understood. We have developed and implemented experiments involving  $^{13}\text{C}$ -labeled AmB both alone and in complex with sterols, in order to understand the detailed conformational rearrangements and structural motifs that endow AmB with these unique biophysical properties.

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PS145

Tue, 12:30-13:00

**Assessing site-specific water accessibility in folded and unfolded proteins using hyperpolarization-enhanced 2D HMQC NMR**

Or Szekely<sup>1</sup>, Gregory L Olsen<sup>2</sup>, Rina Rosenzweig<sup>1</sup>, Lucio Frydman<sup>\*,3</sup>

<sup>1</sup>The Weizmann Institute of Science, <sup>2</sup>University of Vienna,

<sup>3</sup>Department of Chemical and Biological Physics, Weizmann Institute of Science

Hyperpolarized water is a valuable aid in biomolecular NMR. One can utilize it to achieve, under physiologically-like conditions, amide group polarizations that are orders-of-magnitude larger than their thermal counterparts. Suitable experimental procedures can exploit this to deliver 2D <sup>1</sup>H-<sup>15</sup>N NMR correlations, with good resolution and enhanced sensitivity. The resulting signal enhancements depend on the exchange rates between amides and water protons, yielding information about solvent accessibility. This study applied the ensuing ‘HyperW’ NMR method to four proteins, which exhibit a gamut of exchange behaviors. These included PhoA<sup>(350-471)</sup>, an unfolded fragment of Alkaline Phosphatase from *E. coli*; barstar, a folded ribonuclease inhibitor from *Bacillus amyloliquefaciens*; R17, a system possessing folded and unfolded forms under slow interconversion; and drkN-SH3, an N-terminal protein domain where folded and unfolded forms interchange more rapidly and with temperature-dependent population ratios. For the unstructured PhoA4 fragment 2D HyperW sensitivity enhancements were very high,  $\geq 300\times$  over their thermal counterparts, expected due to fast amide exchanges that occur throughout this unfolded protein sequence. Though fully folded barstar also exhibited substantially-enhanced residues; these were not uniform, and reflected what appeared well folded but surface exposed residues. R17 showed the expected superposition of  $\geq 100$ -fold enhancements for its unfolded form, coexisting with more modest enhancement for the folded. *The behavior of drkN-SH3 domain was unexpected: HyperW substantially enhanced both folded and unfolded states -but foremost of all certain sites of the folded protein.* A number of explanations– including cross-correlated relaxation processes, and the possibility of three-site exchange magnetization transfers– were considered to account for these preferential enhancements. Still, the most “reasonable” explanation for larger folded-site enhancements, appears to be that faster exchange rates characterize these sites than their unfolded counterparts. We discuss factors that could bring about such anomalous, hitherto unobserved behavior departing from accepted paradigms relating solvent exposure and protein fold.

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**PS146**

Tue, 10:30-11:05

**Allostery and Dynamics in Ion Channels and Oligomeric Proteins**

Ann McDermott

Allostery in ion channels controls activation coupled inactivation and partly controls mean open time. Solid state NMR experiments on full length wild type channel in proteoliposomes provide evidence for evacuation of ions from the selectivity filter during inactivation and strong coupling between opening and ion affinity. Furthermore, a number of site specific mutants altered in their inactivation properties in the hinge of the inner helix (e.g. F103A) suggest that a group of bulky residues serve as “hotspots” for allostery. The plasticity of ion channels is clearly critical to the many essential processes they carry out in all cells. Methods for characterizing millisecond and microsecond conformational exchange processes in high resolution SSNMR experiments will also be discussed.

The talk will also discuss structures of amyloids involved in human biology, and new NMR methods to sensitize detection of signals. RIPK1:RIPK3 core complex of the necrosome, which initiates TNF-induced necroptosis in the context of immune defense, cancer and neurodegenerative diseases. Using solid-state NMR, we determined the high-resolution structure of the core. RIPK1 and RIPK3 assume serpentine conformations, with short B-segments. Packing analogous to other amyloids results in a hydrophobic core with both hetero and homo hydrophobic contacts, and unusual exposed “ladders” of interacting amino acids. The molecularly detailed structure of a hetero-oligomeric amyloid and provides insights into the mechanisms of signal transduction and of inhibition of necroptosis.

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**PS147** Tue, 11:05-11:30

**A high-resolution description of functional dynamics and allosteric coupling of the  $\beta_1$ -adrenergic receptor from backbone NMR**

Anne Grah<sup>l</sup>\*, Layara Abiko, Shin Isogai, Stephan Grzesiek  
*Biozentrum, University of Basel, Switzerland*

G protein-coupled receptors (GPCRs) are physiologically important transmembrane signaling proteins that elicit intracellular responses upon binding of ligands on the extracellular site. Breakthroughs in crystallography have provided a wealth of static GPCR structures ranging from ligand-bound inactive receptors to fully active receptors in complex with intracellular binding partners such as heterotrimeric G protein and its mimetics. However, dynamical information on the different functional receptor states and their transitions is scarce. Such information is needed to understand the mechanisms of receptor regulation and signal transmission.

We have previously shown that the GPCR response to various ligands can be followed from  $^1\text{H}$ - $^{15}\text{N}$  resonances at virtually any backbone site in a thermostabilized mutant of the turkey  $\beta_1$ -adrenergic receptor ( $\beta_1\text{AR}$ ) [1]. We now provide a detailed analysis of populations and dynamics derived from  $^{15}\text{N}$  chemical shifts and relaxation rates. For this we used the fully thermostabilized and a more native-like mutant of the receptor in binary complexes ranging from antagonists to agonists as well as in the ternary agonist•G protein mimetic complex. This provides new insights into its activation mechanism and key residues involved in allosteric signal transmission.

**References:** [1] Isogai, S., Deupi, X., Opitz, C., Heydenreich, F. M., Tsai, C.-J., Brueckner, F., Schertler, G. F. X., Vepri<sup>n</sup>sev, D. B., Grzesiek, S., *Nature* 2016, 530, 237–241.

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**PS148**

Tue, 11:30-11:55

**<sup>13</sup>C-detected NMR methods to characterise side-chain behaviour in large molecular systems**Ruth B. Pritchard<sup>\*,1</sup>, D. Flemming Hansen<sup>2</sup><sup>1</sup>University of Sussex, <sup>2</sup>ISMB, University College London

The behaviour of side chains is fundamental to the biology and pathology of proteins. They play essential roles in processes as diverse as folding, catalysis, binding and allosteric regulation, and it is clear that in many cases their function is as much linked to their dynamic behaviour as their structure. Despite their significance, methods probing the behaviour of side chains are limited, and mainly focus on small proteins and residues containing methyl groups.

<sup>13</sup>C-detected NMR spectroscopy in a per-deuterated environment provides an excellent means of probing residues and systems intractable to conventional <sup>1</sup>H-detected methods. The presented suite of pulse sequences[1] make use of a single, uniformly-labelled sample to characterise a range of non-methyl- and methyl-bearing side chains. The base residue-specific carbon-carbon correlation experiment has been extended to include elements reporting on both the structure and dynamic behaviour of these side chains. Chemical exchange saturation transfer (CEST) experiments report on conformational exchange on a millisecond time-scale, whilst long-range <sup>13</sup>C-<sup>13</sup>C scalar couplings report on nanosecond to millisecond motions. Most importantly, these experiments have been used to investigate systems as large as 82 kDa. The presented class of methods promises characterisation of side-chain behaviour in large systems and at a level that has so far been reserved for the protein backbone.

**References:** [1] Pritchard & Hansen 2019 *Nature Communications* 10:1747.

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**PS149** Tue, 11:55-12:30

**Inspection of solution-state NMR data to evaluate protein conformational changes**

Rieko Ishima<sup>\*,1</sup>, Ryan Slack, Zhaoyong Xi<sup>1</sup>  
<sup>1</sup>*University of Pittsburgh School of Medicine*

NMR studies of large proteins, over 100 kDa, in solution are technically challenging and thereby of considerable interest in the NMR field. This is primarily due to slowing of molecular tumbling in solution as molecular mass increases. Typical 1H-13C or 1H-15N correlation spectra using 13C- or 15N uniformly labeled proteins show severe line-broadening and signal overlap. It is well known that selective isotope labeling, often concomitant with deuteration, is a useful strategy to reduce signal overlap and line-broadening in biomolecular NMR. However, a reduction in the number of signals is, in turn, disadvantageous in characterization of the overall protein feature. Thus, inspection of solution state NMR data not only of 1H-13C correlation spectra recorded using the selectively-labeled proteins, but also of 1H-15N correlation spectra of uniformly 15N-labeled protein is still useful. We discuss consistency in NMR data recorded using different NMR nuclei for a 66 kDa protein, HIV-1 reverse transcriptase precursor, that forms a homodimer with micro-molar dissociation constant in solution, to understand the structural characteristics. This work is supported by NIH NIGMS and NIAID (GM105401 and GM082251).

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**PS150**

Tue, 12:30-13:00

**Lanthanide-induced relaxation anisotropy**Elizaveta Suturina<sup>\*,1</sup>, Kevin Mason<sup>2</sup>, Carlos Geraldès<sup>3</sup>,Nicholas Chilton<sup>4</sup>, David Parker<sup>2</sup>, Ilya Kuprov<sup>5</sup><sup>1</sup>*University of Bath*, <sup>2</sup>*University of Durham*, <sup>3</sup>*University of Coimbra*,<sup>4</sup>*University of Manchester*, <sup>5</sup>*University of Southampton*

Lanthanide ions accelerate nuclear spin relaxation by two primary mechanisms: dipolar and Curie. Both are commonly assumed to depend on the length of the lanthanide-nucleus vector, but not on its direction. In this communication, we demonstrate experimentally and verify theoretically that this is wrong – careful proton relaxation data analysis in a series of isostructural lanthanide complexes (Ln=Tb, Dy, Ho, Er, Tm, Yb) reveals angular dependence in both Curie and dipolar relaxation. The reasons are:

1. that magnetic susceptibility anisotropy can be of the same order of magnitude as the isotropic part (contradicting the unstated assumption in Gueron's theory of the Curie relaxation process [1]);
2. that zero-field splitting can be much stronger than the electron Zeeman interaction (Bloembergen's original theory of the lanthanide-induced dipolar relaxation process makes the opposite assumption [2]).

These factors go beyond cross-correlation effects; they alter the relaxation theory treatment and make angular dependencies appear in the nuclear spin relaxation rates. Those angular dependencies are impossible to ignore – we demonstrate this both theoretically and experimentally, and suggest that a major revision is needed of the way lanthanide-induced relaxation data is used in structural biology.

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**PS151** Tue, 10:30-11:05

Splitting hairs: Small physical effects in NMR  
Juha Vaara\*  
*University of Oulu*

Computation of conventional NMR parameters has become routine. There remain, however, interesting physics to be explored. I introduce four topics of this kind.

For the first time, measured  $J$ -coupling over a van der Waals (vdW) bond, between  $^{129}\text{Xe}$  and  $^3\text{He}$  in a gas-phase nuclear spin co-magnetometer, can be directly compared with quantitative calculations [1]. The latter involve the second virial coefficient of  $J(^{129}\text{Xe}-^3\text{He})$  from relativistic potential energy and  $J$ -coupling curves.

NMR parameters are magnetic field-dependent. So far, only  $B_0$ -dependent  $^{131}\text{Xe}$  quadrupole coupling has been found [2]. Experiments on  $\text{Co}(\text{acac})_3$ -complex [3], where low-lying d-d excitations render the  $^{59}\text{Co}$  shift significantly  $B_0$ -dependent, are compared with non-linear response theory for the leading  $O(B_0^2)$  terms [4].

Nucleus-specific information can be obtained from changes in light beam polarisation due to interaction with nuclear spins [5]. We predict nuclear spin-induced magnetochiral birefringence and dichroism, as possible reporters of chirality via different indices of refraction when light propagates parallel and antiparallel with spin magnetisation [6].

In a multi-scale study of spin-exchange optical pumping [7], Rb-Xe collisions are extracted from molecular dynamics and analysed using spin dynamics driven by a quantum-chemical spin Hamiltonian. We reproduce the roles of binary collisions and long-lived vdW complexes and, for the first time, see step-wise build-up of  $^{129}\text{Xe}$  polarisation upon vdW oscillations.

**References:** [1] J. Vaara, M. V. Romalis, Phys. Rev. A, in press (2019). [2] T. Meersmann, M. Haake, Phys. Rev. Lett. 81, 1211 (1998). [3] A. M. Kantola, P. Lantto, I. Heinmaa, J. Vaara, J. Jokisaari, in preparation (2019). [4] P. Manninen, J. Vaara, Phys. Rev. A 69, 022503 (2004). [5] I. M. Savukov, S.-K. Lee, and M. V. Romalis, Nature 442, 1021 (2006). [6] L.-j. Fu, J. Cukras, D. Fedotov, S. Coriani, J. Vaara, in preparation (2019). [7] J. Rantaharju, M. Hanni, J. Vaara, submitted (2019).

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**PS152**

Tue, 11:05-11:30

**Second order dispersion by optimised rotation pulses***David Goodwin*<sup>\*1</sup>, *Martin Koos*<sup>2</sup>, *Burkhard Luy*<sup>3</sup><sup>1</sup>Karlsruhe Institute of Technology, <sup>2</sup>Carnegie Mellon University,<sup>3</sup>Institute for Biological Interfaces 4 - Magnetic Resonance and  
Institute for Organic Chemistry, Karlsruhe Institute of Technology

The GRAPE method of optimal control can attempt to find the maximum overlap between a desired rotation propagator and the effective propagator of the pulse sequence [1], termed the *fidelity*. In finding optimal rotation pulses, numerical optimisation methods use the gradient of the fidelity to give super-linear convergence to a maximum overlap [2].

Building on past research that creates broadband pulses performing unitary propagators (BURBOP) [3,4], the research presented in this communication advances a step forward to create a new class of pulses with a defined second order phase dispersion.

One of the problems associated with universal rotation solutions, named BURBOP pulses [3,4], is the resulting high irradiation energy compared with the easier control problem of optimising state-to-state problems. A novel method is presented which will show this energy can be lowered by defining target rotation propagators as a function of phase dispersion. A customised version of the Spinach [5] optimal control toolbox [2,6,7] is used to simulate an ensemble of two-level quantum systems. This new class of pulse is named SORDOR pulses by the authors.

This optimal control method uses a defined quadratic phase dispersion, similar to the chirped pulses, for the targets of optimal control methods to find pulses that produce a rotation around an axis [3,4] at each frequency offset. Results for 90° and 180° SORDOR pulses are compared the achievable fidelity to the equivalent BURBOP pulse.

**References:** [1] J Magn. Reson., 172, 296 (2005). [2] J Magn. Reson., 212, 412 (2011). [3] J. Magn. Reson. 225, 142 (2012). [4] J. Magn. Reson. 216, 78 (2012). [5] J. Magn. Reson., 208, 179 (2011). [6] J. Chem. Phys., 143, 084113 (2015). [7] J. Chem. Phys., 144, 204107 (2016).

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**PS153**

Tue, 11:30-11:55

**First-principles computations of NMR shifts for extended paramagnetic solids:  
significant effects beyond the contact shifts**

Arobeno Mondal<sup>\*1</sup>, Martin Kaupp<sup>2</sup>

<sup>1</sup>Technical University of Munich, Germany, <sup>2</sup>Technische Universität Berlin

NMR is a powerful tool for studying the structural and electronic properties of paramagnetic solids. However, the interpretation of paramagnetic NMR spectra is often challenging as a result of the interactions of unpaired electrons with the nuclear spins of interest. Recently, we reported a novel protocol to compute and analyze NMR chemical shifts for extended paramagnetic solids, accounting comprehensively for Fermi-contact (FC), pseudo-contact (PC), and orbital shifts.[1,2] We combine periodic DFT computation of hyperfine and orbital-shielding tensors with an incremental cluster model for g- and zero-field-splitting (ZFS) D-tensors. The hyperfine tensors are computed with hybrid DFT functionals using the highly efficient Gaussian-augmented plane-wave implementation of the CP2K code. The incremental cluster model allows the computation of g- and ZFS D-tensors by ab initio complete active space self-consistent field and N-electron valence-state perturbation theory methods. We find that <sup>7</sup>Li shifts in the high-voltage cathode material LiCoPO<sub>4</sub> are dominated by spin-orbit-induced PC contributions, in contrast to previous assumptions, changing the interpretation of the shifts fundamentally in terms of covalency. Similar protocols can be applied to the computation of pNMR shifts for clusters with multiple paramagnetic centers. Using such a procedure, <sup>1</sup>H and <sup>13</sup>C shifts have been computed for derivatives of the porous Cr-MIL-101 solid, which contain Cr<sub>3</sub>O clusters with magnetically coupled metal centers within the metal-organic frameworks.[3] A combination of experimental and computational methods has been used to explore the competitive small-ligand binding to these MOFs. The developments described pave the way towards a more-widespread computational treatment of NMR shifts for paramagnetic materials.[4]

**References:** [1] Mondal, A.; Kaupp, M. J. Phys. Chem. Lett., 2018, 9, 1480-1484. [2] Mondal, A.; Kaupp, M. J. Phys. Chem. C, 2019, 123, 8387-8405. [3] Wittmann, T.; Mondal, A.; et al. J. Am. Chem. Soc., 2018, 140, 2135-2144. [4] Mondal, A.; Kaupp, M. Solid State Nucl. Magn. Reson., 2019 (in press).

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**PS154**

Tue, 11:55-12:30

## Computational methods for NMR crystallography of zeolites

Darren Brouwer\*

*Redeemer University College*

Solid-state nuclear magnetic resonance (SSNMR) spectroscopy has emerged as an important technique for structural characterization of solids. Due to the fact that it provides local structural information about the environments of NMR-active nuclei, SSNMR is highly complementary to diffraction techniques whose strength lies in providing information about the long-range periodic structure of a material. By combining solid-state NMR and diffraction techniques with various computational methods (modeling, density functional theory, etc), powerful approaches to structure determination of materials are being developed. These integrated structure determination strategies in which SSNMR spectroscopy plays a crucial role is broadly referred to as *NMR crystallography*. This talk will provide an overview of our NMR crystallography strategies for solving and refining zeolite crystal structures using advanced  $^{29}\text{Si}$  SSNMR methods and employing insights from graph theory, tiling theory, and machine learning.

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**PS155**

Tue, 12:30-13:00

## Methionine renaissance: computing methyl NMR assignments from X-ray structures

Saeed Chasnmiam<sup>1</sup>, Juan Carlos Paniagua<sup>2</sup>,

Joao M.C. Teixeira<sup>2</sup>, Héctor Fuentes<sup>2</sup>, Miquel Pons<sup>\*,2</sup>

<sup>1</sup>University of Barcelona (Spain); Sharif University (Iran), <sup>2</sup>University of Barcelona

Methyl groups are privileged probes for the NMR study of large proteins. Methionine is the least abundant of the methyl-containing amino acids but is often directly connected to functionally important sites. While isotopic labeling with <sup>13</sup>CH<sub>3</sub>-methionine is straightforward, assignment of the resulting spectra is challenging and often requires site-specific mutation of each of the methionines in the protein. Standard methods based on through-bond correlation to assigned backbone resonances or by matching observed and predicted inter-methyl NOEs on the basis of known X-ray structures are not applicable to exclusively methyl-methionine labeled proteins.

In this communication we will show that methionine methyl groups chemical shifts can be predicted from X-ray structures based on fast quantum chemical calculations on simplified models. The use of quantum chemical calculations outperforms the statistics-based predictions in the case of the scarce and flexible methionine residues. The calculated chemical shifts successfully predict the correct assignment or, at least, helps to define the minimal number of mutations needed to complete it.

Methyl methionine NMR has been used to functionally characterize the catalytic domains of calcineurin (345 residues, 7 methionines) and Src (253 residues, 10 methionines).

In the case of calcineurin, methionine methyl signals probe the binding of peptides and the conservation of a cis peptide bond in the mutant in which the native P84 was replaced by alanine [1].

Acknowledgements. Supported in part by grant BIO2016-78006R and by access to the R-LRB, the Spanish NMR facility network.

**References:** [1] Teixeira, J.M.C., Guasch, A., Biçer, A., Aranguren-Ibáñez, A., Chasnmiam, S., Paniagua, J.C., Pérez-Riba, M., Fita, I., Pons, M. Cis-trans proline isomers in the catalytic domain of calcineurin. FEBS J. 286, 1230-1239 (2019)

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**PS156**

Tue, 10:30-11:05

**Defects within Solid Materials Elucidated using  
NMR Spectroscopy – from Local Vacancies to Mesoscale Disorder**

Juergen Senker<sup>\*,1</sup>, Christoph Zehe<sup>1</sup>, Kasper van der Zwan<sup>1</sup>,  
Renée Siegel<sup>2</sup>, Gaël De Paëpe<sup>3</sup>, Klaus Kreger<sup>1</sup>, Hans-Werner Schmidt<sup>1</sup>

<sup>1</sup>University of Bayreuth, <sup>2</sup>Anorganische Chemie III,  
University of Bayreuth, <sup>3</sup>CEA Grenoble

Structure-property relations are essential for designing materials. As many properties are governed by defects and disorder, respectively, elucidating structural details on various length scales is a cornerstone for material science and solid-state chemistry. This lecture will give an overview of our recent progress on using solid-state NMR spectroscopic techniques for studying defects and disorder. Hereby, we will show, that NMR aids the structure elucidation process for materials as diverse as high-pressure minerals, frameworks and supramolecular self-assemblies. It provides central information from local to intermediate length scales by exploiting chemical shifts, connectivities, distances and orientation correlations based on homo- and heteronuclear correlation experiments. NMR spectroscopy is at its best, when used quantitatively as a cost function in addition to scattering and quantum mechanical calculations [1-3].

We will report on the incorporation of water in anhydrous ringwoodite by formation of various hydroxyl defects [2, 3]. Here the quantitative analysis of <sup>1</sup>H 1D and 2D DQ NMR spectra allowed for unravelling a surprisingly rich defect chemistry. In contrast, the key to a deeper understanding of substitution defects within Bridgmanite was an unambiguous assignment of the <sup>27</sup>Al MAS NMR spectra based on STMAS experiments. Finally, to derive a mechanistic picture for the supramolecular self-assembly of benzene trisamides (BTAs) [4] and the role of the resulting nanoobjects within the foaming process of polypropylene, multinuclear (<sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N and <sup>19</sup>F) and multidimensional NMR experiments were carried out. DNP was essential to allow for studying the BTA polymer mixtures down to concentrations of a few hundred ppm.

**References:** [1] H. Grüninger, et. al. *J. Am. Chem. Soc.* **2017**, *139*, 10499. [2] H. Grüninger, et al. *Phys. Chem. Chem. Phys.* **2018**, *20*, 15098. [3] C. S. Zehe, et al., *Angew. Chem. Int. Ed.*, **2017**, *56*, 4432.

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**PS157**

Tue, 11:05-11:30

## 125Te broadband solid-state NMR of the Dirac edge states in ultrathin Bi<sub>2</sub>Te<sub>3</sub> nanoplatelets

Wassilios Papawassiliou<sup>\*,1</sup>, Aleksander Jaworski<sup>2</sup>, Jae Hyuck Jang<sup>3</sup>, Yeonho Kim<sup>3</sup>, Michael Fardis<sup>4</sup>, Hae Jin Kim<sup>5</sup>, Georgios Papavassiliou<sup>4</sup>, Andrew J. Pell<sup>2</sup>

<sup>1</sup>Department of Materials and Environmental Chemistry, Stockholm University,

<sup>2</sup>Department of Materials and Environmental Chemistry, Stockholm University, <sup>3</sup>Electron Microscopy Research Center, Korea Basic Science Institute, <sup>4</sup>Institute of Nanoscience and Nanotechnology, National Center for Scientific Research "Demokritos", <sup>5</sup>2 Nano-Bio Electron Microscopy Research Group, Korea Basic Science Institute

Detection of metallic Dirac electron states on the surface of topological insulators[1] is, to date, restricted to a small number of experimental techniques, such as angle resolved photo-emission spectroscopy and scanning tunneling microscopy. The encroachment of the Dirac states into the bulk interior of a topological insulator is yet to be illuminated experimentally. Getting insight, is crucial in order to further understand the physics of topological materials and probe key properties[2] of this material class. Due to the dependence of the spin orientation of the Dirac electron coupling with the orbital motion and the propagation of this interaction in a crystal, a probe that is sensitive to both spin and orbital motion is necessary. Solid-state Nuclear magnetic resonance (ssNMR) appears to fulfill these requirements, as the nuclear magnetic shielding, and consequently the NMR frequency shift, depends on the spin and orbital magnetic susceptibility at the position of each resonating nucleus. Here, combining advanced, high-resolution broadband[3,4] solid-state <sup>125</sup>Te NMR methods with state-of-the-art density functional theory calculations and scanning transmission electron microscopy on ultra-thin Bi<sub>2</sub>Te<sub>3</sub> nanoplatelets, we demonstrate an excellent atomic-scale probe of the Dirac electrons, mainly through the isotropic NMR Knight shift induced by the Dirac electron orbital currents. In this way, the NMR Knight shift and spin relaxation due to the Dirac electrons at all non-equivalent Te positions were acquired, unveiling the way that Dirac electrons and their excitations spread into the interior of the nanoplatelets.

**References:** [1] M. Z. Hasan, C. L. Kane. Rev. Mod. Phys. 82, 3045-3067 (2010). [2] S. Wu, et al. Science 359, 76–79 (2018). [3] Clement, R. J, et al. J. Am. Chem. Soc. 2012, 134, 17178– 17185. [4] Pell, A. J, et al. State.Prog. Nucl. Magn. Reson. Spectrosc. 111, 1-271, 2019.

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**PS158**

Tue, 11:30–11:55

**Transport of Organic Electrolytes and Ionic Liquids in  
Carbon Materials for Supercapacitors: The High-Gradient NMR Approach**  
Dilara Issayeva<sup>1</sup>, Desirée Leistenschneider<sup>2</sup>, Lars Borchardt<sup>3</sup>, Muslim Dvoyashkin<sup>\*,1</sup>  
<sup>1</sup>*Institute of Chemical Technology, Universität Leipzig*, <sup>2</sup>*Department of Chemistry and  
Food Chemistry, Inorganic Chemistry I, TU Dresden*, <sup>3</sup>*Inorganic Chemistry I,  
Ruhr-Universität Bochum*

The molecular diffusion of ions in energy storage devices, such as, e.g., supercapacitors, is the process enabling their charging and discharging ability. Chmiola et al. demonstrated the strong impact of micropores on the increase of specific capacitance using a series of titanium carbide-derived carbons exhibiting different but precisely uniform pore sizes [1]. An anomalous increase in specific capacitance was observed for those pores being comparable in size to adsorbed electrolyte ions, while the larger pores led to the loss of capacitance. However, under such constraints, one expects kinetic problems caused by confinement-induced obstruction for molecular diffusion [2].

Only very recently, the direct experimental assessment of the ion transport characteristics within the pores of carbon materials became accessible using the quasielastic neutron scattering [3] and the pulsed field gradient (PFG) NMR [4].

Inspired by these recent methodological achievements, we applied the PFG NMR techniques to directly (and selectively) probe the diffusion characteristics of each individual component of organic electrolytes and ionic liquids, that is anion and cation (and solvent, when present), confined to model carbons with uniform and well-defined pore sizes - microporous, mesopores, and with hierarchical pore organization [5]. Quite unexpectedly, it is observed that the presence of a network of mesopores, in addition to smaller micropores—the concept widely used in heterogeneous catalysis to promote diffusion of sorbates—does not necessarily enhance ionic transport in carbon materials.

**References:** [1] J. Chmiola et al., *Science* 313 (2006) 1760–1763. [2] A. Lee et al., *Nanotechnology* 25 (2014) 315401. [3] N. Osti et al., *Phys. Rev. Materials* 1 (2017) 035402. [4] A. Forse et al., *Nat. Energy* 2 (2017) 16216. [5] L. Borchardt et al., *Adv. Energy Mater.* 111 (2018) 1800892.

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PS159

Tue, 11:55-12:30

**Solid-state NMR studies of the electrochemical cycling of  $\text{LiNi}_{0.8}\text{Mn}_{0.1}\text{Co}_{0.1}\text{O}_2$  cathodes**Katharina Märker<sup>\*1</sup>, Chao Xu<sup>1</sup>, Philip J. Reeves<sup>2</sup>, Kent J. Griffith<sup>2</sup>, Clare P. Grey<sup>1</sup><sup>1</sup>University of Cambridge; The Faraday Institution, <sup>2</sup>University of Cambridge

The layered oxide  $\text{LiNi}_{0.8}\text{Mn}_{0.1}\text{Co}_{0.1}\text{O}_2$  (NMC811) is a promising future cathode material for lithium-ion batteries in electric vehicles due to its high specific energy density. The practical use of NMC811 cathodes, however, faces difficulties as they suffer from fast capacity fade. Mitigating this performance fade requires detailed knowledge of the changes of structure and dynamics of NMC811 during charge and discharge.

<sup>7</sup>Li solid-state NMR is a well-suited technique for investigating lithium-ion battery materials as it is sensitive to the local Li environment as well as the Li-ion dynamics. NMC811 is a challenging material for such studies due to the high number of paramagnetic centres ( $\text{Ni}^{2+}$ ,  $\text{Ni}^{3+}$ ,  $\text{Mn}^{4+}$ ), leading to short relaxation times and large hyperfine interactions. The acquisition and interpretation of <sup>7</sup>Li NMR spectra of NMC811 will be demonstrated in this contribution, including data acquired on *ex situ* and *in situ* samples.

*Ex situ* measurements enable the acquisition of NMR spectra under fast magic-angle spinning which yields considerably improved spectral resolution. The *ex situ* <sup>7</sup>Li NMR spectra taken on NMC811 cathodes at different states-of-charge (SOC) reveal a strong increase of Li-ion hopping rates during charge which is confirmed by variable temperature measurements.[1] Modelling of these spectra allows estimating the hopping rates and also reveals that Li mobility decreases drastically at high SOC, which is accompanied by Li/vacancy ordering.[1]

*In situ* <sup>7</sup>Li NMR measurements on NMC811/graphite full-cells are used to simultaneously monitor Li ions in different parts of the cell such as the cathode, the anode, and the electrolyte. We will show a series of measurements at different charging rates and at different temperatures, providing real-time insights into the processes during electrochemical cycling in the whole cell and their contributions to degradation.

**References:** [1] K. Märker, P. J. Reeves, C. Xu, K. J. Griffith, C. P. Grey, Chem. Mater. 2019, 31 (7), 2545–2554.

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**PS160**

Tue, 12:30-13:00

**Surface Structure Determination of Heterogeneous Catalysts by DNP SENS**

Ribal Jabbour<sup>\*1</sup>, Pierrick Berruyer<sup>2</sup>, David Gajan<sup>1</sup>, Marc Renom-Carrasco<sup>3</sup>, Moreno Lelli<sup>4</sup>,  
Christophe Copéret<sup>5</sup>, Lyndon Emsley<sup>2</sup>, Chloé Thieuleux<sup>3</sup>, Anne Lesage<sup>6</sup>

<sup>1</sup>High-Field NMR Center, Université de Lyon, FRE 2034, CNRS/ENS Lyon/ UCB Lyon1, <sup>2</sup>Ecole Polytechnique Fédérale de Lausanne (EPFL), Institut des Sciences et Ingénierie Chimiques,

<sup>3</sup>Université de Lyon, Institut de Chimie de Lyon, LC2P2, UMR 5265 CNRS-CPE Lyon-UCBL, CPE Lyon, <sup>4</sup>Magnetic Resonance Center (CERM), University of Florence, <sup>5</sup>ETH Zürich,

Department of Chemistry and Applied Biosciences, <sup>6</sup>High-Field NMR Center, Université de Lyon, FRE 2034, CNRS/ENS Lyon/ UCB Lyon1

Dynamic Nuclear Polarization (DNP) is one of the promising approaches to overcome the sensitivity limitations of solid-state NMR, and has recently emerged as a powerful technique to amplify the NMR signals of surface species.[1] We have recently demonstrated that the three-dimensional (3D) structure of a model organometallic platinum complex anchored on an amorphous silica can be fully determined by combining DNP Surface enhanced NMR spectroscopy (DNP SENS) with EXAFS data.[2] Here we extend this approach to determine the surface structure of a catalyst containing iridium (Ir) N-heterocyclic carbene (NHC) active sites grafted onto a silica surface.[3] We will first present the 3D structure of the NHC precursor, obtained from 10 internuclear distances, measured from REDOR experiments. This NHC precursor is partially converted into a Ag-NHC intermediate and then to the final Ir-NHC complex, which results in a mixture of different surface species. We will then demonstrate strategies to overcome this challenge, and get access to targeted structural insights on a surface containing multiple well-defined sites. In particular, the implementation of selective REDOR allowed the measurement of several non-ambiguous, long-range <sup>29</sup>Si-{<sup>13</sup>C} distances. They clearly indicate that the structure of the Ag-NHC and Ir-NHC differs from that of the precursor, but also from the Pt complex determined previously. The NMR and EXAFS data point towards the presence of residual organic ligands coordinating the metal center. Establishing fine relationships between the structure and activity of a catalyst is essential in order to develop systems with improved efficiency.

**References:** [1] Berruyer, P. et al, eMagRes. 2018, 7, 93–104. [2] Berruyer, P. et al, J. Am. Chem. Soc. 2017, 139, 849–855. [3] Romanenko, I. et al, Angew. Chemie Int. Ed. 2015, 54, 12937–12941.

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**PS161**

Tue, 10:30-11:05

**Confining and Quantifying Hyperpolarization: 460 GHz-700 MHz DNP NMR  
using Closed-Cycle Helium MAS and Dual Gyrotron Setup**

Yoh Matsuki<sup>\*</sup>, Tomoaki Sugishita, Toshimichi Fujiwara  
*Osaka University*

Sensitivity of magic-angle spinning (MAS) solid-state NMR has been dramatically improved by the advent of high-field dynamic nuclear polarization (DNP) techniques through numerous discussions and breakthroughs made for improving the signal enhancement factor. Beyond the discussions on the sensitivity gain, we here propose two new methods to pursue hitherto under-explored curiosity: a method to confine the hyperpolarization for spatially selective observation of mesoscale molecular domains, and a method that enables quantitation of absolute <sup>1</sup>H polarization amplitude and its spatial distribution around a radical molecule (polarizing agent). The former method utilizes our unique double-gyrotron setup and its ability to switch the microwave frequency back and forth over the range of 0.7 GHz in synchrony to the RF pulses. Each microwave frequency is set to excite the positive or negative DNP effect in turn, producing a sort of “polarization wave” in space around the radical molecule. The second method is based on the use of a closed-cycle helium MAS system for ultra-low temperature DNP, enabling the total sensitivity gain exceeding a factor of 1000 at  $T = 30$  K and  $B_0 = 16.4$  T. In such a case, the high-order spin-correlated term ( $2 I_z S_z$ ) in the quasi-equilibrium spin density operator grows in a significant amplitude and, as we show, is observable separately from the lowest-order Zeeman term ( $S_z$ ) for the polarization quantitation. The method does not require evaluation of “microwave-off” signal as well as un-doped reference sample, and is also unaffected by the quenching and depolarization effects, providing an accurate and efficient way for the polarization quantitation. Potential applications will also be discussed.

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**PS162**

Tue, 11:05-11:30

**Multi-Sample Dissolution DNP with a Cryogen-Free Polariser**Adam Gaunt<sup>\*,1</sup>, Tian Cheng<sup>1</sup>, Arnaud Comment<sup>1</sup>*University of Cambridge*

Dissolution Dynamic Nuclear Polarisation (DNP) has shown great potential in providing large signal enhancement to metabolites of interest in low gamma metabolic magnetic resonance imaging. Originally DNP polarisers were based on pumped-helium cryostats, which provide a high cooling power to contain the extra heat load introduced during the dissolution. However, these systems are not efficient at running at low temperatures for extended periods of time; neither are they cost effective due to the rising price of helium.

We present a closed-cycle cryogen-free 7T polariser which requires no input of liquid helium or any other cryogens. The polariser is based on a modified commercial dilution refrigerator. The closed system can run continuously at 1.4K for many weeks without interruption, useful for solid state measurements of samples with prolonged longitudinal relaxation times as well as making it a highly interesting system for dissolution DNP. Liquid-state <sup>13</sup>C polarization larger than 40% were obtained on different samples.

Traditional methods of sample dissolution are not suitable for a cryogen-free system due to the need to introduce warm helium to pressurise the sample space prior to introduce the dissolution apparatus into the cryostat. Instead, in order to minimise the heat load introduced during the dissolution process, we used a fluid path.

The sample space in the polariser is sufficiently large to house a maximum of 4 fluid paths meaning up to 4 mL of sample can be polarised at once. We implemented an insert that allows polarising two samples in parallel and dissolving them consecutively within an interval of 20min. These consecutive dissolutions can be carried out without significant deleterious heat-loads at the cooling stages of the cryostat. Limiting temperature increases on any of the cooling stages in this way allows a rapid recovery of the base temperature and prevents a potential quench of the magnet.

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**PS163**

Tue, 11:30-11:55

**Catalyzing the progress in parahydrogen-based NMR hyperpolarization**Igor V. Koptyug\*, Kirill V. Kovtunov*International Tomography Center, SB RAS*

Among the hyperpolarization (HP) techniques, parahydrogen-based methods are the simplest and technically least demanding. Because such techniques (PHIP, SABRE) rely heavily on catalysis, some of the unsolved problems in both fields are rather similar. One major trend in modern catalysis is a broad search for approaches to combine advantages of homogeneous and heterogeneous catalysts, namely the well-defined structure of the active catalytic center of homogeneous catalysts for high reaction selectivity, and the ease of solid heterogeneous catalyst removal after the reaction. Similarly, it would be highly advantageous to combine the ability of a soluble transition metal complex to add  $H_2$  to a substrate in a pairwise manner, and the feasibility of rapid filtration of the reaction mixture to yield a metal-free HP fluid for biomedical use. Indeed, HP effects have been demonstrated lately with the use of several concepts of modern catalysis, including immobilization of transition metal complexes on porous solids, the use of single-site and single-metal-atom heterogeneous catalysts, and active site isolation in metal alloys and bimetallic structures. Heterogeneous catalysis is also suitable for production of HP propane as a promising agent for gas-phase imaging. Another recent breakthrough in modern catalysis is the demonstration that certain metal-free systems are able to activate small molecules such as  $H_2$ . PHIP effects with the use of amine-borane frustrated Lewis pairs and other metal-free systems demonstrated recently may provide an alternative way to produce biocompatible HP solutions. Thus, the implementation of the achievements of modern catalysis in HP research can lead to a substantial progress in parahydrogen-based NMR signal enhancement, as will be illustrated with several recent examples. Furthermore, the HP techniques can be highly useful in addressing many challenges of modern catalysis research, to be exemplified with the mechanistic insight into the heterogeneous hydrogenation of cyclopropane and MRI of model hydrogenation reactors.

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**PS164**

Tue, 11:55-12:30

**Utilizing hyperpolarized noble gas T1 relaxation contrast for  
MRI in biomedical and engineering applications.**

Thomas Meersmann\*

*University of Nottingham*

Longitudinal ( $T_1$ ) relaxation is usually considered as disadvantageous for MRI with hyperpolarized (hp) spin systems as it leads to depolarization and hence to a loss in the observable signal. However, it has been demonstrated previously that quadrupolar  $T_1$  relaxation of the hyperpolarized noble gas isotope  $^{83}\text{Kr}$  (nuclear spin  $I = 9/2$ ) can be utilized to probe surfaces that are in contact with the noble gas. For example, surface quadrupolar relaxation (SQUARE)  $T_1$  maps of hp  $^{83}\text{Kr}$  are indicative of an emphysema model in excised rodent lungs [1]. MRI at the very low resonance frequency of  $^{83}\text{Kr}$  (i.e. 11.5 MHz at 7 T) requires hyperpolarization through spin exchange optical pumping (SEOP) similar to that for the hp  $^{129}\text{Xe}$  production. However, as a consequence of quadrupolar relaxation, hp  $^{83}\text{Kr}$  cannot be concentrated from buffer gases of the laser pumping process through cryogenic separation or through membranes without depolarization. Therefore, a new production methodology was developed that uses molecular hydrogen as buffer gas during SEOP and its subsequent removal through catalytic combustion [2]. Currently, novel instrumentation is being developed to make this approach feasible for clinical applications.

Similar to  $^{83}\text{Kr}$  MRI SQUARE contrast, paramagnetic relaxation of hp  $^{129}\text{Xe}$  can be applied to study surfaces, in particular for chemical engineering and materials science applications. Generally, MRI of fluid flow can probe the structure-transport relationship [3], and we use hp  $^{129}\text{Xe}$  to study gas transport and reactive zones in diesel catalysts that consist of materials with hierarchical pore structure. The accessibility of catalytic and paramagnetic centers can be probed through  $^{129}\text{Xe}$  relaxation measurements provide insights into catalytic activity in these systems.

**References:** [1] DML Lilburn et al., J. R. Soc. Interface, 12, (2015), 20150192. [2] NJ Rogers et al., Proc. Nat. Acad. Sci., 113, (2016), 3146-3168. [3] GE Pavlovskaya et al, Physical Review Fluids, 3, (2018), 044102\_1-20.

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**75% Liquid-State  $^1\text{H}$  Polarization for Hyperpolarized Water**  
Arthur C. Pinon\*, Andrea Capozzi, Jan Henrik Ardenkjær-Larsen  
*Technical University of Denmark*

Hyperpolarization via dissolution Dynamic Nuclear Polarization (dDNP) is without doubt the most widespread technique to overcome the low sensitivity in the liquid state Magnetic Resonance.[1] Hyperpolarized water is a versatile tool with possible applications ranging from biomedicine to chemistry. For instance, it can be used to acquire high resolution angiographic and perfusion images without employing any metal-ion contrast agent (e.g.  $\text{Gd}^{3+}$ ) in animals,[2] or probe proton exchange in proteins.[3]

Nevertheless, exploiting the  $^1\text{H}$  nuclei enhanced signal is not as straightforward as for  $^{13}\text{C}$  or other low-gamma nuclei: not only is the  $T_1$  of pure water intrinsically too short on the dDNP scale (3-4 s),[4] but the strong interaction between water protons and paramagnetic agents in solution provides a fatal relaxation rate contribution during dissolution and transfer.

UV-induced non-persistent radicals have been employed to efficiently polarize  $^{13}\text{C}$  and other low-gamma nuclei via dDNP.[5] Generated by UV-irradiation of a frozen solution containing a fraction of pyruvic acid or its derivatives, these radicals are stable as far as the sample temperature is below 190 K. The UV-radicals natural quenching at the moment of dissolution alleviates from the radical elimination step, and drastically reduces relaxation processes during dissolution and transfer.

Here we show that pyruvic acid derivates UV-irradiated for 10 min at 77 K can generate water solid-state  $^1\text{H}$  polarizations higher than 90% within 20 min at 6.7 T and 1.1 K, leading to radical-free liquid-state water  $^1\text{H}$  polarizations of ~ 75 % with liquid-state  $^1\text{H}$   $T_1$ s higher than 40 s at 9.4 T and 313 K.

**References:** [1] J. H. Ardenkjær-Larsen et. al, *Proc Natl Acad Sci*, **2003**. [2] K. W. Lipsø et. al, *Mag Res Med*, **2017**. [3] Q. Chappuis et. al, *J Phys Chem Lett*, **2015**. [4] K. Krynicky, *Physica*, **1966**. [5] A. Capozzi et. al, *Nat Com*, **2017**.

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**PS166**

Tue, 16:15-16:50

**Cancer Drug Discovery Using Fragment-Based Methods and Structure-Based Design**

Stephen Fesik\*

*Vanderbilt University School of Medicine*

Cancer is a devastating disease that affects the lives of almost everyone, and its effective treatment still remains an important unmet medical need. In order to discover new cancer drugs, we are applying fragment-based methods and structure-based design to identify and optimize small molecules that inhibit highly validated cancer targets. Although many of these targets are technically challenging and thought to be undruggable, fragment-based methods offer several advantages over more conventional approaches which suggest that it may be possible to achieve success. In this presentation, examples will be given of how this methodology can be used to discover small molecules that bind to highly validated but technically challenging cancer targets.

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**PS167** Tue, 16:50-17:15

**Modulation of aggregating proteins studied by  
NMR and beyond in neuro- and cellular degeneration**

Sergey Ryazanov<sup>1</sup>, Leif Antonschmidt<sup>1</sup>, Riza Dervisoglu<sup>1</sup>, Kris Runge<sup>1</sup>, Stefan Becker<sup>1</sup>,  
Andrei Leonov<sup>1</sup>, Loren B Andreas<sup>1</sup>, Armin Giese<sup>2</sup>, Christian Griesinger<sup>\*1</sup>  
<sup>1</sup>*MP1bpc*, <sup>2</sup>*MODAG*

In Parkinson’s disease (PD), a-Synuclein aggregates to Lewy bodies, which are connected to neuronal dysfunction and death, similar to Abeta and tau in Alzheimer’s (AD), prion protein in Creutzfeldt Jacob and IAPP in Type II diabetes mellitus (T2DM). Using structural biology derived predictions [1], a-Synuclein is shown to form non-toxic intrinsically disordered monomers and non-toxic fibrils, while immediate toxicity is exerted by oligomers [2]. Prevention of formation of these toxic oligomers by small molecules, specifically anle138b, observed in vitro and in vivo (3) using ultracentrifugation or superresolution imaging, specifically anle138b, leads to neuroprotection and restoration of functionality of the neurons in all mentioned diseases, specifically in PD [3], MSA [4], AD based on tau [5] or Abeta42 overexpression [6] and T2DM [7]. Biophysical characterization of anle138b with target proteins will be discussed [5,8] in solution and in membranes with NMR spectroscopy.

**References:** [1] Bertoncini, CW; et al. PNAS, 102 (5): 1430-1435. [2] Karpinar, DP; et al. Embo Journal, 28 (20): 3256-3268. [3] Wagner, J; et al. Acta Neuropathol. 125, 795-813 (2013); Levin, J; et al. Act. Neuropath. 127, 779-780 (2014); Giese, A; Bertsch, U; Kretschmar, H; Habeck, M; Hirschberger, T; Tavan, P; Griesinger, C; Leonov, A.; Ryazanov, S; Weber, P; Geissen, M; Groschup, MH; Wagner, J. (2010) WO/2010/000372; A. Giese, F. Schmidt, C. Griesinger, A. Leonov, S. Ryazanov: WO2017/102893; Wegrzynowicz M et al. Acta Neuropathol.<https://doi.org/10.1007/s00401-019-02023-x> (2019). [4] Heras-Garvin, A. et al. Movement Disorders in press. [5] Wagner, J. et al. Act. Neuropath.130, 619-631 (2015). [6] Martinez Hernandez, A; et al. EMBO Mol. Med. 10, 32-47 (2018). [7] J. Hoppener et al. “Glucose and HbA1c normalization and insulin sensibilization in a HIAPP overexpression model in T2DM” in preparation. [8] Deeg, A.A. et al. Biochim. Biophys. Act. 1850 (9), 1884-1890 (2015); Reiner, A.M. et al. Biochim. Biophys. Acta Gen. Subj. 1862, 800-807 (2018).

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**PS168**

Tue, 17:15-17:40

**NMR as a tool for defining cyclotide membrane binding:  
applications in medicine and agriculture**

David Craik\*

*University of Queensland*

Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia

Naturally occurring as well as ‘designer’ cyclic peptides offer great potential as leads for drug design or crop protection agents in agriculture. This talk will focus on one class of cyclic peptides known as cyclotides, which are topologically unique in that they have a head-to-tail cyclized peptide backbone and a cystine knotted arrangement of three conserved disulfide bonds. This makes cyclotides exceptionally resistant to chemical, thermal or enzymatic degradation and, indeed, cyclotides are amongst nature’s most stable proteins. They occur in plants from the Rubiaceae (coffee), Violaceae (violet), Solanaceae (nightshade), Fabaceae (legume) and Cucurbitaceae (cucumber) families of plants where their natural function is presumed to be in host defence. This presentation will describe the membrane binding properties of cyclotides and how the delineation of these properties by NMR and other biophysical techniques has assisted in the understanding of their natural defense functions and pharmaceutical applications. In particular, solid phase synthesis has allowed us to make a range of modified cyclotides to probe structure-activity relationships. A cyclotide-containing product was recently approved for insect control in cotton and macadamia nut crops, marking the first commercial application of cyclotides in agriculture, and there are more than two dozen published examples of cyclotide-based drug leads.

Acknowledgments: Work in our laboratory is supported by the Australian Research Council and the National Health & Medical Research Council

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**PS169** Tue, 16:15-16:50

**Conformations of Tau in Dynamic Assemblies**  
Markus Zweckstetter\*  
*German Center for Neurodegenerative Diseases (DZNE)*

The microtubule-associated protein Tau plays a key role in Alzheimer’s disease (AD). In healthy conditions, Tau binds to tubulin and microtubules, promotes tubulin polymerization and regulates microtubule dynamics in neurons. However, during the course of AD, Tau aggregates into oligomers and amyloid fibrils, which further associate into neurofibrillary tangles in the intracellular space. The appearance and distribution of Tau aggregates correlates with the loss of neurons and cognitive functions in AD.

We use NMR spectroscopy in combination with other biophysical tools to study the structure and dynamics of Tau in different physiological and pathological states. I will report on our recent findings regarding:

- Liquid-liquid phase separation of Tau
- Molecular recognition of Tau by the human Hsp90 chaperone system

**References:** [1] Oroz J, Kim JH, Chang BJ, Zweckstetter M. Nat Struct Mol Biol. 2017 24:407-413. [2] Oroz J, Chang BJ, Wysoczanski P, Lee CT, Pérez-Lara Á, Chakraborty P, Hofele RV, Baker JD, Blair LJ, Biernat J, Urlaub H, Mandelkow E, Dickey CA, Zweckstetter M. Nat Commun. 2018 9:4532. [3] Ambadipudi S, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. Nat Commun. 2017 8:275. [4] Ambadipudi S, Reddy JG, Biernat J, Mandelkow E, Zweckstetter M. Chem Sci. 2019 DOI: 10.1039/c9sc00531e. [5] Ukmar-Godec T, Hutten S, Grieshop MP, Rezaei-Ghaleh N, Cima-Omori M-S, Biernat J, Mandelkow E, Söding J, Dormann D, Zweckstetter M. Nat Commun. 2019 in press.

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**PS170**

Tue, 16:50-17:15

**Multivalency and phase separation of measles virus replication machinery.**Serafima Guseva<sup>\*,1</sup>, Sigrid Milles<sup>2</sup>, Damien Maurin<sup>2</sup>,Malene Ringkjøbing Jensen<sup>2</sup>, Rob Ruigrok<sup>3</sup>, Martin Blackledge<sup>2</sup><sup>1</sup>*Viral Replication Machines Group & Protein Dynamics and Flexibility by NMR Group,**Institut de Biologie Structurale (IBS), CEA, CNRS, University Grenoble Alpes,**Grenoble, France,* <sup>2</sup>*Groupe Flexibilité et Dynamique des Protéines par RMN,**Institut de Biologie Structurale (IBS), CEA, CNRS, University Grenoble Alpes, Grenoble,**France,* <sup>3</sup>*Viral Replication Machines Group, Institut de Biologie Structurale (IBS),**Univ. Grenoble Alpes, CEA, CNRS, Grenoble 38000, France*

RNA viruses tend to concentrate their replication machinery within so called “viral factories”. This membraneless compartment, formed by phase separation, has liquid properties and has been shown for several viruses *in vivo* [1,2]. Measles virus (MeV) is the cause of measles, it infects T-cells and macrophage cells, belongs to *Paramyxoviridae* family. Its genome consists of non-segmented negative-strand RNA, which encodes eight proteins from six genes (N, P, L, H, F and M). The MeV replication machinery consists of viral RNA covered by nucleoproteins (N), and protecting the genome against the host cell immune system, Large protein (L), the viral RNA-polymerase, and its essential co-factor, Phosphoprotein (P) [3]. As has been shown for other single strand RNA viruses there are N and P are essential for phase separation [1,2]. As shown by NMR, N and P interact with each other via long intrinsically disordered domains during their viral cycle [4,5].

Here we show for the first time phase separation of MeV N and P proteins *in vitro*, identify phase separation scaffold and characterise its liquid behaviour. Using NMR we describe multiple interactions between P and N disordered regions which are essential for phase transition and regulation of its biophysical parameters. In addition, we could show a non-stationary stoichiometry between N and P within droplets and propose the model on the basis of observations.

**References:** [1] Brunel J. et al., J Virol., 2014, 88(18):10851-63. [2] Nikolic J. et al., Nat Com, 2017, 8(58):00102-9. [3] Heinrich B. et al., mBio, 2018, 9(5):02290-17. [4] Jensen et al., PNAS, 2011, 108(24):9839-44. [5] Milles et al., Sci Adv, 2018, 22:4(8):eaat7778.

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**PS171** Tue, 17:15-17:40

**A Molecular View of the Liquid to Gel Phase Transition of  
Heterochromatin Protein HP1 $\alpha$**

Bryce Ackermann, Galia Debelouchina\*  
*University of California, San Diego*

Heterochromatin protein 1 $\alpha$  (HP1 $\alpha$ ) plays a central role in the organization of nuclear content and in the regulation of gene expression and chromatin compaction. Recent work has shown that this protein can phase separate into liquid droplets and gels in vitro, properties that have vast implications for the mechanism of heterochromatin formation and regulation in cells. Despite the tremendous amount of interest in this process, the amorphous, dynamic and viscous nature of the gel condensates has precluded high-resolution analysis of the molecular interactions that underlie HP1 $\alpha$  transitions. Using magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy, here we present for the first time a molecular description of the liquid to gel transition of phosphorylated HP1 $\alpha$ . This methodology has allowed us to follow in real time the rigidification of the molecular interaction network during gelation and to identify specific residues that contribute to gel formation. Furthermore, the addition of physiologically relevant chromatin polymers disrupts the gelation process while preserving the conformational dynamics within individual HP1 $\alpha$  molecules. Our results suggest an important role for chromatin in determining the material properties of HP1 $\alpha$  condensates and in establishing the complex dynamics within heterochromatin compartments. Our methodology can be applied to other protein systems that undergo phase separation and thus provide atomic resolution details of an elusive biological process.

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PS172

Tue, 16:15-16:50

**New approaches for J-coupling measurement and  
five-membered ring conformation analysis**

Davy Sinnaeve\*

CNRS / Université de Lille

For any conformational analysis based on spectroscopic data, both the amount of data extracted and the accuracy of the theoretical relation between data and conformation are key. In this presentation, new developments for both the measurement of  $^1\text{H}$ - $^1\text{H}$   $J$ -couplings and their use for conformational analysis of five-membered rings will be discussed.

Measuring  $J$ -couplings in compounds that are mostly aliphatic can be problematic, as the limited chemical shift dispersion and the presence of many couplings often results in intricate and overlapping multiplets. Also strong coupling conditions are common, which complicates accurate  $J$ -coupling extractions. A number of strategies will be presented that build on the PSYCHEDELIC experiment [1] that can resolve these issues.

Five-membered rings are widely occurring in biological and synthetic compounds, and the preferred ring conformation is often crucial for function. In contrast to six-membered rings, five-membered rings are generally flexible, sampling a distribution of conformers, making their analysis challenging. Experimentally, vicinal  $J$ -couplings are ideal for conformational analysis of ring systems, as they are sensitive to dihedral angles. However, a key issue is that the available Karplus relations are not always appropriate for a given system. For instance, for difluorinated prolines [2,3], existing Karplus relations for  $^1\text{H}$ - $^1\text{H}$  couplings turn out to be insufficiently accurate, while no appropriate relations exist for  $^1\text{H}$ - $^{19}\text{F}$  couplings. Also non-bonded electronic interactions across the five-membered ring have significant impact on the  $J$ -coupling. We therefore resorted to an alternative approach that is based on extensive mapping of the full conformational space of model compounds using DFT, followed by  $J$ -coupling calculation.

**References:** [1] Sinnaeve D. *et al.*, *Angew. Angew. Chem. Int. Edit.* (2016), 55, 1090. [2] Hofman G.J. *et al.*, *Chem. Commun.* (2018), 54, 5118. [3] Hofman G.J. *et al.*, *J. Org. Chem.* (2019), 84, 3100.

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**PS173**

Tue, 16:50-17:15

**A boost in Drug Discovery with Secondary-labeled Hyperpolarized Ligands**Olivier Cala<sup>\*1</sup>, Quentin Chappuis<sup>1</sup>, Morgan Ceillier<sup>1</sup>, Samuel François Cousin<sup>1</sup>, Houssein Eddine Boughazi<sup>1</sup>, Amélie Beck<sup>1</sup>, Basile Vuichoud<sup>1</sup>, Aurélien Bornet<sup>2</sup>, Sami Jannin<sup>1</sup><sup>1</sup>Université de Lyon, Université Claude Bernard Lyon 1, ENS de Lyon, CNRS, CRMN FRE 2034, <sup>2</sup>Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Hyperpolarization by dissolution DNP[1] provides a route to enhancing <sup>13</sup>C MR sensitivity by more than four orders of magnitude on a wide range of small molecules. However, many potential applications of dDNP (metabolomics, drug discovery, etc.) would highly benefit from a higher efficiency, throughput, and repeatability of the method.

In this context, we had demonstrated in 2016 that high levels of polarization were reachable in short times (60% in 8 min)[2] and with high repeatability (CV=3.6%)[3].

One important drawback still remains in dDNP which is that it generally relies on low natural abundance <sup>13</sup>C spins (1.1%). In 2009, Wilson et al. proposed an approach where amine groups in amino acids were labeled with [1,1-<sup>13</sup>C] acetic anhydride[4], and subsequently hyperpolarized. Though very promising, this approach had not been taken up by the dDNP community till 2018 where we revisited this secondary labeling approach in the context of NMR drug screening[5]. We have showed how ligands could be secondary labeled, used to probe interactions with target proteins through <sup>13</sup>C NMR.

We are now working at combining this concept with dDNP with the aim of decreasing experiment time and sample concentration by orders of magnitudes compared to the classical approach (STD / WaterLOGSY). This has the potential to considerably improve the detection and identification of ligands, and shorten the discovery time of new drug candidates.

**References:** [1]. Ardenkjaer-Larsen, J. H. et al. Proc. Natl. Acad. Sci. USA 100, 10158–10163 (2003). [2]. Bornet, A. et al. Phys. Chem. Chem. Phys. 18, 30530–30535 (2016). [3]. Bornet, A. et al. Anal. Chem. 88, (2016). [4]. Wilson, D. M. et al. Proc. Natl. Acad. Sci. 106, 5503–5507 (2009). [5]. Cala, O. et al. Euromar 2018 Nantes France.

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**PS174**

Tue, 17:15-17:40

**Monitoring Oxygen Levels in Microfluidic Devices using  $^{19}\text{F}$  NMR**Sylwia Ostrowska<sup>\*,1</sup>, Bishnubrata Patra<sup>1</sup>, Ciara Nelder<sup>1</sup>, Manvendra Sharma<sup>1</sup>, Marcel Utz<sup>2</sup><sup>1</sup>University of Southampton, <sup>2</sup>School of Chemistry, University of Southampton

We report an in-situ, non-invasive approach to quantify oxygen partial pressure in microfluidic lab-on-a-chip (LoC) devices. LoC systems provide a versatile platform to culture biological systems. As they allow a detailed control over the growth conditions, LoC devices are finding increasing applications in the culture of cells, tissues and other biological systems [1]. Integrated microfluidic NMR spectroscopy [2] allows non-invasive monitoring of metabolic processes in such systems. Quantification of oxygen partial pressure would help ensuring stable growth conditions, and provide a convenient means to assess the viability of the cultured system. However, oxygen, one of the most important metabolites, cannot be quantified using either proton or carbon NMR spectroscopy.

As is well known, the oxygen partial pressure can be determined by MRI in vivo by measuring the  $^{19}\text{F}$  spin-lattice relaxation time of perfluorinated agents [3]. Here, we show that the oxygen partial pressure in microfluidic devices of  $2.5\ \mu\text{l}$  can be quantified using the  $^{19}\text{F}$  spin-lattice relaxation rate of perfluorinated tributylamine. The compound is added to the aqueous perfusion medium in the form of micrometer-sized droplets. Our set up comprises a microfluidic device and a PDMS layer sandwiched between two 3D printed holders. The droplet emulsion is delivered via a syringe pump and carbogen is delivered through a separate channel. The semi-permeable PDMS layer acts as a diffusion bridge between the liquid and gas channels, allowing for oxygen to diffuse into the emulsion.  $T_1$  is obtained through standard inversion recovery experiments detected using a home-built transmission-line probe.[2] Due to the non-toxic nature of droplet emulsion, it can be easily incorporated into the perfusion fluid allowing for quantification of tissue oxygen levels.

**References:** [1] Gracz et al., Nature Cell Biology 17, 340–349, 2015. [2] M.Sharma, M.Utz, J.Mag.Res 303, 75-81, 2019. [3] R.Manson et al., Magnetic Resonance in Medicine 18, 71-79, 1991.

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**PS175** Tue, 16:15-16:50

**Studying structure and function of sialic acid TRAP transporters from pathogenic bacteria by pulsed EPR, FRET and X-ray crystallography**  
Martin Peter, Janin Glaenger, Gavin Thomas, Gregor Hagelucken

Many pathogens such as *Vibrio cholerae* use tripartite ATP-independent periplasmic (TRAP) transporters to scavenge N-acetyl- neuraminic acid (sialic acid) from host organisms. The sialic acid is then incorporated into the bacterial cell wall, as a disguise to protect against detection by the human immune system. TRAP transporters are a structural and functional mix between ABC transporters and secondary active transporters. The substrate binding proteins (SBP) of TRAP transporters are the best studied component and are responsible for initial high-affinity substrate binding. To better understand the dynamics of the ligand binding process, pulsed electron-electron double resonance (PELDOR, also known as DEER) spectroscopy and FRET were applied to study the conformational changes in the N-acetylneuraminic acid-specific SBP VcSiaP. The protein is the SBP of VcSiaPQM, a sialic acid TRAP transporter from *Vibrio cholerae*. Spin-labeled double-cysteine mutants of VcSiaP were analyzed in the substrate-bound and -free state and the measured distances were compared to crystal structures of the labelled protein. The data were compatible with two clear states only, which are consistent with the open and closed forms seen in TRAP SBP crystal structures. Substrate titration experiments demonstrated the transition of the population from one state to the other with no other observed forms. Mutants of key residues involved in ligand binding and/or proposed to be involved in domain closure were produced and the corresponding PELDOR experiments reveal important insights into the open-closed transition. Further, PELDOR distance measurements on the whole transporter in lipid nano discs were used to evaluate molecular models of the transporter.

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**PS176**

Tue, 16:50-17:15

**When stronger magnets don't help: Methods for disentangling overlapping high-field EPR spectra illustrated in record organic solar cell blend PBDB-T:ITIC**Melissa Van Landeghem<sup>\*,1</sup>, Wouter Maes<sup>2</sup>, Etienne Goovaerts<sup>1</sup>, Sabine Van Doorslaer<sup>1</sup><sup>1</sup>*Department of Physics, University of Antwerp*, <sup>2</sup>*Institute for Materials Research, Design & Synthesis of Organic Semiconductors, Hasselt University*

Spectral overlap, even at high field, is a problem generally encountered in many EPR studies. In the specific case of bulk-heterojunction (BHJ) organic solar cells (OSCs), the paramagnetic species of interest are light-induced radicals which are created as a pair after charge transfer at the interface between the donor polymer and molecular acceptor regions making up the BHJ blend. Hence, the similar g-values expected for the positive and negative organic radicals often lead to strong spectral overlap complicating the unambiguous assignment of the light-induced (LI) EPR spectrum.

The donor-acceptor combination studied here, PBDB-T:ITIC, was the first fullerene-free OSC to recently achieve >11% efficiency, challenging the state-of-the-art polymer-PC<sub>71</sub>BM devices [1]. For this blend, the two-component structure of the LI-EPR spectrum could not even be resolved at W-band frequency (94 GHz). Therefore we separated the two contributions to the total EPR spectrum by exploiting two different properties of the charge-transfer radicals, namely the (small) difference in their longitudinal ( $T_1$ ) relaxation times and the presence of a unique magnetic nucleus, <sup>14</sup>N, in ITIC. For the  $T_1$ -based method, we applied an inversion-recovery filter to selectively suppress one component in the spin echo analogously to the relaxation-filtered hyperfine spectroscopy (REFINE) technique first proposed by Maly et al. [2]. Sensitive detection of the <sup>14</sup>N hyperfine couplings at W-band frequency was achieved by means of electron-electron double resonance (ELDOR)-detected NMR (EDNMR). Here we demonstrate the application of EDNMR-induced EPR to obtain an EPR spectrum containing only contributions from the ITIC radical [3]. Both approaches are validated by LI-EPR spectra on related blends which yield better-resolved spectra of the individual PBDB-T and ITIC radicals.

**References:** [1] W. Zhao et al., *Adv. Mater.*, 2016, 28, 4734. [2] T. Maly, T. F. Prisner, *J. Magn. Reson.*, 2004, 170, 88. [3] M. Van Landeghem et al., *J. Magn. Reson.*, 2018, 288, 1.

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**PS177**

Tue, 17:15-17:40

**Non-magnetic magnetic resonance**

Gunnar Jeschke<sup>\*,1</sup>, Mantas Šimėnas<sup>2</sup>, Daniel Klose<sup>1</sup>, Maciej Ptak<sup>3</sup>,  
Kęstutis Aidas<sup>2</sup>, Mirosław Mączka<sup>3</sup>, Jūras Banys<sup>2</sup>, Andreas Pöpl<sup>4</sup>

<sup>1</sup>ETH Zurich, <sup>2</sup>Vilnius University, <sup>3</sup>Polish Academy of Sciences, <sup>4</sup>Leipzig University

Magnetic resonance observes spin transitions whose frequencies depend on magnetic field because spin is associated with magnetic moment. Allowed transitions involve a unit change of the magnetic quantum number. If the magnetic quantum number is not a good quantum number, other transitions can be partially allowed. The transition moment of such “forbidden” transitions depends on magnetic field. All this is taken for granted. Yet, in three-pulse electron spin echo envelope modulation (ESEEM) experiments on a Mn(II)-doped  $[(\text{CH}_3)_2\text{NH}_2][\text{Zn}(\text{HCOO})_3]$  metal-formate framework, we have recently observed features whose frequencies and normalized intensities did not change in the magnetic field range between 0.325 and 3.35 T [1]. By a series of isotope substitution experiments these signals could be attributed to the methyl groups of the dimethylammonium cation. Here we demonstrate that they derive from hyperfine-perturbed methyl tunnel splitting. We discuss how a nearby electron spin breaks  $C_3$  symmetry of the methyl group and why this can lead to polarization transfer to the tunnel splitting transition. We show that, by manipulating solely the electron spin by microwave pulses, the tunnel-split states can be coherently superimposed and the phase of this coherence can again be read out by the electron spin. Spectra and signal buildup are in good agreement with simulations, whereas rotation barriers derived from the tunnel splitting for two frameworks are in fair agreement with predictions by density functional theory. We discuss the possibility of observing the hyperfine-unperturbed tunnel splitting in an ESEEM experiment and the potential of the effect for dynamic nuclear polarization.

**References:** [1] Šimėnas, M., Macalik, L., Aidas, K., Kalendra, V., Klose, D., Jeschke, G., Mączka, M., Volkel, G., Banys, J. & Poppl, A. Pulse EPR and ENDOR Study of Manganese Doped  $[(\text{CH}_3)_2\text{NH}_2][\text{Zn}(\text{HCOO})_3]$  Hybrid Perovskite Framework. *J. Phys. Chem. C* **121**, 27225-27232 (2017).

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**PS178**

Tue, 16:15-16:50

**Dissolvable inserts for achieving performance enhanced resonators**Jessica Kelz, Garabedian Alexandra, Rachel Martin\**University of California, Irvine*

Dissolvable 3D-printed templates can be used to produce NMR transceiver coils of a particular geometry. Wire coils such as variable-pitch solenoids are wound on dissolvable 3D printed forms, which are then dissolved away in solvent. The use of high-temperature resin, with the appropriate solvent, enables annealing to be performed before dissolving the template. This approach allows facile, reproducible production of coils even in the hands of inexperienced researchers. The ability to design a coil and its corresponding template in a CAD program, simulate the magnetic and electric fields in its vicinity, print out the template, and make it greatly facilitates the process of testing coil designs and sharing successful ones with other laboratories. This approach can also be used to enable production of more complicated designs that are not easily made with hand winding. I will describe strategies for using 3D printed coil forms in conjunction with simulation-based optimization to produce high-homogeneity rf coils. Simulations and experimental (benchtop and NMR) measurements using different resonator designs will be discussed, along with future applications to biomolecular NMR.

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**PS179**

Tue, 16:50-17:15

## Rapid scan EPR-on-a-chip

Silvio Küstner\*<sup>1</sup>, Anh Chu<sup>2</sup>, Boris Naydenov<sup>1</sup>, Jens Anders<sup>2</sup>, Klaus Lips<sup>3</sup>

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Electron paramagnetic resonance (EPR) is the method of choice to investigate and quantify paramagnetic states in e.g. semiconductor devices, proteins, catalysts and molecular nanomagnets. Common EPR spectrometers use microwave (mw) resonators, where the sample is inserted. This design, however, limits the versatility for in situ / operando measurements. Here, we present an improved design of a miniaturised EPR spectrometer, implemented on a single microchip (EPR-on-a-chip). Instead of an mw resonator, an array of coils, each from a voltage-controlled oscillator (VCO), with a diameter of a few hundred micrometer is used simultaneously as mw source and detector, replacing the entire microwave bridge. Similar to EPR microresonators, the filling factor of the EPRoC is high by design, leading to a better absolute spin sensitivity than conventional EPR. The usage of the VCO allows to sweep the microwave frequency, instead of magnetic field as in the conventional EPR, thus enabling operation with a permanent magnet. Due to its compactness, EPRoC can be incorporated into conventional thin-film growth reactors, (electro)chemical cells, batteries or in UHV environments.

Frequency sweeps in combination with the intrinsically high  $B_1$  render EPRoC perfect for rapid frequency scan EPR (rsEPRoC) with scan rates up to 2 000 THz/s. Rapid scan EPR can lead to a signal-to-noise improvement especially for samples with long relaxation times, which would otherwise be saturated in continuous wave EPR. We demonstrate the increased sensitivity of rsEPRoC, by investigating a few micrometer thick layers of amorphous silicon (a-Si) on quartz.

In this talk, we will review the recent advances in rsEPRoC, show first results on amorphous silicon samples, and discuss applications that will benefit from the increased sensitivity.

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**PS180**

Tue, 17:15-17:40

**Rheology and  $^{23}\text{Na}$  Multiple Quantum Filtered (MQF)  
rheo-NMR and MRI of Bile Salt Micelles**

Galina Pavlovskaya<sup>\*,1</sup>, Alisha Hallwood<sup>1</sup>, Samuel Holmes<sup>1</sup>,  
Thomas Meersmann<sup>2</sup>, Fioretta Asaro<sup>3</sup>

<sup>1</sup>University of Nottingham, <sup>2</sup>Sir Peter Mansfield Imaging Centre,  
School of Medicine, University of Nottingham, <sup>3</sup>University of Trieste

Bile or gall is a dark green to yellowish brown fluid, produced by the liver of most vertebrates and aids the digestion of lipids in the small intestine. The composition of bile is mostly water (97%) however, it also contains small amount (0.7%) of bile salts, as well as fats and inorganic salt ions. Bile salts are complex molecules that tend to form micellar aggregates in solutions if their and/or salt concentration increases. This process affects rheology of the bile thus making its viscosity shear-dependent during flow in a bile duct. Pathological bile extracted from gallbladder and liver patients is non-Newtonian [1], and is hypothesized that the formation of micellar aggregates in the bile during flow through the duct contributes to this behaviour. This currently is being explored for potential clinical benefits for the use in  $^{23}\text{Na}$  whole body MRI. One of the principal components of human bile is taurodeoxycholic (TDC) acid that forms a sodium salt, NaTDC, in the excess of  $\text{Na}^+$  [2]. We studied temperature and shear effects on the micellar formation in 0.2M NaTDC /0.25M NaCl and in 0.2M NaTDC /0.5M NaCl systems using rheology and  $^{23}\text{Na}$  MQF rheo-NMR and MRI. Double quantum filtered magic angle (DQF MA) and triple quantum filtered (TQF) rheo-NMR [3-5] was performed at multiple shear rates and temperatures. The formation of shear-induced phase was clearly demonstrated by  $^{23}\text{Na}$  rheo-NMR and it was found that  $^{23}\text{Na}$  MQF rheo-NMR methods were able to detect and characterise the formation of the shear-induced phase more efficiently than bulk rheometry methods.  $^{23}\text{Na}$  MRI with MQF filters allowed to map zones where shear-induced phase was formed and to characterise molecular alignment in the gap. This demonstrates the potential of  $^{23}\text{Na}$  MQF MRI contrast for the in-vivo molecular mechanics for clinical benefits.

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**PS181** Wed, 10:30-11:05

**Integrative structural biology of non-coding RNA-protein complexes:  
telomerase and 7SK**

Juli Feigon\*  
*University of California Los Angeles*

Telomerase and 7SK are essential eukaryotic RNA-protein complexes (RNPs) involved in telomeric DNA synthesis and regulation of mRNA transcription, respectively. Each comprises a non-coding RNA and constitutively and/or transiently associated proteins. Telomerase maintains the DNA at the ends of linear chromosomes, thereby preventing genomic instability. Its catalytic core is a non-coding telomerase RNA (TER) and a unique telomerase reverse transcriptase (TERT); other associated proteins are involved in biogenesis, assembly, recruitment to telomeres, and recruitment of other proteins of the DNA synthesis machinery. TERT uses a template complementary to ~1.5 telomere repeats in TER to repetitively synthesize the telomere repeat sequence at the 3' end of the DNA (TTGGGG in ciliates, TTAGGG in vertebrates), but this template alone is insufficient for activity with TERT. Multiple steps of single-stranded telomeric DNA template binding/realignment, nucleotide addition, strand separation, and template translocation are required for synthesis of a single telomere repeat and telomere repeat addition processivity (RAP). We have been using an integrative structural biology approach combining NMR spectroscopy, X-ray crystallography, mass spectrometry, and electron microscopy to study the structure and function of telomerase from the ciliate *Tetrahymena* and from humans. I will discuss how our NMR studies of telomerase RNA structure and dynamics have been combined with cryo electron microscopy to help elucidate the roles of TER and proteins in this remarkable enzyme. I will also present recent NMR and X-ray crystallography results on the human long-noncoding RNA 7SK and two of its protein partners Larp7 and methylphosphate capping enzyme (MePCE).

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**PS182**

Wed, 11:05-11:30

**A Case of Domain Cooperation in a Multidomain Protein Interaction at Telomeres.**

Simona Miron, Guillaume Gaullier, Marie-Hélène Le Du,

Sophie Zinn-Justin, Philippe Cuniassé\**Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay*

Telomeres are specialized structures located at the ends of linear chromosomes essential for cell viability and genome integrity. Their protective function is due to the formation of the Shelterin complex, that caps the end of the DNA. In the mammalian complex, Telomeric Repeat-binding Factor 2 (TRF2) interacts with TRF2-interacting protein 1 (RAP1).

We previously showed by ITC that TRF2-RAP1 interaction involves a complex biphasic mechanism [1]. RAP1, as TRF2, is a multidomain protein, comprising a N-terminal domain with sequence similarity to BRCT domains (RAP1[BRCT]), a central pseudo-Myb domain and a C-terminal domain that binds with high affinity with the so-called RAP1-binding domain of TRF2. The function of the RAP1[BRCT] is not yet assigned.

Here we report a structural analysis of the binding between the different domains of RAP1 and TRF2. First, we solved the solution structure of RAP1[1-114] that belongs to BRCT domain family. The conformation of the YRLGP sequence in RAP1[1-114] is well defined and similar to that adopted in a 14-mer peptide crystallized in complex with the dimeric TRFH domain of TRF2 [1]. This led us to build a RAP1[1-114]-TRFH dimer model. However, we showed by NMR that the isolated RAP1[1-114] domain is not able to interact with TRFH. This observation seemed a priori in contradiction with ITC data previously obtained to characterize the interaction of the full form of RAP1 with TRF2. However, re-examination of ITC and NMR interaction data obtained with truncated forms of the two proteins permitted to reconcile these data in the light of the divalent thermodynamics interaction model [3]. The cooperative mechanism revealed by this analysis could contribute to regulate the interaction of TRF2 with partners interacting via YRLGP-like peptides.

**References:** [1] Gaullier G., S. Miron, S. et al. (2016). *Nucleic Acids Research* 44: 1962. [2] Kane, R., S. (2010). *Langmuir* 26:8636.

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**PS183** Wed, 11:30-11:55

**Solution structure of Upstream-of-N-Ras, a 116 kDa multi-domain RNA binding protein**

Nele Merret Hollmann<sup>1</sup>, Pravin Kumar Ankush Jagtap<sup>1</sup>, Tanit Guitart<sup>2</sup>, Pawel Masiewicz<sup>1</sup>,  
Lara Sweetapple<sup>1</sup>, Bernd Simon<sup>1</sup>, Fátima Gebauer<sup>2</sup>, Janosch Hennig<sup>\*,1</sup>

<sup>1</sup>*Structural and Computational Biology Unit, EMBL Heidelberg.* <sup>2</sup>*Stem Cells and Cancer Programme, Gene Regulation, Centre for Genomic Regulation (CRG) Barcelona*

The protein Upstream-of-N-Ras (Unr) is a highly conserved and abundant RNA binding protein with elevated expression levels in several cancer types. Here, it is supposed to bind certain mRNAs to regulate their translation. In *Drosophila*, Unr acts an RNA chaperone, where it binds to long non-coding RNA RoX2 to promote formation of the dosage compensation complex. In all hitherto publications concerning Unr it has been proposed that it features five cold shock domains (CSDs), which are known for binding single-stranded RNAs. These domains are presumably connected with flexible linkers.

However, our construct optimizations and NMR analysis revealed the presence of altogether nine CSDs. Interestingly, the predicted canonical CSDs are interspersed with novel non-canonical CSDs, which share the same fold with the canonical ones, but lack their otherwise conserved trademark RNA binding residues. Structure determination of several tandem and triple domain constructs by NMR and X-ray crystallography revealed that there are no flexible linkers and that the domains have indeed a fixed orientation towards each other. This indicates that Unr, although binding to single-stranded RNAs via its CSDs, achieves also RNA structure specificity due to the fixed orientations and distances of canonical CSDs.

Complementing the structures with small-angle X-ray scattering and further NMR data we were able to obtain a high-resolution structural ensemble of the full-length protein. Additional mutational analysis using biochemical, biophysical and cellular assays confirm the importance of inter-domain arrangements for RNA specificity and function.

This study presents a new paradigm of how a general single-stranded RNA binding protein achieves RNA structure specificity.

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**PS184**

Wed, 11:55-12:30

## High molecular-weight complexes in the regulation of gene expression: a view by integrative structural biology

Nataliya Danilenko, Lukas Lercher, John Kirkpatrick, Frank Gabel, Teresa Carlomagno

The Regulator of Ty1 Transposition protein 106 (Rtt109) is a fungal histone acetyltransferase required for histone H3 K9, K27 and K56 acetylation. These acetylation sites have been linked to processing and folding of nascent H3 and play an integral role in replication- and repair-coupled nucleosome assembly. Rtt109 is unique in its activation, performed by two structurally unrelated histone chaperones, Asf1 and Vps75. These proteins stimulate Rtt109 activity via different mechanisms. Rtt109 - Asf1 association has been proposed to be responsible for K56 acetylation, while the Rtt109-Vps75 interaction is required for K9 acetylation.

In our work we find that Rtt109, Vps75 and Asf1 are capable of assembling as a previously uncharacterized complex onto the substrate H3-H4 dimer. Using an integrative structural biology approach based on a powerful combination of solution state NMR and small angle neutron scattering (SANS) we solve the structure of this complex and provide a structural basis for the efficiency and selectivity of acetylation at the at the H3 K9, K27 and K56 sites.

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**PS186**

Wed, 11:05-11:30

**Squeeze It Until It Breaks: In-situ NMR at Geophysically Relevant Conditions**Thomas Meier<sup>\*,1</sup>, Leonid Dubrovinsky<sup>1</sup>, Natalia Dubrovinskaia<sup>2</sup>, Florian Trybel<sup>1</sup>,Saiana Khandarkhaeva<sup>1</sup>, Sylvain Petitgirard<sup>3</sup>, Timofey Fedotenko<sup>2</sup><sup>1</sup>*Bavarian Geoinstitute*, <sup>2</sup>*Material Physics and Technology at**Extreme Conditions, Laboratory of Crystallography*, <sup>3</sup>*ETH Zürich*

Recent developments in magnetic flux tailoring techniques paved the way for NMR in high pressure diamond anvil cells at multi-megabar pressures. Using a combination of physical vapor deposition and focused ion beam milling techniques, NMR resonator structures based on the principles of recently developed Lenz lenses can be realized with 1  $\mu\text{m}$  spatial resolution. These structures have been demonstrated to combine the mechanical robustness and spin sensitivity required for geophysically extreme conditions, i.e. pressures well above 100 GPa and temperatures above 1000 K.

Here we present our work of the last 3 years at the Bavarian Geoinstitute which led to this major technological advancement.

**PS187**

Wed, 11:30-11:55

**Microscale NMR-spectroscopy with femtomole sensitivity using diamond quantum sensors**

Dominik Bucher\*

*Technical University of Munich*

Nuclear magnetic resonance (NMR), one of the most powerful analytical techniques in chemistry and life science, is typically limited to macroscopic volumes due to its inherent low sensitivity. This excludes NMR spectroscopy from analysis of microscopic samples sizes such as in single-cell biology or in microfluidic applications. In recent years, it has been shown that NMR signals can be detected from nano- to microscale volumes by a new sensor class – quantum sensors based on defects in the diamond lattice - the nitrogen-vacancy (NV) center. However, these experiments were limited by a low spectral resolution and to pure samples with high viscosity, which precludes practical applications in chemistry. Here, I will present our recent results where we could overcome these basic problems. First, I will describe how NV-centers can be used to detect NMR signals from picoliter sample volumes on the surface of the diamond chip with high spectral resolution ( $\sim 1$  Hz). Second, I will discuss our newest results on improving the molecular sensitivity of this approach by hyperpolarizing nuclear sample spins. This technique combines microscopic-scale NV-NMR with a fully integrated Overhauser dynamic nuclear polarization scheme which reaches femtomole sensitivity. I will provide an overview of this rapidly developing technology and discuss potential applications, such as single cell metabolomics.

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**PS188**

Wed, 11:55-12:30

## Solid State NMR Probes for 1.5 GHz Spectrometer

Peter Gor'kov\*, Wenping Mao, Jason Kitchen, Ilya Litvak,

Ivan Hung, Zhehong Gan, William Brey

*National High Magnetic Field Laboratory*

We report on design of solid-state NMR probes for the 1.5 GHz NMR magnet at NHMFL facility in Florida.[1] This magnet is open to external NMR users, offering sensitivity and resolution enhancements, and the new opportunities in NMR of quadrupolar and low- $\gamma$  nuclei. We will discuss strategy for making higher-field solid-state NMR probes for materials and biological applications, while preserving the large sample volumes that NMR community grew accustomed to in the sub-GHz NMR range. We constructed multi-resonant direct-detection NMR probes, MAS and static, with sample sizes in 2, 3, 4, 5 mm range. These probes are part of the 1.5 GHz Bruker NEO spectrometer. We will report performance and NMR spectra.

Both  $^1\text{HXY}$  and  $^1\text{HX}$  probes use Low-E coils designs that separate high- and low-frequency RF circuits[2]. This allows efficient direct detection at higher  $^1\text{H}$  frequencies with far less trade-off in sample size or sensitivity. The triple-resonance  $^1\text{HXY}$  circuit is arranged on tune cards<sup>1</sup> for quick interchange of X/Y isotopes. The static probe has modular slide-in sample coils that accommodate different sample shapes and sizes: 3, 4, 5 mm round and 4x4 mm square profiles.

Each probe required additional hardware to perform NMR in the hybrid magnet consisting of series-connected superconducting coil and resistive DC insert.<sup>1</sup> Active field-regulation compensates  $B_0$  fluctuations, enabling signal averaging and 2D NMR experiments. An inductive sensor tracks  $B_0$  field and drives an outer correction coil to cancel fast 60 Hz fluctuation and harmonics. An external  $^7\text{Li}$  NMR field lock is incorporated to correct slower  $B_0$  drift. A  $\text{MnCl}_2$ -doped LiCl solution serves as the lock sample. Spatial  $B_0$  inhomogeneity of  $< 0.9$  ppm over  $1\text{cm}^3$  DSV is achieved by combining active shims with the passive ferroshims in the probehead.

**References:** [1] Gan Z. et al., J.Magn.Reson. 2017; 284, 125. [2] Gor'kov P. et al., J.Magn.Reson. 2007; 185, 77.

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**PS189**

Wed, 10:30-11:05

**Boosting the NMR characterization of small- to medium-sized molecules**

Katalin E. Kövér\*

*University of Debrecen*

The recently introduced CLIP-COSY[1] experiment providing homonuclear correlation spectrum with high quality clean in-phase multiplets expedites the assignment of scalar coupled proton spin network, aiding the structure elucidation of small- and medium-sized molecules. The resolution of COSY spectra is, however, limited by the inherently small chemical shift dispersion of proton resonances.

Last year we devised HSQC-variant of the CLIP-COSY experiment[2] for enhancing the resolving power of the method by utilizing the increased chemical shift range of heteronuclei. Herein, it will be demonstrated that the performance of the original HSQC-CLIP-COSY experiment can be further boosted with incorporation of heteronuclear spin echo block(s) in the pulse sequence, allowing phase-editing of crosspeaks of different types. The edited HSQC-CLIP-COSY experiments providing well-resolved spectra have great promise, enabling straightforward NMR assignments for carbohydrates and peptides and for other types of molecules.

The applicability of the CLIP-COSY approach can be extended for binding studies using a combined STD-CLIP-COSY(relayed) experiment. The resulting well-resolved, high quality 2D spectrum makes feasible to separate overlapping signals of 1D STD, allowing quantitative assessment of binding.

In small-molecule NMR there has always been a need for an experiment to distinguish between two- and three-bond correlations for quaternary  $^{13}\text{C}$ -s in a sensitive and reliable way. Last year we introduced a simple, non-selective experiment, SEA XLOC[3], which is capable to distinguish these correlations in a novel way and is applicable for all  $^{13}\text{C}$  multiplicities.

In the talk the scope and limitations of these experiments will be illustrated by applications to spins systems of varying complexity.

**References:** [1] Koos, MRM, Kummerlöwe, G, Kaltschnee, L, Thiele, CM, Luy, B, *Angew. Chem., Int. Ed.*, 55, 7655-7659, 2016. [2] Gyöngyösi, T, Timári, I, Haller, J, Koos, MRM, Luy, B, Kövér, KE, *ChemPlusChem*, 83, 53-60, 2018. [3] Gyöngyösi, T, Nagy TM, Kövér, KE, Sørensen, OW, *Chem. Commun.*, 54, 9781-9784, 2018.

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**PS190**

Wed, 11:05-11:30

## Prebiotic Organization of Biomolecules on Mineral surfaces

Hagop Abadian, Jean-Francois Lambert, Christel Gervais

The structural organization of the interface characterizing binding, assembly and recognition of biomolecules on inorganic surfaces has attracted considerable attention in the domains of catalysis and prebiotic chemistry. In the context of origins-of-life chemistry, this study is focused on the catalytic effect of a silica surface on amino acids condensation, more precisely on two amino acids, Leucine and Glutamic acid, adsorbed on silica nanoparticles. It takes into consideration the important effect of hydration on the system. Solid state NMR is a choice technique for surface characterization and observation of bonding at the atomic level, as well as local proximities between the amino acid and the silica surface sites.

$^{13}\text{C}$  and  $^{15}\text{N}$  CP-MAS NMR and 2D-HETCOR experiments allowed to characterize the structure of adsorption complexes at the interface, involving amino acids, surface groups, and water molecules. At high surface coverages, both crystalline and adsorbed Leucine exist in the samples, while only adsorbed forms of leucine were observed at rather low surface coverage (3%Leu/SiO<sub>2</sub>). The same type of approach was applied to Glutamic Acid, but the adsorbed form was predominant only at much lesser loadings (0.3%Glu/SiO<sub>2</sub>) than those observed for Leucine. The sensitivity of NMR compared to other characterization techniques is low; however, we were able to use  $^{13}\text{C}$  and  $^{15}\text{N}$ -enriched amino acids which gave us information on the evolution of chemical shifts, after adsorption and upon drying in careful conditions, even for such low loadings.

Hydration was a key parameter in our experiments and samples with varying degrees of hydration were studied experimentally by NMR, and also by computational methods such as DFT simulation. Water turned out to mediate the interaction of molecules with silanols of the silica surface.

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**PS191**

Wed, 11:30-11:55

**Recent advances in polypeptidic thermoresponsive alignment media for organic compounds**

Christina M. Thiele\*

*Technische Universität Darmstadt*

Anisotropic NMR parameters become increasingly important in organic structure elucidation for the determination of conformations and relative configuration of natural products, synthesized compounds and catalysts.[1]

For anisotropic NMR parameters to be obtained suitable alignment media are necessary. The use of lyotropic liquid crystals from helically chiral polymers is especially intriguing in that respect as they additionally allow for enantiodiscrimination.[2]

We have recently synthesized several homopolypeptides[3], which form lyotropic liquid crystals, are suitable for the measurement of anisotropic NMR observables, show excellent enantiodiscrimination and furthermore induce different orientations at different temperatures. They are thus considered thermoresponsive.

The possibility to induce different orientation at different temperatures thus alleviates the need to use more than one alignment medium in cases of ambiguity.

The intriguing properties of these new thermoresponsive alignment media will be described in this presentation.

**References:** [1] For reviews see: C. M. Thiele, *Eur. J. Org. Chem.* **2008**, 5673-5685; V. Schmidts, *Magn. Reson. Chem.* **2017**, *55*, 54-60. [2] For review see: P. Lesot, J. Courtieu, *Prog. Nucl. Magn. Reson. Spectrosc.* **2009**, *55*, 128-159. [3] M. Schwab, D. Herold, C. M. Thiele, *Chem. Eur. J.* **2017**, *23*, 14576-14584; M. Schwab, V. Schmidts, C. M. Thiele, *Chem. Eur. J.* **2018**, *24*, 14373-14377; S. Jeziorowski, C. M. Thiele, *Chem. Eur. J.* **2018**, *24*, 15631-15637.

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**PS192**

Wed, 11:55-12:30

**Fast quantitative 2D NMR for metabolomics**

Patrick Giraudeau

*Université de Nantes*

NMR is a major tool in metabolomics thanks to its non-destructive and highly reproducible character. NMR metabolomics include untargeted analysis where spectral fingerprints are analyzed with statistical tools to highlight potential biomarkers, and targeted methods which aim at accurately quantifying multiple metabolites. Most studies rely on 1D NMR which suffers from ubiquitous spectral overlap that hampers the accurate determination or quantification of biomarkers.

In this lecture, we will try to answer the following question: can we replace **1D by 2D spectroscopy in NMR metabolomics**? We illustrate, through several examples, how 2D NMR can advantageously replace 1D spectra in both targeted and untargeted workflows, provided that fast and reproducible methods are employed to fit the high-throughput requirements of metabolomics [1].

For untargeted methods, a variety of accelerated pulse sequences can be used, such as those relying on **ultrafast 2D NMR**. These 2D methods lead, after statistical analysis, to a **better determination of biomarkers**, as we recently showed on a food chemical safety example [2]. When peak overlap is critical –for instance in spectra recorded on a benchtop spectrometer– 2D NMR can even yield a **better separation between sample groups** [3].

In the case of targeted methods, tailored solutions are needed so that 2D NMR can be used for the simultaneous quantification of multiple analytes in complex samples. We will describe different strategies –all including accelerated acquisition methods– to quantify metabolites from 2D NMR spectra, either relying on analytical chemistry approaches [4] or on the design of “**intrinsically quantitative**” 2D experiments [5].

**References:** [1] J. Marchand, et al., Curr. Op. Biotechnol. 2017, 43, 49-55. [2] J. Marchand, et al., Metabolomics 2018, 14, 60. [3] B. Gouilleux, et al., Food Chemistry 2018, 244, 153-158. [4] T. Jézéquel, et al., Metabolomics 2015, 11, 1231-1242. [5] J. Farjon, et al., Anal. Chem. 2018, 90, 1845-1851.

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**PS193**

Wed, 10:30-11:05

**The True Tales of the Flexible Tails – Interaction of  
J-domain Protein with Hsp70 chaperones**

Rina Rosenzweig\*

*Weizmann Institute of Science*

Hsp70s are ubiquitous chaperones tasked with safeguarding proteins throughout their entire lifecycle, from synthesis to degradation, and are thus critical for maintaining protein-homeostasis. The ATPase cycle of Hsp70s, which is allosterically coupled to the binding and release of their substrates, is, in turn, regulated by a large set of dedicated co-chaperones consisting of nucleotide-exchange-factors and of J-domain proteins. Humans contain multiple such J-domain-proteins (JDPs), all of which interact through their conserved J-domain with Hsp70s in an ATP dependent manner. The most abundant JDPs in the cell belong to very structurally-similar Class-A and Class-B families and contain, in addition to the J-domain, an adjacent glycine-rich-region, two client binding domains (CTDI and CTDII), and a dimerization domain.

Despite the many similarities, however, Class-A and Class-B J-proteins still exhibit significant differences in both structure and function. We were, therefore interested to see whether the two classes of JDPs also display differences in their interactions with Hsp70 chaperones.

To this end, we used solution NMR spectroscopy to test the interaction of Hsc70 with the two classes of DnaJ chaperones, and monitored DnaJ-dependent Hsc70 activation via functional assays.

Our results uncover that Class-A and Class-B DnaJs, in fact, interact with Hsc70 in a different manner. While both classes of DnaJs bind Hsc70 through their J-domain, with this weak interaction activating Hsc70 catalytic activity, Class-B DnaJs also contain an additional Hsc70 binding site. When in complex with Hsc70, we have identified that DnaJB1 interacts both via its N-terminal J-domain and its CTDI substrate-binding-domain. The binding of the Hsc70 C-terminal region to DnaJB1 CTDI causes a conformational change that exposes the DnaJB1 J-domain for Hsc70 binding, with only this second interaction leading to activation of Hsc70 catalytic activity. This, then points to a new regulatory interaction between class-B DnaJ chaperones and Hsc70, that is absent in class-A DnaJs.

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**PS194** Wed, 11:05-11:30

**Dynamic regulation of human Hsp70 chaperone functional cycle by its co-chaperones and client protein**

Guillaume Mas\*, Johanna Ude, Sebastian Hiller  
*Biozentrum, University of Basel*

BiP is the only member of the Hsp70 chaperone family in the human endoplasmic reticulum [1]. Its chaperone activity is driven by ATP binding and hydrolysis that trigger the conformational change regulating the docking and undocking of its nucleotide-binding domain (NBD) and substrate-binding domain (SBD). To achieve its many functions, BiP is regulated by several co-chaperones including the nucleotide-exchange factor (NEF) and J-domain protein (JDP). Although structure of intermediate states of BiP have been determined by x-ray crystallography [2] and specific segments of its functional cycle have been explored by NMR [3, 4] and FRET studies [5], characterization of the entire mechanism driving BiP function requires studies at the atomic level under working conditions in presence of its co-chaperone network.

Here, we report a study by real-time methyl NMR, under physiological conditions, of the BiP functional cycle in presence of its major cochaperones (JDP and NEF) and a native client protein. We demonstrate that the co-chaperones speed up the BiP functional cycle by tuning the equilibrium between its docked and undocked conformation, via regulating the allosteric communication between NBD and SBD. Furthermore, we reveal how the dynamic network of co-chaperones and client protein regulate BiP activity, providing for the first time at atomic resolution a time-resolved description of the BiP functional cycle. This study opens up new perspectives to understand how dysfunction in the regulation of BiP by its co-chaperones is linked to a broad range of BiP-related diseases such as cancer, cardiovascular and neurodegenerative disease.

**References:** [1] Wang, J., et al. (2017). *Gene* 618, 14–23. [2] Yang, J., et al. (2015). *Structure* 23, 2191–2203. [3] Wieteska, L., et al. (2017). *eLife* 6, 18966. [4] Rosenzweig, R., et al. (2017). *eLife* 6, 199. [5] Rosam, M., et al. (2018). *NSMB* 25, 90–100.

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**PS195**

Wed, 11:30-11:55

### NMR Informed Molecular Modeling to Capture Transient Chaperone-Substrate Interactions

Loïc Salmon<sup>\*1</sup>, Scott Horowitz<sup>2</sup>, Logan Ahlstrom<sup>3</sup>, Philipp Koldewey<sup>3</sup>,  
Charles Brooks<sup>4</sup>, James Bardwell<sup>3</sup>

<sup>1</sup>Centre de RMN à Très Hauts Champs (CNRS/ENSL/UCBL), <sup>2</sup>University of Denver,  
<sup>3</sup>HHMI and University of Michigan, <sup>4</sup>University of Michigan

Nuclear Magnetic Resonance (NMR) Spectroscopy is a unique tool to study complex systems such as biological macromolecules due to its ability to probe molecular structure and dynamics at atomic resolution and on a wide range of timescales. During the last decades major progresses have been made towards the description of biomolecular dynamics and protein folding. However, fundamental questions remain open.

Chaperone proteins are key elements in regulating protein folding and aggregation prevention. Despite their critical biological role, the mechanisms by which chaperone proteins interact with their substrates remain quite elusive, in part due to the major role played by conformational disorder.

To investigate this question and more generally propose a strategy to study transient biomolecular interactions between flexible partners, we developed an approach combining NMR spectroscopy and coarse grain modeling, where the information obtained on the two partners is used to implement a system specific force-field encoding the accessible experimental knowledge on that system.

With this approach we depicted the interaction between Spy, a recently discovered chaperone, and Im7, an in vivo substrate. The approach provided us a detailed picture at the residue level of a chaperone-substrate interaction allowing for a detailed description of the role of conformational disorder in chaperone action. The results were in agreement with multiple other experimental information including from x-ray crystallography, attesting the robustness of the method.

**References:** [1] Salmon, Ahlstrom et al. J. Am. Chem. Soc., 138, 9826–9839 (2016). [2] Horowitz, Salmon, Koldewey et al. Nat. Struct. Mol. Biol., 23, 691–697 (2016). [3] Salmon et al. Meth. Mol. Biol., vol 1764 (2018).

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**PS196** Wed, 11:55-12:30

**Atomic Insight into the Function and Activity of Molecular Chaperones**  
Charalampos Kalodimos\*  
*St Jude Children's Research Hospital*

Molecular chaperones are necessary for maintaining a functional proteome in the cell by preventing the aggregation of unfolded proteins and/or assisting with their folding. Despite the central importance of the binding of chaperones to unfolded substrates, the structural basis of their interaction remains poorly understood. The scarcity of structural data on complexes between chaperones and unfolded client proteins is primarily due to technical challenges originating in the dynamic nature of these complexes.

I will discuss how NMR spectroscopy can be used as an extremely powerful tool to determine the structural and dynamic basis for the recognition and interaction of unfolded proteins by molecular chaperones.

**PS197** Wed, 10:30-11:05

**ESR microfluidics with picoliter samples**  
Aharon Blank\*, Nir Dayan, David Cristea, Yakir Ishay, Yaron Artzi  
*Technion - Israel Institute of Technology*

Microfluidics is a well-established technique to process, synthesize and analyse small amounts of materials for chemical, biological, medical, and environmental applications. Typically, it involves the use of reagents with volume that is smaller than ~1 microliter – ideally even nano- or pico-liter. Conventional electron spin resonance (ESR), is typically carried out with ~ 1 ml of sample, thereby making it incompatible with most microfluidics applications. Here we show that by using a new class of miniature surface resonators, combined with photolithography to prepare microfluidics patterns, ESR can be applied to measure small samples, down to picoliter volume, without sacrificing concentration sensitivity. Our experiments with resonators having mode volume of ~ 1 nano-liter can obtain good signal from solutions with ~ 1 mM spin concentration, while smaller resonators can be used to measure even smaller volumes, but for higher spin concentrations. All our experiments are performed at room temperature, making our technique compatible with future microfluidics applications that would employ compact resonators, microfluidic chips, miniature magnet and a compact ESR-on-a-chip spectrometer. This could result in a complete new approach to process and measure paramagnetic liquid samples, applicable for a variety of chemical, biological, medical and environmental applications.

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**PS198**

Wed, 11:05-11:30

**Spin-labeled nanobodies as proteins' conformational reporters towards in-cell EPR applications.**Laura Galazzo<sup>\*,1</sup>, M. Hadi Timachi<sup>1</sup>, Gianmarco Meier<sup>2</sup>, Cedric A.J. Hutter<sup>2</sup>, Markus A. Seeger<sup>2</sup>, Enrica Bordignon<sup>1</sup><sup>1</sup>*Ruhr University Bochum*, <sup>2</sup>*Institute of Medical Microbiology, University of Zürich*

Nanobodies (i.e. single-domain antibodies) are promising new tools for in-cell applications due to their low molecular weight, protein- and state- specificity, nano- or sub-nano-molar affinity to their target and the possibility to be inserted into cells. We propose here the use of spin-labeled nanobodies as conformational reporters of wild type unlabeled proteins via DEER spectroscopy.

We focused on a set of spin-labeled nanobodies targeting ABC transporters, proteins that couple the energy deriving from the binding and hydrolysis of ATP to large conformational changes that enable substrate translocation across membranes. These molecular machines have been studied in different environments (as detergent, liposomes and nanodiscs) via different techniques[1,2]; in this framework, the challenge ahead is the investigation of their conformational plasticity in a native environment.

We show here[3] the applicability of the use of gadolinium-labeled nanobodies against the heterodimeric exporter TM287/288: thanks to a “cocktail” of state- and non-state-specific nanobodies binding to different sites of the protein, we were able to obtain a fingerprint distance of the outward-facing state of the transporter and follow the conformational cycle of the unlabeled wild type protein. Orthogonal labels attached to the transporter were also used to corroborate and strengthen the findings. With this proof of principle, we then used a non-state-specific nanobody showing high affinity for both nucleotide binding domains of the homodimeric exporter MsbA to gather structural information in detergent, proteoliposomes, nanodiscs and inside-out vesicles. Our results show a strong structural dependency on the environment, proving the need of an investigation in a native environment. Reliable structural information at low micromolar protein concentrations were obtained, paving the way for the use of biocompatible Gd-labeled nanobodies in cells.

**References:** [1] Mi, W. et al., 2017, *Nature*, 549, 233-237. [2] Borbat, P.P. et al., 2007, *PloS biology*, 5, e271. [3] Galazzo, L. et al., 2019, in preparation.

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**PS199**

Wed, 11:30-11:55

**Quantitative sub-micromolar pulse dipolar EPR spectroscopy evidences high copper(II) labeling efficiency for double-histidine motifs**Joshua Wort<sup>1</sup>, Katrin Ackermann<sup>1</sup>, Angeliki Giannoulis<sup>1</sup>, Alan Stewart<sup>1</sup>, David Norman<sup>2</sup>, Bela Bode<sup>\*1</sup><sup>1</sup>University of St Andrews, <sup>2</sup>University of Dundee

Electron paramagnetic resonance (EPR) distance measurements provide highly accurate and precise geometric constraints. These have made valuable contributions to studies of the structures and conformations of biomolecules. Recently, application of double-histidine (dHis) motifs, coupled with Cu<sup>II</sup> spin-labels has shown promise in even higher precision distance measurements.[1] However, the non-covalent Cu<sup>II</sup>-coordination approach is vulnerable to low binding-affinity. Earlier estimations of dissociation constants ( $K_D$ ) revealed micromolar to low millimolar  $K_D$ s under EPR distance measurement conditions.[1] As many challenging biomolecular targets are only stable at or below low micromolar concentration higher  $K_D$ s are likely to limit the usefulness of this approach.

We have investigated the binding affinity directly from primary pulse dipolar (PD) EPR data.[2] By combining spectroscopically orthogonal Cu<sup>II</sup> and nitroxide spin-labels and performing RIDME (relaxation-induced dipolar modulation enhancement) distance measurements the uncertainty from speciation of the Cu<sup>II</sup> spin-label is largely mitigated. By exploiting the superb sensitivity of this experiment and label combination we have demonstrated significant loading of dHis sites at submicromolar concentrations. This study demonstrates that the affinity for Cu<sup>II</sup>-chelators is not limiting for PDEPR studies in the low micromolar range and that the combination of RIDME and the orthogonal spin-labels Cu<sup>II</sup> and nitroxide makes submicromolar PDEPR experiments feasible.[3]

**References:** [1] (a) T.F. Cunningham, M.R. Putterman, A. Desai, W.S. Horne, S. Saxena, *ACIE* **2015**, *54*, 6330; (b) S. Ghosh, M.J. Lawless, G.S. Rule, S. Saxena, *JMR* **2018**, *286*, 163. [2] (a) K. Ackermann, A. Giannoulis, D.B. Cordes, A.M.Z. Slawin, B.E. Bode, *ChemComm* **2015**, *51*, 5257; (b) A. Giannoulis, M. Oranges, B.E. Bode, *ChemPhysChem* **2017**, *18*, 2318; (c) A. Giannoulis, K. Ackermann, P. Spindler, C. Higgins, D.B. Cordes, A.M.Z. Slawin, T.F. Prisner, B.E. Bode, *PCCP*, **2018**, *20*, 11196. [3] J.L. Wort, K. Ackermann, A. Giannoulis, A.J. Stewart, D.G. Norman, B.E. Bode, *under review*.

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**PS200**

Wed, 11:55-12:30

**High-Frequency Electron-Nuclear Double Resonance to Study Biomolecules**

Marina Bennati\*

*MPI for Biophysical Chemistry & University of Göttingen*

Electron-nuclear double resonance (ENDOR) and dynamic nuclear polarization (DNP) are two techniques based on polarization transfer between electron and nuclear spins. Despite differences in the experimental realization, their similarities rely on the detailed mechanism of hyperfine interactions. The lecture will give an overview of our recent developments in these two methods in solids (ENDOR) and solution (Overhauser DNP) to study biological systems. To this end, design and implementation of coupled EPR/NMR experiments at various microwave frequencies, particularly in the high-frequency/high-field EPR regime, has been in focus.

We have recently implemented a spectrometer to perform routine ENDOR spectroscopy at 263 GHz/9.4 Tesla. Spectrometer design, performance as well as the demonstration of unprecedented spectral resolution in ENDOR for studies of protein radicals is presented. Moreover, we illustrate that high frequency ENDOR in combination with a new  $^{19}\text{F}$ /nitroxide labelling strategy can be used to measure interspin distances in the range 0.5 – 1.5 nm. Finally, expansion of  $^{13}\text{C}$  dynamic nuclear polarization in the liquid state towards high magnetic fields is discussed. We could recently measure  $^{13}\text{C}$  NMR signal enhancements on small molecules up to fields of 9.4 Tesla.

**PS201**

Thu, 10:30-11:05

**In-Cell NMR: Past, Present and Future**

Phil Selenko\*

*Weizmann Institute of Science*

Recent breakthroughs in optical and electron microscopy have changed the fields of Cell and Structural Biology in a most profound manner, with ever more detailed information about the inner workings of cells becoming available. Complementary to the advancements, novel *in situ* methods are beginning to emerge as powerful tools in Cellular Structural Biology. Here, I discuss how recent developments in in-cell NMR and EPR contribute to our understanding of basic biological processes in live cells. Specifically, I outline how these techniques provide time-resolved atomic-resolution information about intracellular protein structures and functions, which cannot be obtained with any other method at this time.

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**PS202**

Thu, 11:05-11:30

**In-cell PELDOR of spin-labelled RNA duplexes**Alberto Collauto<sup>\*1</sup>, Subham Saha<sup>2</sup>, Dnyaneshwar B. Gophane<sup>2</sup>,Snorri Th. Sigurdsson<sup>2</sup>, Thomas F. Prisner<sup>1</sup><sup>1</sup>*Institute of Physical and Theoretical Chemistry and Center for Biomolecular Resonance,*<sup>2</sup>*Science Institute, Department of Chemistry, University of Iceland*

Structural investigation of nucleic acids is usually carried out either in diluted buffered solutions or on crystals of these biomolecules. These environments markedly differ from the native one in which these molecules are found, where effects as macromolecular crowding or intermolecular interactions can play a significant role on the conformation.

Aim of the present study was to investigate whether the structures of short RNA duplexes would be different when placed in a buffered solution vs inside *Xenopus laevis* oocytes. The structural information was obtained as a set of distance constraints between paramagnetic tags covalently linked to the biomolecule under study; these constraints were obtained from 4-pulse PELDOR measurements.

In this work two different labelling strategies were used to label a uridine with an isoin-doline-derived spin label, differing both by the flexibility of the linker and by the point of attachment. In the first case, the spin label was post-synthetically conjugated to the ribose sugar ring *via* a thiourea linkage. In the second case, the spin label was attached to the uracil base through a C-C bond; an intramolecular hydrogen bond between the label and the uracil restricts the rotation around the C-C bond, resulting in a semi-rigid label. For both strategies, the paramagnetic centre was protected against the reducing environment of the cell by replacing the normal *gem*-dimethyl groups, flanking the nitroxide moiety, with *gem*-diethyl groups.

The results showed a clear, reproducible shift of the mean inter-spin distance to shorter values upon internalisation inside cells. In order to understand these findings, the duplexes were further investigated under different *in-vitro* conditions, such as in cytoplasmic extract from the *Xenopus laevis* oocytes as well as in the presence of protein crowders (BSA and lysozyme). The results of these experiments indicate an involvement of electrostatic interactions in the observed conformational changes.

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**PS203**

Thu, 11:30-11:55

**In-cell DNP Supported Solid-State NMR on Soluble Proteins**Siddarth Narasimhan\*, Alessandra Lucini Paioni,

Johan van der Zwan, Gert Folkers, Marc Baldus

*Utrecht University*

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Increasing evidence suggests that the highly complex and dynamic environment of the cell interior and its physiochemical setting imposes critical control on cellular functions, which is hardly reproducible under in vitro conditions. In-cell solution-state NMR can track such structural and dynamical interactions at the atomic level provided that proteins or other molecular units are small and tumble rapidly. On the other hand, solid-state NMR (ssNMR) has been used to probe proteins and large protein complexes in bacterial cells and at the cell membrane periphery of human cells. However, extending such studies to investigate proteins and molecular complexes inside human or bacterial cells poses additional challenges. Firstly, sample preparation schemes must be designed that achieve molecule-specific isotope labeling of biomolecules in cells at endogenously relevant protein concentrations. In addition, non-conventional NMR concepts must be used to overcome the inherent low sensitivity of such cell preparations. Dynamic Nuclear Polarization (DNP), has been widely used to boost sensitivity in ssNMR experiments. However, the strong reducing environment inside the cells can be deleterious to DNP radicals, thus precluding protein studies inside cells. We have developed tailored biochemical and solid-state NMR approaches that allow studying protein structure inside cells at atomic level under high-sensitivity DNP conditions. We demonstrate our methods on both Prokaryotic and Eukaryotic systems, thus opening up a plethora of applications for NMR-based cellular structural biochemistry.

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**PS204** Thu, 11:55-12:30

**Structure determination of antimicrobial peptides in  
model membranes and live bacteria**

Frances Separovic\*  
*University of Melbourne*

Resistance to antibiotics is a growing health concern worldwide. Antimicrobial peptides (AMPs) present an alternative to conventional antibiotics but details of their mechanism of action and the basis for differences in their potency observed between different bacterial strains remain unclear. Structural information is crucial for defining the molecular mechanism by which these peptides recognize and interact with a particular lipid membrane. Nuclear magnetic resonance (NMR) structural investigations of cationic AMPs from Australian tree frogs in a range of different lipid systems will be discussed. Although these AMPs are unstructured in aqueous solution, they are alpha-helical in hydrophobic or membrane-like environments. The degree of helicity depends on membrane curvature and surface charge with a greater helical stretch in phospholipid bilayer membranes compared to micelles. Molecular dynamics simulations of the AMP, maculatin 1.1, show N-terminal exposure to the solvent and indicated that the peptide bends to adapt to the micelle curvature. Maculatin induced greater headgroup and acyl-chain perturbations for anionic phospholipids, which are found in bacterial membranes. The AMP appeared to lie on the surface of charged lipid membranes but insert in a transmembrane fashion with zwitterionic bilayer membranes. Solid-state NMR and dynamic nuclear polarization (DNP) studies of maculatin in live bacteria also support a transmembrane orientation. DNP NMR using spin-labelled peptides in combination with specifically <sup>13</sup>C and <sup>15</sup>N labelled maculatin give insight into pore formation and how AMPs self-assemble within bacterial membranes. The results of these structural studies could be used to design more potent AMPs for therapeutic applications.

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Thu, 12:30-13:00

## Fluxomic studies by in cell and in vitro

## Dissolution-Dynamic Nuclear Polarization NMR

David Guarin<sup>\*,1</sup>, Jessie Mosso<sup>1</sup>, Mathieu Baudin<sup>1</sup>, Dennis Kurzbach<sup>2</sup>,  
 Emeric Miclet<sup>3</sup>, Daniel Abergel<sup>1</sup>

<sup>1</sup>Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure,  
 PSL University, Sorbonne Université, CNRS, 75005 Paris, France, <sup>2</sup>University Vienna,  
 Faculty of Chemistry, Institut of biological chemistry, Whringer Straße 38, 1090 Vienna,  
 Austria - Vienna, <sup>3</sup>Sorbonne Université, École normale supérieure, PSL University, CNRS,  
 Laboratoire des biomolécules, LBM, 75005 Paris, France

Since the invention of dissolution Dynamic Nuclear Polarization (DDNP)[1] and the dramatic signal enhancement it provides in solution-state NMR, a wide range of applications have emerged. In particular, this improvement opens new avenues for the study of fast dynamical processes such as chemical reactions, with a time resolution smaller than a second. DDNP has proven of particular interest in the study of such metabolic processes as enzymatic reactions that are affected by many factors, such as dietary or environmental. These reactions can now be studied *in vitro* and *in cell*, providing access to the nature and degree of possible cell impairment.

We have initiated kinetic studies of enzymatic reactions using DDNP on the oxidative stage of the Pentose Phosphate Pathway (oxPPP)[2], one of the crucial metabolic pathways in cells. OxPPP produces NADPH, one of the main sources of reductive power in the cell. It comprises three enzymes: Glucose-6-Phosphate dehydrogenase (G6PDH), 6-Phosphogluconolactonase and 6-Phosphogluconic acid dehydrogenase.

By studying the enzymatic cascade involving Hexokinase and G6PDH, we could extract the relevant kinetic parameters involved in the process. The complexity of the kinetic pathway and the large number of model parameters makes it a particularly demanding task that requires the repeatability of the experiments. Fitting the signal build-up curves of the multiple metabolites allowed us to extract the relevant kinetic parameters. This showed satisfactory correlation with the enzyme activities used in the experiments.

In parallel, *in cell* experiments performed on *E. coli* allowed to provide a global and quantitative view of Glucose metabolism in function of the cell-growth and cell stress conditions. In particular, the differential  $\alpha / \beta$  anomeric Glucose uptake by the cells appeared as an indicator of the relative activities of Glycolysis and PPP.

**References:** [1] Ardenkjær-Larsen et al. PNAS, (2003), 10158–10163. [2] A. Sadet et al. Chem.Eur.J. (2018), 4, 5456–5461.

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**PS206**

Thu, 10:30-11:05

## Dynamic Phenomena in Fuel Cells and Batteries Investigated by Various NMR Techniques

Oc Hee Han\*

*Korea Basic Science Institute*

For mechanistic investigation of energy conversion/storage systems such as fuel cells and batteries, integral understanding of not only electrochemical phenomena but also chemical reactions and dynamics of chemical species in the systems is essential. We have employed various NMR techniques to reach the goal and some results will be presented.

The water and proton dynamics in Nafion polymer electrolyte membrane (PEM) will be introduced first. PEM is a main constituent, separating cathode and anode electrodes, in low temperature fuel cells such as hydrogen fuel cells and direct alcohol fuel cells (DAFC). For this study, Overhauser dynamic nuclear polarization as well as slow magic angle spinning was employed. The results indicated that hydrophobic surface enhances water dynamics. Multiscale water/proton dynamics in PEM will be discussed together with non-MR data in addition.

Secondly, the reaction mechanism of DAFC will be discussed in terms of cons and pros of toroid cavity detector and flow NMR techniques employed for this study.

Thirdly, the role of histidine additive in vanadium redox flow battery (VRFB) investigated with multinuclear solution NMR and first-principles calculations will be explained. The results showed that histidine additive dynamically interacts with vanadium ions forming an outer-sphere  $[\text{VO}_2^+(\text{V})\text{-histidine}_2^+]$  complex. This dynamic complexation was found to prevent the aggregation of  $\text{VO}_2^+(\text{V})$  ions and subsequent growth of  $\text{V}_2\text{O}_5$ , resulting in suppression of  $\text{V}_2\text{O}_5$  precipitation. This leads to the improved stability and performance of VRFB.

Finally, challenges and promising aspects of MR techniques for investigation of dynamic phenomena occurring in electrochemical systems and their constituents will be summarized.

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**PS207**

Thu, 11:05-11:30

**Bulk Hyperpolarization of Inorganic Materials**Snaedis Björgvinsdóttir\*, Brennan J. Walder, Pinelopi Moutzouri,

Nicolas Matthey, Lyndon Emsley

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We have recently shown how the bulk of proton-free inorganic solids can be hyperpolarized using dynamic nuclear polarization, resulting in sensitivity enhancements in MAS experiments.[1] This is achieved by hyperpolarizing nuclei near the particle surface with impregnation DNP and then allowing slow spontaneous spin diffusion between weakly magnetic nuclei to relay the hyperpolarization towards the bulk.

Pulse cooling is a version of this method that uses multiple contact cross-polarization for bulk hyperpolarization. We show how to maximize sensitivity gains in pulse cooling by optimization of the pulse parameters and delays. In addition, we show how to improve sensitivity by modulating the MAS rate during the experiment, which can provide gains of up to a factor 3.5 for the  $^{119}\text{Sn}$  spectra of  $\text{SnO}_2$ , compared to a constant MAS rate.[2] We also show how multidimensional experiments can be used to probe the pathway of spin diffusion, particularly for the spectra of compounds with more than one bulk chemical shifts.

**References:** [1] Bjorgvinsdottir, S.; Walder, B. J.; Pinon, A. C.; Emsley, L., Bulk Nuclear Hyperpolarization of Inorganic Solids by Relay from the Surface. *J Am Chem Soc* 2018, 140 (25), 7946-7951. [2] Bjorgvinsdottir, S.; Walder, B. J.; Matthey, N.; Emsley, L., Maximizing nuclear hyperpolarization in pulse cooling under MAS. *J Magn Reson* 2019, 300, 142-148.

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**PS208**

Thu, 11:30-11:55

**Room-temperature triplet dynamic nuclear polarization in nanoporous materials and in water**

Nobuhiro Yanai\*

*Kyushu University*

Nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) are powerful and versatile methods in modern chemistry and biology fields. Nevertheless, they suffer from intrinsically limited sensitivity due to the low nuclear spin polarization at ambient temperature. One of the promising methods to overcome this limitation is dynamic nuclear polarization (DNP) that transfers spin polarization from electrons to nuclei. In particular, DNP based on photo-excited triplet (triplet-DNP) is promising, since it allows the hyperpolarization at room temperature. In typical scheme of triplet-DNP, the spin-selective intersystem crossing (ISC) produces the large electron spin polarization in the excited triplet state sublevels, and this polarization is effectively transferred to nuclear spins by a pulsed microwave irradiation for satisfying Hartmann-Hahn condition, so-called integrated solid effect (ISE).

Previous studies of triplet-DNP have been limited to dense crystalline and amorphous materials, and it remains difficult to hyperpolarize biology-relevant probes. To overcome this limitation, we introduce the chemistry of metal-organic frameworks (MOFs) to the field of triplet-DNP (J. Am. Chem. Soc., 2018, 140, 15606). The nanoporous structure of MOFs allows the accommodation of polarizing agents as well as other guest molecules. This work paves the way towards the hyperpolarization of various probe molecules at room temperature for imaging applications.

Another important challenge of triplet-DNP is to develop air-stable polarizing agents. Since the first report of room-temperature triplet-DNP in 1990, pentacene has been the only and best option of triplet polarizing agents. However, the poor air-stability of pentacene has severely limited the applicability of triplet-DNP. We demonstrate the first example of air-stable polarizing agents with high polarizing ability comparable to pentacene (J. Phys. Chem. Lett., 2019, 10, 2208).

We also show the first example of triplet-DNP in water by downsizing the conventional bulk crystals to nanocrystals.

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**PS209**

Thu, 11:55-12:30

**DNP Polarizing Agents for High-Field, Fast-MAS and Variable Temperature**

Moreno Lelli\*

*University of Florence*

MAS DNP is increasingly establishing itself as a powerful technique to boost sensitivity of NMR. It makes it possible to run, in minutes, experiments that would take weeks, or simply would not be possible otherwise because they are too insensitive. At 9.4 T (400 MHz) and 100 K, DNP enhancements of around 250 are now possible with several polarizing agents (PA) (AMUPol1, TEKPol2, and more recently(3) SPIROPOL,(4) PyPolPEG2OH,(5) bcTol,(6) AsymPolPOK,(7)...). Despite excellent performance at 9.4 T, these dinitroxide biradicals are much less efficient at 18.8 T, both in terms of enhancement and overall sensitivity gain. The need for more efficient PA stimulated the research of biradical with a narrow EPR line,(8) such as for trityl in TEMTriPOL.(9)

Here we show how either monoradical like BDPA, based on the Overhauser effect, or hybrid biradicals, based on the Cross-Effect mechanism and designed by coupling BDPA with a nitroxide unit, provide very high DNP enhancements and overall sensitivity gains at 18.8 T, with performance significantly exceeding dinitroxides at this field. Overhauser DNP with BDPA in OTP shows enhancements of over 100 at 18.8 T and 40 kHz MAS with 1.3 mm rotors at 100 K, with no depolarization or quenching effects.<sup>10</sup> The enhancement also persists at higher temperature, with values of around 30 at  $-30\text{ }^{\circ}\text{C}$ !<sup>(11)</sup>

Hybrid BDPA-nitroxide biradical show enhancements up to 185 at 18.8 T, 100K and 40 kHz MAS, which is so far the highest enhancement and sensitivity gain at high magnetic field.<sup>(12)</sup> We will discuss how the DNP performance of all these systems depends on a combination of several factors, from the magnetic properties of the polarizing agent to the role played by spin-diffusion. The potential of these polarizing agents for the characterization of pharmaceutical and functionalized material surfaces will be presented.

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**PS210**

Thu, 12:30-13:00

**Improving bis-nitroxides' geometry for MAS-DNP**

Frederic Mentink-Vigier\*

*National High Magnetic Field Laboratory, Florida State University*

Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) is becoming a main-stream method to increase the sensitivity of solid-state NMR experiments. By irradiating a sample with a strong microwave ( $\mu\text{w}$ ) source, nitroxide biradicals are used to enhance the proton polarization via the cross-effect (CE) mechanism.[1]

To increase the enhancement factor (the NMR signal ratio with/without  $\mu\text{w}$  irradiation,  $\epsilon_{\text{on/off}}$ ), the biradical structures underwent extensive optimization.[2, 3] In particular, the nitroxides' g-tensors relative orientation ( $\alpha, \beta, \gamma$ ) is essential for efficient CE under MAS.[4]

From the early biradical design the role of  $\beta$  was clearly identified experimentally and was recently confirmed theoretically by scanning the complete angular space.[5]

Nonetheless both approaches (experimental and theoretical) are incomplete. First, improving  $\epsilon_{\text{on/off}}$  may not correlate with increasing proton polarization as biradical induces nuclear depolarization without  $\mu\text{w}$ . Biradicals with lower  $\epsilon_{\text{on/off}}$  can provide equal or better polarization performance.[6] Second, the simulations neglected the relative orientation's impact on the enhancement vs magnetic field.[7] Therefore, the question "Can bis-nitroxide be improved?" is still debatable.

In this presentation, using a new quantitative theoretical approach,[7] this question is discussed and an ideal orientation is presented. A single parameter is sufficient to evaluate the potential performance of a bis-nitroxide structure. Finally, the method is illustrated on the bTurea series to explain recent experimental observation of AMUPol,[2] bcTol-M,[3] and HydrOPol.[8]

**References:** [1] Rosay et al., Phys. Chem. Chem. Phys. 12, (2010). [2] Sauvée et al., Chem. - A Eur. J. 22, (2016). [3] Geiger et al., Chem. - A Eur. J. 24, (2018). [4] Mentink-Vigier et al., J. Magn. Reson. 258, (2015). [5] Perras et al., ChemPhysChem. 18, (2017). [6] Mentink-Vigier et al., J. Am. Chem. Soc. 140, (2018). [7] Mentink-Vigier et al., Phys. Chem. Chem. Phys. 21, (2019). [8] Stevanato et al., 60<sup>th</sup> ENC, Asilomar (2019).

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**PS211**

Thu, 10:30-11:05

**New Discoveries on HBV infection by Metabolomics**

Yulan Wang\*

*Singapore Phenome Center, Lee Kong Chian School of Medicine,  
Nanyang Technological University*

Hepatitis B Virus (HBV) is a double stranded DNA virus and belongs to hepadnaviridae family. HBV infection causes a severe liver infectious disease and has become a global problem affecting human health. At least 250 million people are chronically infected with HBV, with an estimated 650,000 deaths per year from HBV associated hepatocellular carcinoma, mainly in Asia. Current clinical strategy is aimed at inhibiting the HBV replication. Research in HBV infection is also hindered by the factor that there is no great animal models. We employed metabolomics approach and investigated both human circulating metabolites and cell model. We found that HBV replication induces the promotions of central carbon metabolism, biosynthesis of nucleotides and total fatty acids; HBV up-regulates the biosynthesis of hexosamine and phosphatidylcholine through activating glutamine-fructose-6-phosphate amidotransferase 1 (GFAT1) and choline kinase  $\alpha$  (CHKA), respectively. Furthermore, we demonstrate that GFAT1 and CHKA are two potential targets for treating HBV infection. To elucidate the relationship between HBV replication and host downstream lipid metabolism, we measured 10 classes of phospholipids in HBV infected patients and cells and we found that the levels of phosphatidylcholine (PC), phosphatidylethanolamine, and lyso-phosphatidic acid were increased in HBsAg (+) group compared with HBsAg (-), while phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, and sphingomyelin were decreased, which were confirmed in HBV infected HepG2.2.15 cell line. We further evaluated the levels of enzymes of PC pathways and found that PCYT1A and LPP1 for PC synthesis were up-regulated after HBV infection. Moreover, the HBV replication was inhibited when PCYT1A and LPP1 were knocked down. These results indicated that the PC synthesis in HBV infected host are regulated by PCYT1A and LPP1, which suggests that PCYT1A, LPP1 could be new potential targets for HBV treatment.

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**Slice-selective, high-resolution MAS NMR of intact tissue biopsies gives improved spatial resolution of metabolic distributions**

Elisabeth V. Vonhof<sup>\*,1</sup>, Martial Piotto<sup>2</sup>, Jeremy K. Nicholson<sup>3</sup>,  
Elaine Holmes<sup>4</sup>, John C. Lindon<sup>1</sup>, Jia V. Li<sup>1</sup>

*<sup>1</sup>Division of Integrative Systems and Digestive Medicine, Department of Surgery and Cancer, Imperial College London, <sup>2</sup>Bruker BioSpin, <sup>3</sup>Australian National Phenome Centre, Murdoch University, <sup>4</sup>Division of Integrative Systems and Digestive Medicine, Department of Surgery and Cancer, Imperial College London; Australian National Phenome Centre, Murdoch University*

Biological tissue biopsies are often heterogeneous in cell type and structure and the recognition of this heterogeneity is crucial for many diagnostic studies. For example, differentiation between involved and uninvolved tissue is paramount for the determination of exact tumour margins [DeFeo et al., 2010]. Conventional HR-MAS-NMR spectroscopy is an excellent tool to study metabolites and their abundances within such tissue biopsies [Beckonert et al., 2010; Lindon et al. 2009].

However, the resulting HR-MAS-NMR spectrum is an average over the entire biopsy and any information about underlying biological tissue heterogeneity lost.

We therefore aimed to implement a HR-MAS-NMR-based approach to explore an aspect of spatial heterogeneity of tissue biopsies.

We demonstrate that the previously established [Sarou-Kanian et al., 2015] combination of a gradient-assisted, slice-selective pulse sequence with the conventional HR-MAS-NMR setup and tissue sample preparation yields spatially resolved spectra within a single tissue biopsy of interest.

Slice-selective (SS) HR-MAS-NMR was established on a tissue biopsy of known spatial heterogeneity, namely chicken thigh muscle with skin, characterized by two distinct layers.

Despite short acquisition times of 2 min per selected slice, spectral resolution and sensitivity were excellent with a typical peak linewidth for alanine of ca. 1 Hz and an SNR of around 400 for a 28 scan experiment.

Further, we show that changes in intensity of individual metabolites can be tracked throughout the sample with respect to spatial origin. This allowed us to create a metabolite-specific, one-dimensional abundance profile throughout individual biopsies.

Resulting SS-HR-MAS-NMR spectra clearly show a distribution of metabolites within single tissue biopsies. Spectra from slices containing muscle clearly differed from those containing mostly skin, particularly so in lipid content.

Without modification to the standard hardware or sample preparation methods, SS-HR-MAS-NMR can be used to spatially resolve tissue biopsies with powerful implications for future studies far beyond cancer research.

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**PS213**

Thu, 11:30-11:55

### Advanced Analysis of Chronic Kidney Disease by NMR Derived Metabolomic Fingerprints

Helena Zacharias<sup>1</sup>, Michael Altenbuchinger<sup>2</sup>, Ulla T. Schultheiss<sup>3</sup>, Claudia Samol<sup>2</sup>,  
Fruzsina Kotsis<sup>4</sup>, Inga Poguntke<sup>4</sup>, Peggy Sekula<sup>4</sup>, Mustafa Büyüközkan<sup>5</sup>, Jan Krumsiek<sup>6</sup>,  
Anna Köttgen<sup>4</sup>, Rainer Spang<sup>2</sup>, Peter J. Oefner<sup>2</sup>, Wolfram Gronwald<sup>\*,7</sup>

<sup>1</sup>*Institute of Functional Genomics, University of Regensburg, Regensburg, Germany,*  
<sup>2</sup>*Institute of Functional Genomics, University of Regensburg, Germany,* <sup>3</sup>*Institute of Genetic  
Epidemiology, Department of Biometry, Epidemiology, and Medical Bioinformatics, Faculty  
of Medicine and Medical Center, University of Freiburg, Germany,* <sup>4</sup>*Institute of Genetic  
Epidemiology, Department of Biometry, Epidemiology, and Medical Bioinformatics,  
Faculty of Medicine and Medical Center, University of Freiburg, Freiburg, Germany,*  
<sup>5</sup>*Institute of Computational Biology, Helmholtz Zentrum München, Germany,* <sup>6</sup>*Institute for  
Computational Biomedicine, Englander Institute for Precision Medicine, Department of  
Physiology and Biophysics, Weill Cornell Medicine, New York, United States,* <sup>7</sup>*Institute of  
Functional Genomics, University of Regensburg, Germany*

Identification of chronic kidney disease patients at risk of progressing to end-stage renal disease (ESRD) is essential for treatment decision-making and clinical trial design. Here, we show that proton nuclear magnetic resonance (NMR) spectroscopy of blood plasma specimens together with techniques from machine learning improves the currently best performing kidney failure risk equation, the so-called Tangri score. Our NMR study cohort comprises 4640 participants from the German Chronic Kidney Disease (GCKD) study, of whom 185 (3.99%) progressed over a mean observation time of  $3.70 \pm 0.88$  years to ESRD requiring either dialysis or transplantation. In this context, we also introduce mixed graphical models to reveal important associations between NMR derived metabolic features, demographic and drug features and variables measured by standard clinical chemistry. Results show important associations between chronic kidney disease and for example gout. In summary, we demonstrate that NMR substantially improves the analysis of chronic kidney disease.

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**PS214** Thu, 11:55-12:30

**Improving survival predictability and biological insight through NMR based metabolomics of Acute Respiratory Distress Syndrome (ARDS)**

Neeraj Sinha<sup>\*1</sup>, Akhila Viswan<sup>1</sup>, Afzal Azim<sup>2</sup>

<sup>1</sup>Centre of Biomedical Research, <sup>2</sup>Department of Critical Care Medicine, SGPGIMS

Acute Respiratory Distress Syndrome (ARDS), as characterized by the onset of clinically significant hypoxemia and diffuse pulmonary infiltrates, has been a challenge to the critical care physicians due to high death toll rate. Categorization of the severity of ARDS is based on degree of hypoxemia enumerated by partial pressure of oxygen to the fraction of inspired oxygen (PaO<sub>2</sub>/FIO<sub>2</sub>) ratio and chest X-ray. ARDS diagnostic criteria is based on Berlin definition and classified as mild ARDS (P/F between 200-300), moderate ARDS (P/F between 100-200), severe ARDS (P/F between <100). Due to complex etiology of ARDS, efforts are required to apply system biology tools to understand disease progression and to improve survival prediction. In this direction, we have applied nuclear magnetic resonance (NMR) based metabolomics to understand heterogeneous biology of ARDS. The NMR spectroscopy of mini – bronchoalveolar lavage fluid (mBALF) was optimised and several small molecular weight metabolites were identified which are indicator of lung pathology. NMR spectroscopy of human serum samples helps in identifying the metabolites associated with ARDS severity. Further sub classifying the progression, outcome and the metabolites contributing to pulmonary and non-pulmonary causes of ARDS, mBALF and serum samples were being used in larger sample size for which initial model was tested with respect to control showing good separation and accuracy. The sensitivity and specificity of individual serum metabolites and mBALF metabolites as resultant serum and mBALF endotypes were used further to determine their clinical predictability when combined with clinical APACHE and SOFA score. The accuracy increased to AUROC 1 indicating the clinical relevance of the above determined metabolic endotypes. Pathway analysis of serum endotype and mBALF endotype predictive of mortality gave important metabolic pathway symbolic of ARDS correlated changes in metabolism.

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**PS215**

Thu, 12:30-13:00

**The effect of osmolytes in biomolecular stability as investigated by NMR spectroscopy. Lessons from halophilic proteins**

Oscar Millet\*

*CIC bioGUNE*

Protein folding is usually driven by the hydrophobic core while the role of the surface residues is considered to be marginal. Intimately ligated to protein folding, protein stability establishes the energy required to unfold a protein and the equilibrium populations of the folded and unfolded conformations at a given temperature. Proteins from halophilic organisms challenge this concept since they are often unfolded at low salt conditions while they become folded only in the presence of high salt concentrations. This is possible thanks to a severe modulation of the amino acid composition in the surface of the protein. This halophilic signature is general and conserved and constitutes a biological meter for protein quinary structure (the protein stability and folding when considering the cosolute).

In here, we use NMR spectroscopy and other biophysical techniques to investigate the mechanisms contributing to protein haloadaptation (i. e. stabilization in KCl) and their potential interplay with the stabilization mechanism induced by osmolytes (Sarcosine, Taurine, TMAO, Sucrose, Glycine, Betaine and Trehalose), using several halophilic and mesophilic proteins as reporters. Our data suggest co-evolution of the surface residues to become stabilized not only by salt, but also by osmolytes as well.

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**PS216**

Thu, 10:30-11:05

## Spinning faster: Developments and Applications to Biological NMR

Beat H Meier<sup>\*,1</sup>, Alexander Malär<sup>1</sup>, Maarten Schledorn<sup>1</sup>,  
Anahit Torosyan<sup>1</sup>, Thomas Wiegand<sup>1</sup>, Denis Lacabanne<sup>1</sup>, Callon Morgane<sup>1</sup>,  
Niels-Alexander Lakomek<sup>2</sup>, Rajdeep Deb<sup>1</sup>, Susanne Penzel<sup>1</sup>, Vlastimil Jirasko<sup>2</sup>,  
Pfister Sara<sup>1</sup>, Lauriane Lecoq<sup>3</sup>, Matthias Ernst<sup>1</sup>, Anja Böckmann<sup>3</sup>  
<sup>1</sup>ETH Zurich, <sup>2</sup>ETH Zurich, <sup>3</sup>IBCP Lyon

### Solid-state Protein NMR: Resolution and Sensitivity for Fast MAS Experiments

Beat H. Meier[1], Alexander Malär[1], Maarten Schledorn[1], Anahit Torosyan[1],  
Susanne Penzel, Thomas Wiegand[1], Denis Lacabanne[2], Albert A. Smith[1], Nils-  
Alexander Lakomek[1], Alons Lends, Vlastimi[1] Jirasko, Lauriane Lecoq[2], Matthias  
Ernst[1], Anja Böckmann[2]

Fast magic-angle spinning allows for highly resolved protein proton spectra and gives access to site-specific relaxation data in solid proteins. Fast MAS requires small rotor diameters and correspondingly small sample amounts in the order of 100 picoliters, limiting the signal-to-noise ratio. The limitations encountered depend on the linewidths and coherence lifetimes and will be discussed in detail.

Spectra at 150 kHz MAS (and hopefully higher) will be discussed and compared to spectra around 100 kHz.

Applications presented include dynamical aspects of the helicase DnaB, a bacterial, ATP-driven enzyme that unwinds double-stranded DNA during DNA replication. Conformations mimicking the pre-hydrolytic state, the transition state and a post-hydrolytic state are arrested and then investigated by 3D NMR spectroscopy. In addition, spectra of viral capsids, membrane proteins, and of the Rpo4/7 protein complex will be discussed.

**References:** [1] Laboratory of Physical Chemistry, ETH Zürich, Vladimir-Prelog-Weg 2, 8903, Zurich, Switzerland. [2] Institut de Biologie et Chimie des Protéines, Bases Moléculaires et Structurales des Systèmes Infectieux, Labex Ecofect, UMR 5086 CNRS, Université de Lyon, 7 passage du Vercors, 69367 Lyon, France.

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**PS217**

Thu, 11:05-11:30

**Sensitivity-Enhanced Protein Solid-state NMR using Ultra-fast MAS  
and Structural Studies of Alzheimer's Amyloid- $\beta$**

Yoshitaka Ishii<sup>\*1</sup>, Takayuki Kamihara<sup>1</sup>, Isamu Matsuda<sup>1</sup>, Yiling Xiao<sup>2</sup>

<sup>1</sup>*Tokyo Institute of Technology*, <sup>2</sup>*Univ. Illinois at Chicago*

This work involves two separate topics on our ongoing progress of protein SSNMR methods using ultra-fast MAS and solid-state NMR (SSNMR) applications to amyloid proteins. First, we discuss resolution and sensitivity enhancement in <sup>1</sup>H-detected biomolecular SSNMR under ultra-fast magic angle spinning (UFMAS) conditions ( $\geq 80$  kHz) in a high magnetic field (<sup>1</sup>H frequency: 750-900 MHz).[1,2] Our data on protein microcrystal GB1 and amyloid- $\beta$  (A $\beta$ ) fibril show that traditionally time-consuming 3-5D biomolecular SSNMR is feasible for signal assignments and structural examination of proteins in a nano-mole-scale with this approach. Our discussion will include drastic sensitivity enhancement by novel polarization-transfer schemes and other methods for multi-dimensional SSNMR using ultra-fast MAS. We briefly introduce our nation-wide effort to construct a 1.3 GHz NMR at RIKEN.

Second, we examine structures, kinetics, and functions of amyloid- $\beta$  using solid-state NMR (SSNMR). Increasing evidence suggests that formation and propagation of misfolded aggregates of 42-residue A $\beta$ 42, rather than the more abundant 40-residue A $\beta$ 40, provokes the Alzheimer's cascade. Our group recently presented the first detailed atomic model of A $\beta$ 42 amyloid fibril based on SSNMR data.[3] The result revealed a unique structure that was not previously identified for A $\beta$ 40 fibril. Based on the results and additional SSNMR data, we discuss how amyloid fibril structures affect "prion-like" propagation across different A $\beta$  isoforms, including WT A $\beta$ 40 and E22G pathogenic mutant of A $\beta$ 40.[4] We also discuss SSNMR-based structural analysis of toxic spherical assembly of A $\beta$ , including one that was identified from brains affected by AD.[5] The results provide insight into amyloid misfolding of A $\beta$ 42 in Alzheimer's disease.

**References:** [1] Wickramasinghe, N.et al. Nat. Methods 2009, 6, 215. [2] Ishii, Y.et al. J. Magn. Reson. 2018, 286, 99. [3] Xiao, Y.et al. Nat. Struct. Mol. Biol. 2015, 22, 499. [4] Yoo, B.et al. JACS 2018, 140, 2781. [5] Parthasarathy, S.et al. JACS 2015, 137, 6480.

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**PS218** Thu, 11:30-11:55

**Atomic resolution characterization of a folding intermediate by pressure-jump NMR**  
Joseph Courtney, Cyril Charlier\*, Philip Anfinrud, Adriaan Bax  
*NIH*

Understanding how proteins fold into stable structures without external assistance remains one of the major open questions in biophysics. The ability of NMR to report atomic resolution structural information for both folded and unfolded proteins makes it a uniquely powerful to study protein folding. However, typical experiment times of hours to days are incompatible with the typical microsecond to second timescale of folding. Here, we use a new hardware which rapidly and repeatedly switches sample pressure from native (1 bar) to unfolded (2.5 kbar) conditions in less than 3 ms to study the folding of a pressure-sensitized mutant of Ubiquitin. Recently, we demonstrated the presence of two parallel folding pathways where one involves a meta-stable intermediate. Initially, we developed a set of experiments to obtain site-specific <sup>15</sup>N and <sup>13</sup>C' chemical shifts of this intermediate state using stroboscopic chemical shift measurement or a reverse-sampled indirect evolution period. In addition, as in this case the intermediate has a relatively long lifetime, we have modified standard 3D experiments to measure the first chemical shift evolution (<sup>1</sup>H, <sup>15</sup>N, <sup>13</sup>Ca, <sup>13</sup>C') ~60 ms after the pressure drop, which allows direct observation of cross peaks to intermediate state frequencies. Complemented with other NMR observables such as pressure-jump NOEs and RDCs, we derived a structural model for the intermediate. The model reveals a structure that is similar to the native state of wild type Ubiquitin but but contains multiple non-native H-bonds. This method opens the way for the structural characterization of folding intermediates at atomic resolution.

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**PS219**

Thu, 11:55-12:30

**Monitoring phosphorylation events at the  
interface between the nuclear envelope and chromatin**

Camille Samson<sup>1</sup>, Agathe Marcelot<sup>1</sup>, Ambre Petitalot<sup>1</sup>, Florian Celli<sup>1</sup>, Nada Essawy<sup>1</sup>,  
Virginie Ropars<sup>1</sup>, Brigitte Buendia<sup>2</sup>, François-Xavier Theillet<sup>1</sup>, Sophie Zinn-Justin<sup>\*,1</sup>

<sup>1</sup>*Institute for Integrative Biology of the Cell, CEA, CNRS, Université Paris-Saclay,  
CE-Saclay, Gif/Yvette, France,* <sup>2</sup>*Unité de Biologie Fonctionnelle et Adaptative,  
CNRS, Université Paris Diderot, France*

The molecular mechanisms that regulate genome organization in the mammalian interphase nucleus are largely unclear. At the interface between the nuclear membrane and chromatin, the inner nuclear envelope contains both nucleoskeleton filaments (lamins) and transmembrane proteins (NETs). Lamins tether heterochromatin to the nuclear envelope and modulate chromosome territory positions. Tissue specific expression of NETs also influences genome organization. Phosphorylation regulates localization and interactions of the nuclear envelope proteins during cell cycle and after a mechanical stress. We focused on a complex formed by lamin A/C, emerin (one of the best characterized NETs) and the chromatin binding protein BAF. These proteins contain intrinsically disordered regions (IDRs) that are highly phosphorylated in cells. We showed that BAF dimer mediates the interaction between lamin A/C and emerin, we solved the 3D structure of the complex [1], and we analysed the impact of cell cycle-dependent BAF phosphorylation by the kinase VRK1 on BAF structure and complex assembly. Emerin exhibits a large IDR that is responsible for self-assembly and binding to structural proteins (lamin, actin, tubulin) [2]. We also described the impact of mechano-dependent emerin tyrosine phosphorylation by the Src kinase on emerin structure and binding properties. Finally, we identified defective phosphorylation and binding events associated to muscular dystrophy [3] and premature ageing syndromes [1].

**References:** [1] Samson et al., *Nucleic Acids Res.* 46, 10460-10473 (2018). [2] Samson et al., *FEBS J.* 284, 338-352 (2017). [3] Herrada et al., *ACS Chem Biol.* 10, 2733-2742 (2015).

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## PS220

Thu, 12:30-13:00

### Protein phase diagrams determined by high-pressure NMR

Andi Klamt, Matthias Dreydoppel, Heiner Raum, Ulrich Weininger, Jochen Balbach\*  
*Martin-Luther-University Halle-Wittenberg*

The accessible free energy landscape is a generic property of proteins, which determines both their protein folding pathways and their biological function. This landscape can be explored by determining the thermodynamic stability of proteins at different pressures and temperatures. We combine these variations with NMR spectroscopy to gain molecular resolution. For two proteins (apoKti11 [1] and GB1 [2]) we could determine the pressure-temperature phase diagram, which allowed to explain the stabilization of the proteins at elevated pressures in thermodynamic (volume and entropy changes) and structural terms (conformation plasticity and pKa value changes). For apoKti11, for the first time we could disclose a hyperbolic pressure-temperature phase diagram. Pressure induced changes in the protein folding rates of GB1 derived from CPMG relaxation dispersion allocated the stabilization effect to the native state while the transition and unfolded states remained unaffected.

## PS221

Thu, 10:30-11:05

### High-Field and Fast-MAS Solid-State NMR: Enabling Application to Pharmaceuticals and Supramolecular Assembly

Steven Brown\*  
*University of Warwick*

Applications of advanced solid-state NMR methods for probing intermolecular interactions, notably hydrogen bonding are presented: Homonuclear 1H-1H double-quantum (DQ) experiments reveal proximities (typically under 3.5 Angstroms) among pairs of hydrogen atoms, for example distinguishing between ribbon-like or quartet-like self assembly in guanosine supramolecular structures [1-2] or pushing the limit of detection for a minority solid-state form of a pharmaceutical molecule [3]. 14N-1H spectra show one-bond NH connectivities or additionally longer-range NH proximities depending on the recoupling time employed: Applications to guanosine self assembly [4], a pharmaceutical [5] and to probe the stability of a fumarate salt [6] are shown.

**References:** [1] Peters, G. M. et al J. Am. Chem. Soc. 2015, 137, 5819. [2] Reddy, G. N. M. et al Chem. Eur. J. 2017, 23, 2315. [3] Maruyoshi, K. et al J. Pharm. Sci. 2017, 106, 3372. [4] Reddy, G. N. M. et al Cryst. Growth Des. 2016, 15, 5945. [5] Tatton, A. S. et al Cryst. Growth Des. 2018, 18, 3339. [6] Corlett, E. K. et al CrystEngComm 2019, in press.

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Thu, 11:05-11:30

**Protein resonance assignment without spectral analysis:****five-dimensional spectroscopy of immobilized proteins at ultrafast MAS**

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Tanguy Le Marchand<sup>3</sup>, Rafal Augustyniak<sup>1</sup>, Diane Cala-De Paepe<sup>3</sup>, Isabella C. Felli<sup>4</sup>,  
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The difficulty to automate data acquisition and analysis of complex protein spectra has been one of the major bottlenecks for the widespread use of NMR spectroscopy in structural biology. A promising approach are spectra of high dimensionality (>3) which yield multiple nuclear correlations within fewer experiments, provide high resolution and unambiguous sequential resonance assignment, thus are prone to automation.

Multidimensional spectroscopy (5D-7D) has been explored in solution NMR, however, the concept suffers from a severe inherent contradiction: a satisfactory performance of multiple coherence-transfer experiments is only observed for globular proteins with molecular sizes smaller than about 20 kDa (fast tumbling) or by intrinsically disordered proteins. The deadlock is nowadays removed in proton-detected solid-state NMR at fast magic-angle spinning (MAS). Efficient multiple coherence transfers, narrow proton signals and high detection sensitivity, can be obtained, independently from molecular mass, employing high magnetic fields and ultrafast MAS. The application scope of high-dimensional spectroscopy is thus radically increased.

Here we employ Automated Projection Spectroscopy (APSY), which allows direct inference of a high-dimensional peak list from a number of lower order projection spectra (2D or 3D). We demonstrate the approach with two complementary 5D <sup>1</sup>H<sup>N</sup>-detected experiments that evolve all traversed backbone nuclei: (H)NCOCANH and (H)NCACONH. We show that sensitive five-dimensional correlations are feasible on microcrystalline and fibrillar proteins at 60 and 110 kHz MAS. APSY, now embedded natively in Bruker TopSpin, not only handles data collection but also entirely bypasses spectral analysis. It delivers an output that directly contains the positions of all resonances. It is coupled to a flexible resonance assignment algorithm FLYA, yielding effortlessly expeditious resonance assignments. The protocol, automated from data collection up to resonance assignment, is in principle amenable to widespread access even by inexperienced spectroscopists, and may push forward the size limits of the proteins amenable to site-specific NMR studies.

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**PS223**

Thu, 11:30-11:55

**Solid-state NMR strategies towards speed and resolution**Kshama Sharma<sup>\*1</sup>, Asif Equbal<sup>2</sup>, Nielsen Niels. C. <sup>3</sup>, Kaustubh R. Mote<sup>1</sup>, Madhu P. K. <sup>1</sup><sup>1</sup>TIFR Centre for Interdisciplinary Sciences, <sup>2</sup>UC Santa Barbara, <sup>3</sup>Aarhus Univeristy, Denmark

Solid-state NMR is a very flexible and powerful technique for the elucidation of geometry and dynamics information on a variety of samples. However, there is still a need to overcome sensitivity and resolution aspects along with the necessity to carry out multidimensional experiments in a short span of time. In order to overcome these challenges, we have made use of two approaches.

First approach involves the improvement of heteronuclear spin decoupling efficiency at high magic-angle spinning (MAS) frequencies. For this, a unified strategy of two-pulse based heteronuclear decoupling for high-spinning frequencies and low-power radio-frequency irradiation in solid-state MAS NMR is presented which incorporates simultaneous time and phase modulation. Decoupling sequences like TPPM, XiX and rCW turn out to be specific solution of this approach. This approach not only highlights the existing solutions but also generates new solutions for efficient decoupling.

Secondly, to speed up the data acquisition process, process, pulse sequences that implement sequential acquisition strategies on one and two radio radiofrequency channels with a combination of proton and carbon detection to record multiple experiments under MAS have been coded. These strategies are expected to work better with <sup>1</sup>H detection under fast-magic angle spinning due to low RF amplitude requirements. <sup>1</sup>H detection under fast MAS regime demands heteronuclear decoupling on <sup>13</sup>C or <sup>15</sup>N channel in order to achieve the maximum resolution. We show that the longitudinal <sup>15</sup>N polarisation survives decoupling and can be used to perform multiple sequential experiments at fast MAS. Using multiplex phase cycling, we carried out numerous residue linking experiments in a single experimental block that is alone sufficient for obtaining assignments.

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**PS224**

Thu, 11:55-12:30

**Lab-Frame 1H-1H-Spin Diffusion in Fully Protonated  
Solids at Fast Magic Angle Spinning NMR**

Vipin Agarwal\*

*Tata Institute of Fundamental Research*

Spin-diffusion (SD) was amongst the first methods proposed to spatially transfer polarization between dipolar-coupled nuclear spins. Lab-frame proton spin-diffusion (PSD) has proved particularly useful in structural characterization of a large variety of molecules. Perturbation theory treatment of the SD phenomenon states that during SD, the rate constant of magnetization exchange between two nuclei depends on the dipolar couplings and their chemical shift difference, manifested through the zero-quantum lineshape. Usually attempts are made to minimize chemical shift differences in order to ensure exclusively dipolar transfer that in turn can be rationalized in terms of distances. At slow MAS, the chemical shift differences in protons are non-existent due to the strong dipolar-coupling network of the protons and the spin-part of the dipolar Hamiltonian comprises of two-spin flip flop terms. At fast MAS (>60kHz), the two-spin flip-flop term is averaged out and PSD is dominated by three-spin terms (based on average Hamiltonian theory description). Under our experimental conditions of fast MAS and high static magnetic fields, lab-frame PSD shows unusual polarization transfer profiles. These transfer profiles cannot be explained using the current model of lab-frame PSD. We use theory and numerical simulations to explain the origin of these unusual polarization transfer profiles and highlight the general conditions under which such transfers can be observed. This appears to be a general mechanism of polarization exchange during PSD at fast magic angle spinning and could potentially have a bearing on proton NMR in solids.

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**PS225** Thu, 12:30-13:00

**Biomolecular structural conversion processes probed with  
DNP-enhanced, millisecond time-resolved solid state NMR**  
Robert Tycko\*, Jaekyun Jeon  
*National Institutes of Health*

We have developed experimental methods for initiating nonequilibrium structural conversion processes (e.g., protein folding, peptide self-assembly, ligand/receptor complex formation, etc.) by rapid mixing and for trapping intermediate states by rapid freezing after a defined time interval, on the millisecond time scale. When combined with low-temperature dynamic nuclear polarization, selective isotopic labeling, and solid state NMR techniques, these methods allow us to characterize the time-dependence of multiple aspects of molecular structure during a rapid structural conversion process. As an example, I will describe results for the folding and self-assembly of the 26-residue peptide melittin after a rapid pH jump. The data indicate that unstructured melittin monomers at low pH adopt helical conformations and self-assemble into antiparallel dimers at neutral pH in a cooperative manner on the 6-9 ms time scale. Melittin tetramers then form quickly, but become fully structurally ordered more slowly, on the time scale of about 60 ms. The latest results from other applications will also be described. Overall, this approach to studies of nonequilibrium structural conversions has broad applicability, providing information that is not readily available in as comprehensive a manner from alternative approaches such as optical spectroscopies.

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**PS226**

Thu, 16:15-16:50

**A Tale of Two Sugars:  $^{13}\text{C}$  NMR Tracking of the  
Metabolic Fates of Glucose and Fructose in Cancer**

Lloyd Lumata\*

*University of Texas at Dallas*

Increased dietary consumption of sugar has been implicated in a number of clinical pathologies, including obesity and other metabolic diseases. High fructose corn syrup, a sugar mixture of about 40% glucose and 60% fructose, is a ubiquitous sweetening additive in a number of drinks and food. In this study, we have investigated the metabolism of these two types of sugar in SfXL glioblastoma and HuH7 hepatocarcinoma cell lines.  $^{13}\text{C}$  NMR spectroscopy was used in this study due to high specificity courtesy of the wide chemical shift dispersion of carbon-13. The goal of this study was to investigate the metabolism of fructose and glucose in brain and liver cancer, given the ubiquity of these two sugars in Western diet and the high sugar addiction of these cancers. The main finding of this preliminary work is that, despite the same caloric content of these two sugars, fructose and glucose metabolized quite differently in brain and liver cancer cells. In the absence of glucose in the media, there was no indication of metabolism of  $[\text{U-}^{13}\text{C}]$ fructose in SfXL cells. In the presence of unlabeled glucose in DMEM, we have observed metabolism of  $[1\text{-}^{13}\text{C}]$ fructose into  $[3\text{-}^{13}\text{C}]$ lactate. However, lactic acid production rate from  $[1\text{-}^{13}\text{C}]$ fructose is found to be relatively slower compared to lactic acid production from  $[\text{U-}^{13}\text{C}]$ glucose. On the other hand, substantial lactic acid production from  $[\text{U-}^{13}\text{C}]$ fructose was observed in HuH7 liver cancer cells due to the presence of specialized hepatic enzymes that can metabolize fructose. Metabolic kinetics of these two sugars as well as the NMR results of co-administered  $^{13}\text{C}$ -fructose and  $^{13}\text{C}$ -glucose will be presented.

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**PS227** Thu, 16:50-17:15

**Metabolic Pathway Profiling (MPP) with stable-isotope tracing**  
Thomas Brendan Smith<sup>1</sup>, Kamlesh Patel<sup>1</sup>, Laura Ferrante<sup>1</sup>, Jay Nath<sup>2</sup>,  
Andrew Ready<sup>2</sup>, Mark Jeeves<sup>1</sup>, Christian Ludwig<sup>\*,1</sup>  
<sup>1</sup>University of Birmingham, <sup>2</sup>University Hospitals Birmingham

Stable isotope tracers such as <sup>13</sup>C are increasingly being used to study metabolism in high resolution. However, determining the metabolic fluxes within the system remains technically challenging due to both the complexity of the metabolic network and the sophisticated methods required to analyse the complex spectra derived from NMR and mass spectrometry. Here we present three tools to aid and expand the analysis of tracer based metabolism studies.

Firstly we have recently developed an algorithm (CANMS) that allows the simultaneous analysis of NMR and MS data, which yields a model-free isotopomer distribution, thus allowing a better understanding of metabolic mechanisms. We also demonstrate improved NMR experiments that allow more rapid data acquisition of 2D-<sup>1</sup>H,<sup>13</sup>C-HSQC NMR spectra and the determination of per carbon <sup>13</sup>C percentage incorporation via <sup>12</sup>C filtered 1D-<sup>1</sup>H-NMR spectra. These experimental approaches allow us to accurately determine the amount of <sup>13</sup>C incorporated into metabolites from a single sample. This is vital in order to be able to reliably use this methodology in a clinical setting or in vivo.

Secondly, the manual analysis of 2D-NMR spectra is a laborious process which can lead to biased and inconsistent results. To retrieve meaningful information, we have developed a data-driven algorithm to annotate and analyse multiplets in 2D-<sup>1</sup>H,<sup>13</sup>C-HSQC NMR spectra arising from <sup>13</sup>C-<sup>13</sup>C scalar couplings. The algorithm performs accurate metabolite pick-peaking and multiplet analysis, determining the contribution of each multiplet component to the metabolite signal.

Lastly, we present improved pulse sequences that allow the detection of certain quaternary carbon nuclei thus increasing the information content available and therefore reducing the probability of over-fitting the data. Using our innovative approach, we are able to correlate tracer data with metabolic pathway activity in our cell line model, which is then compared with the clinical outcome of tracing perfused, pre-transplant kidneys.

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PS228

Thu, 16:15-16:50

**Imaging Human Brain Function and Connectivity over Multiple Spatial Scales**

Kamil Ugurbil\*

*University of Minnesota*

An extraordinary feature of brain function is the encoding of information at multiple spatial and temporal scales, going from the cellular level in the form of action potentials to coordinated activity over billions of neurons spanning large parts of the brain, if not the entire brain, to achieve perception and behavior. Bridging and spanning these multiple scales of organization is an essential, but a daunting task necessary for understanding brain function and ultimately dysfunction. Rapid developments in instrumentation for RF transmission and signal detection, a push to exploit unique advantages available at very high magnetic fields (achieving 7 Tesla in 1999 and currently at 10.5T for human imaging), despite the major challenges of imaging at the correspondingly high RF frequencies, and a plethora of novel imaging acquisition techniques that increase spatiotemporal sampling has been bringing transformative changes into our ability to map human brain function and connectivity. These developments complemented by other non-MR imaging methods hold the promise that in the near future it will be feasible to integrate information from the level of a single synapse to whole brain networks that define behavior.

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**PS229** Thu, 16:50-17:15

**Deuterium Metabolic Imaging for in-vivo monitoring of pregnancy in mice at 15.2 T.**  
Stefan Markovic<sup>\*1</sup>, Tangi Roussel<sup>2</sup>, Lucio Frydman<sup>3</sup>

<sup>1</sup>Weizmann Institute of Science, Department of Chemical and Biological Physics, Rehovot, Israel, <sup>2</sup>Center for Magnetic Resonance in Biology and Medicine, Marseille, France, <sup>3</sup>Department of Chemical and Biological Physics, Weizmann Institute of Science

The roster of molecular imaging methods has been recently extended by the introduction of deuterium metabolic imaging (DMI), whereby after administration of a deuterated precursor in rodents or humans, deuterium MRS and MRSI is used to examine metabolic products such as glutamine/glutamate or lactate when applied to brain studies. Here we examine its use for imaging pregnancy-related conditions such as preeclampsia and intrauterine growth restriction. Late-term pregnant ICR mice at around day 19 of gestation were administered with either uniformly deuterated glucose or glucose selectively doubly deuterated on C6 position intravenously at a dose of 2.3 g/kg body-weight. Deuterium chemical-shift imaging (CSI) and non-localized MRS were then performed on a Bruker-Biospec at 15.2T with a “sandwich setup” whereby a 20x45 mm Bruker 1H butterfly surface coil was placed underneath the mouse abdomen, and a customized 20 mm single-loop surface coil tuned to deuterium at 99.8 MHz placed on top of the mouse belly. Slice-selective 1H anatomical and 2H CSI data were then acquired; the latter delivered one 3D data set every 7 min with a FA 90 deg, TR 100 ms, slice thickness 7 mm, FOV 45x45 mm, and a matrix size of 8x8, which was interpolated to 32x32 elements.

Deuterated glucose was predominantly observed localized in the maternal kidney, dropping significantly after 40 min while having its peak in the entire fetal tissue. As a main product of metabolism HDO was observed, formed mostly in the fetuses and with an intensity that grew even 3h after injection. Although of low S/N, lactate maps indicate maximum lactate production 50-70 min after injection localized in fetal livers.

We conclude that DMI may offer new ways of prolonged molecular imaging for monitoring of pregnancy conditions at thermal polarization. Further animal studies of these models as well as of cancer models are ongoing.

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**PS230**

Thu, 16:15-16:50

**Guanine-rich DNA regions and their amazing structures**

Janez Plavec\*

*Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia, Slovenia*

Double helix is the most known structure of DNA. It can account for transfer of genetic information. However, DNA can fold into a wide range of structures that are associated with its unique biological roles and functions. G-rich DNA segments adopting to  $d[G_{\geq 3}N1-7G_{\geq 3}N1-7G_{\geq 3}]$  motif are populated in hundreds of thousands and have the potential to form a G-quadruplex structure. G-rich fragments from the PLEKHG3 gene can form tetrahelical structures that differ significantly from G-quadruplexes, despite containing the G-quadruplex folding motif  $d[G3NG3NG3NG3]$ , where  $N=AGCGA$ . These sequences adopt tetrahelical cores of AGCGA repeats, connected with edge-type loops of G-G base pairs. A marked difference between G- and AGCGA-quadruplexes is their opposing response to changes in water activity. While the former become stabilized with decreasing water activity, the reverse is true for the latter (and B-DNA).

Another intriguing case when relying on sequence details alone to predict G-quadruplex structure was reported recently on a G-rich sequence found in the regulatory region of the RANKL gene, associated with homeostasis of bone metabolism. An oligonucleotide with four G-tracts of three successive guanine residues folds into a two-quartet basket-type G-quadruplex.

$d[(G4C2)3G4]$  implicated in neurological disorders ALS and FTD forms two major G-quadruplex structures. Structural characterization of the G-quadruplex named AQU revealed an antiparallel fold composed of four G-quartets and three lateral C-C loops. Two C•C base pairs are stacked on the nearby G-quartet and are involved in a dynamic equilibrium between symmetric N3-amino and carbonyl-amino geometries and protonated C+•C state.

**Selected References:** Angew. Chem. Int. Ed. 2019, 58, 2387. Angew. Chem. Int. Ed. 2018, 57, 15395. Nucleic Acids Res. 2018, 46, 11605. Nucleic Acids Res. 2019, 47, 2641. J. Am. Chem. Soc. 2019, 141, 2594. Nucleic Acids Res. 2018, 46, 4301. J. Am. Chem. Soc. 2018, 140, 5774.

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**PS231**

Thu, 16:50-17:15

## Unique quadruplex structure and anti-disease activity of RNA aptamer, and in-cell NMR of nucleic acids

Masato Katahira\*

Kyoto University

We previously identified an RNA aptamer against a prion protein, r(GGAGGAGGAGGA) (R12). We showed that R12 forms a unique quadruplex structure and reduces a level of the abnormal prion protein, PrP<sup>Sc</sup>, in the mouse neuronal cells, implying its therapeutic potential as to prion diseases [1,2]. We also utilized R12 to develop the K<sup>+</sup>-responsive ribozyme [3] and RNA aptamer against HIV-1 Tat protein [4], using its quadruplex formation in response to K<sup>+</sup>. Here, we reveal that RNA with analogous sequence to R12 can reduce the level of PrP<sup>Sc</sup> much more efficiently. Structure determination rationalizes the higher anti-prion activity of this new RNA [5].

Some recent studies suggested that amyloid beta (A $\beta$ ) forms soluble oligomers, protofibrils and fibrils; the A $\beta$  oligomers being more toxic than the fibrils. The A $\beta$  oligomers reportedly bind to prion protein (PrP), which acts as a receptor on the cell membrane, possibly resulting in Alzheimer's disease (AD) [6]. Thus, it is thought that compounds that can disrupt the formation of the prion-A $\beta$  oligomer complex may prevent AD. Here, we demonstrate that R12 inhibits the interaction of PrP with A $\beta$ , which implies therapeutic potential of R12 to AD [7].

In-cell NMR is a promising method to obtain the information on the structure, dynamics and interaction of biomolecules. We succeeded in observing NMR signals of DNA/RNA in living human cells for the first time [8]. The observed signals directly suggested the formation of DNA/RNA hairpin structures in living human cells. Further development of in-cell NMR studies of nucleic acids in human cells will be presented.

**References:** [1] Nucleic Acids Res., 2013. [2] Nucleic Acids Res., 2014. [3] Chem. Commun., 2015. [4] Chem. Commun., 2017. [5] submitted. [6] Nature, 2009. [7] FEBS J., 2019. [8] PCCP, 2018.

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**PS232**

Thu, 16:15-16:50

**Muti-Extreme THz ESR -Recent Developments and Future-**Hitoshi Ohta<sup>\*1</sup>, Susumu Okubo<sup>1</sup>, Eiji Ohmichi<sup>2</sup>, Takahiro Sakurai<sup>3</sup>, Hideyuki Takahashi<sup>1</sup><sup>1</sup>Kobe University, Molecular Photoscience Research Center, <sup>2</sup>Kobe University, Graduate School of Science, <sup>3</sup>Kobe University, Research Facility Center for Science and Technology

THz ESR under multi-extreme conditions, which covers the frequency region between 0.03 and 7 THz[1], the temperature region between 1.8 and 300 K[1], the magnetic field region up to 55 T[1], and the pressure up to 1.5 GPa[2], has been developed in Kobe. Firstly, we will show our recent developments of the torque magnetometry[3] and mechanically detected ESR[4] measurements using a commercially available membrane-type surface stress sensor, which is the extension from our micro-cantilever ESR[5], and its application to the microliter solution sample (myoglobin)[6]. Secondly, we will show that the pressure region is extended to 2.7 GPa using the hybrid-type pressure cell[7]. Recent development of high-pressure THz ESR with the 25 T superconducting magnet[8] and its application to  $\text{Cs}_2\text{CuCl}_4$  will be discussed[9].

**References:** [1] H. Ohta et al., J. Low Temp. Phys. **2013**, 170, 511. [2] T. Sakurai et al., Rev. Sci. Instr. **2007**, 78, 065107. [3] H. Takahashi et al., J. Phys. Soc. Jpn. **2017**, 86, 063002 (**Editor's Choice**). [4] H. Takahashi et al., Rev. Sci. Instrum. **2018**, 89, 036108. [5] H. Ohta et al., AIP Conf. Proceedings **2006**, 850, 1643; E. Ohmichi et al., Rev. Sci. Instrum. **2008**, 79, 103903; E. Ohmichi et al., Rev. Sci. Instrum. **2009**, 80, 013904; E. Ohmichi et al., J. Mag. Res. **2013**, 227, 9; H. Takahashi, E. Ohmichi, H. Ohta, Appl. Phys. Lett. **2015**, 107, 182405. [6] T. Okamoto et al., Appl. Phys. Lett. **2018**, 113, 223702 (**Editors Picks**). [7] K. Fujimoto et al., Appl. Mag. Res. **2013**, 44, 893; H. Ohta et al., J. Phys. Chem. B **2015**, 119, 13755; T. Sakurai et al., J. Mag. Res., **2015**, 259,108; T. Sakurai et al., J. Phys. Soc. Jpn. **2018**, 87, 033701. [8] T. Sakurai et al., J. Mag. Res. **2018**, 296, 1-4. [9] S. A. Zvyagin et al., Nature Communications, **2019**, 10,1064.

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**PS233** Thu, 16:50-17:15

**High-Q Photonic Band Gap Resonators for mm-wave  
EPR of Lossy Aqueous Samples and Thin Films**

Alexander Nevzorov, Sergey Milikisiyants, Antonin Marek, Alex Smirnov\*  
*North Carolina State University*

Water and other polar molecules are known to absorb electromagnetic radiation and the absorption is particularly strong in the mm-Wave (mmW) range. Metal surfaces are also becoming increasingly lossy. These high dielectric losses represent the major challenge for constructing EPR and also DNP NMR probeheads suitable for accommodating samples with the maximum volume. While large samples can be fitted into non-resonant mmW structures, resonator cavities offer significantly higher mm-wave B1 fields – an essential condition for DNP. High-Q resonators also provide the best EPR concentration sensitivity at X- and Q-band. Last year we described a radically new line of EPR resonators that are based on one-dimensional photonic band gap (PBG) dielectric crystals. PBG crystals were assembled from  $\lambda/4$  low-loss dielectric layers with alternating dielectric constants and demonstrated experimental  $Q \approx 520$  at 94.3 GHz. Anodic aluminum oxide nanoporous disc of 50  $\mu\text{m}$  in thickness was employed as an aqueous sample holder allowing for ca. 2-3  $\mu\text{l}$  sample volume. Here we report on significant improvements of PBG EPR resonators in both sample volume and experimental Q-factors while minimizing dielectric losses even for liquid aqueous samples. A series of smooth and corrugated 95 GHz transitions were tested to improve flatness of the mmW front. The resonator Q-factor was further improved by increasing the sample diameter from 12 to 36 mm yielding 9-fold sample volume increase. The best experimental  $Q \approx 3,300$  has been observed for 8 alternating  $\lambda/4$  layers of alumina and air. Experimental tests of the new resonators for aqueous and thin film samples are also reported. Finesse of 200 GHz PBG resonator for 300 MHz (1H) DNP was improved by forming photonic crystals from dielectric layers with high  $\epsilon_1/\epsilon_2$  ratio. Supported by NIH R21EB024110 and R01GM130821.

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Up-conversion of NMR signals from radio-frequency to  
optical regimes through a mechanical transducer

Kazuyuki Takeda\*

Kyoto University

Conventional reception of NMR signals relies on electrical amplification of the electromotive force caused by nuclear induction. In general, the signals cannot be transported without noise being added through the process of amplification before being acquired.

Here, we report a different approach that potentially leads to much less noise added through signal transduction. The idea is to employ up-conversion of radio-frequency NMR signals to an optical regime using a high-stress silicon nitride *membrane* that interfaces the NMR-probe circuit and an optical cavity. In this approach that we call Electro-Mechano-Optical NMR, or *EMO NMR* [1-2], A metal layer coated on the membrane serves both as an electrode of a *capacitor* and a *mirror* of an optical cavity.

- the nuclear induction signal is transcribed to the vibration of the membrane through the electro-mechanical coupling.
- In turn, the displacement of the membrane modulates the light in the optical cavity due to the opto-mechanical coupling.

In this way, optical NMR detection is realized without sacrificing the versatility of the traditional nuclear induction approach. Theories predicts that the added noise through the EMO scheme can be made smaller compared to that in the conventional NMR.

In the presentation we show demonstration of EMO NMR as well as our current efforts toward its extension, including:

- Rf-to-light signal up-conversion using a High-temperature superconducting rf resonator,
- New design and fabrication of a compact rf-to-light transducer that would fit inside the bore of a superconducting magnet, and
- Development toward combination to EMO MRI and MAS.

**References:** [1] K. Takeda, K. Nagasaka, A. Noguchi, R. Yamazaki, Y. Nakamura, E. Iwase, J.M. Taylor, K. Usami, *Optica*. 5 (2018) 152. doi:10.1364/OPTICA.5.000152. [2] Y. Tominaga, K. Nagasaka, K. Usami, K. Takeda, *J. Magn. Reson.* 298 (2019) 6–15. doi:10.1016/j.jmr.2018.11.003.

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### Algorithmic cooling by using long-lived singlet states

Christian Bengs<sup>1</sup>, Lynda Brown<sup>1</sup>, Konstantin Ivanov<sup>2</sup>, Alexey Kiryutin<sup>3</sup>,  
Alex Pop<sup>1</sup>, Kirill Sheberstov<sup>4</sup>, Alexandra Yurkovskaya<sup>2</sup>, Richard CD Brown<sup>1</sup>,  
Bogdan Rodin<sup>\*2</sup>, Malcolm Levitt<sup>1</sup>

<sup>1</sup>University of Southampton, <sup>2</sup>International Tomography Center, <sup>3</sup>International  
Tomography Center SB RAS, <sup>4</sup>Johannes Gutenberg University of Mainz

Algorithmic cooling is a relatively new method to increase overall spin-polarization in NMR, which is based on manipulations of coupled slow-relaxing and fast-relaxing spins. The method enables increasing the magnetization of slow-relaxing spin by using the ability of fast-relaxing spins to pump entropy into the environment. Here, we suggest a new method to increase spin polarization by using long-lived spin order. In the simplest case of a two-spin system, such a spin order is singlet order, while the fast-relaxing order is represented by spin magnetization.

We have developed a novel approach to algorithmic cooling by using a strongly-coupled spin pair, in which the long-lived singlet order can be sustained even in the absence of spin-locking. Experiments are performed for a naphthalene derivative having a pair of <sup>13</sup>C labels with extremely long-lived singlet order. Algorithmic cooling is achieved by performing selective singlet-to-T<sub>1</sub> and singlet-to-T<sub>2</sub> conversion steps multiple times and allowing magnetization relaxes between subsequent conversions.

Firstly, we achieved the efficiency of singlet order formation of 0.82 (measured in units of thermal magnetization), which is greater than 2/3, which is the maximal theoretically allowed value assuming spin evolution given by unitary transformations. Secondly, we managed to increase the magnetization of the two-spin system by a factor of 1.23. For achieving such a conversion efficiency, we optimized methods used for singlet order generation, M2S, APSOC, and adiabatic SLIC.

To describe the new phenomena, we implemented a theoretical approach. In the simplest variant, we assume that lifetime of singlet order much longer than the T<sub>1</sub>-relaxation time and calculate the maximal theoretically allowed efficiency of algorithmic cooling. A more advanced superoperator-based approach enables quantitative modeling of the experiments: the theoretical result is in a good agreement with experimental findings.

The reported study was funded by RFBR according to the research project №19-32-80004

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**PS236**

Fri, 10:30-11:05

**Investigating the dynamic conformational landscape of  
G-protein-coupled receptors (GPCRs)**

Daniel Nietlispach\*

*University of Cambridge, Department of Biochemistry*

GPCRs belong to a family of ca. 850 plasma membrane-embedded proteins, which as molecular signalling switches control a wide range of physiological processes in health and disease. Activation of the individual receptor proteins is initiated via extracellular stimuli, such as photon uptake, or binding of small molecules, peptides, proteins, ions, lipids etc. This initial stimulus leads to conformational changes in the GPCR that favour intracellular binding partners to interact at the cytoplasmic receptor side. This then triggers a variety of downstream signalling cascades.

GPCRs represent the largest and most intensely studied class of drug targets and it is becoming increasingly clear that beyond the canonical activation through orthosteric ligands there is influence from many additional molecular factors, revealing the regulation of these receptors to be highly allosteric.

A wealth of structure data has provided a detailed static view of the basic conformational features of receptor activation. Yet, GPCRs are highly dynamic and adaptable entities that populate a complex and changing energy landscape. Not surprisingly therefore, the exact details of how signal propagation progresses from the extracellular side of the receptor to the cytoplasm are still unclear and the mechanistic determinants that allow a receptor to differentiate between the different interaction partners are still poorly understood.

Various NMR studies have provided experimental evidence on the dynamic nature of GPCRs. Here we investigate the class A receptor  $\beta_1$  adrenergic receptor ( $\beta_1$ AR) using a range of complementary  $^{13}\text{C}$  and  $^{19}\text{F}$  NMR techniques that reveal the response of the ligand binding pocket as well as the cytoplasmic side of the receptor to probing with ligands, nanobodies and mini-G protein. Our studies show the receptor existing as a mixture of low populated intermediates that interconvert with each other, with populations as well as exchange kinetics influenced by ligand binding.

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**PS237** Fri, 11:05-11:30

**Light-induced changes in the conformational dynamics of a reversibly photo-switchable fluorescent protein revealed by solution NMR spectroscopy**  
Nina-Eleni Christou\*, Virgile Adam, Dominique Bourgeois, Bernhard Brutscher  
*Institute de Biologie Structurale, UGA, CNRS, CEA*

Reversibly photo-switchable fluorescent proteins (RSFPs) are important tools for microscopy and other biotechnological applications. They are currently routinely used for Near-Field Super Resolution Microscopy techniques, e.g. RESOLFT [1]. Their characteristic switching between a fluorescent “on” state and a non-fluorescent “off” state, combined with signal processing algorithms has allowed to improve the resolution of cellular imaging down to of a few nanometers. Crystallographic studies of such RSFPs have provided crucial insights on their atomic structure, that has guided the field of fluorescent protein engineering in the search for better tags. However, the crystal forms of such proteins studied at cryogenic temperatures does not provide a realistic picture of the conformational dynamics, and how they influence the photophysics of the RSFP. So far, only a single NMR study for the RSFP Dronpa has been reported in the literature [2]. Here, we present a comprehensive NMR study of rsFolder, a green negative RSFP [3]. Using a portable in-situ laser illumination device, we were able to perform NMR assignments and derive local dynamic information for both, the fluorescent “on” and “off” states of rsFolder, aided by Laser-driven Exchange NMR experiments. After photo-switching, rsFolder experiences significantly enhanced millisecond time scale dynamics, affecting mostly the environment of the chromophore as detected by extensive line broadening of backbone and side chain resonances. H/D exchange measurements also revealed a global destabilization of the  $\beta$ -barrel region facing the phenol ring of the endogenous chromophore, as well as the chromophore-connecting helical structures. Single-point mutants of rsFolder have been used in an attempt to make correlations between the NMR-observed features and the different photophysical properties of these mutant RSFPs.

**References:** [1] Hell, S.W., 2003. Nat.Biotechnol. 21, 1347–1355. [2] Mizuno, H. et al, 2010. J.Biomol.NMR. 48, 237-246. [3] El Khatib, M. et al, 2016. Sci.Reports. 6:18459(1-12)

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**PS238**

Fri, 11:30-11:55

**Probing transition state conformations of  
RNA refolding reactions by NMR spectroscopy**

Boris Fürtig\*, Heidi Zetzsche, Katharina Hohmann, Stefanie Rückriegel  
*Goethe University*

In contrast to most proteins, RNAs can adopt more than one conformational state of similar stability. The different stable conformations are directly linked to the RNA's biological function. Examples for such functional multi-state RNAs are riboswitches, ribozymes and RNA thermometers, where either external factors such as small molecular metabolites and temperature or the intrinsic base-pairing potential modulate the equilibrium of these long-lived conformational states. The interconversion of these functional states is referred to as RNA refolding. This refolding process is slow as it is represented by a transition state with a characteristic high free energy.

Here, we present our endeavors in the characterization of bi- and multistable RNA molecules by NMR spectroscopy in order to elucidate the structure of RNA transition states. Besides standard multi-dimensional static NMR techniques, we employ  $^{15}\text{N}$ - and  $^{13}\text{C}$ -CEST as well as laser-assisted real-time NMR. The exchange based experiments are applied on unmodified RNA systems containing different isotope-labelling schemes. In contrast the real-time experiments start under a non-equilibrium condition and monitor the relaxation towards the equilibrium state, therefore the utilization of RNA sequences modified by photo-labile protecting groups is required.

Our experiments lead us to an in-depth characterization of the RNA conformations of in total five different multistable RNA systems (from small bistable RNAs to purring sensing riboswitches). It further allowed a detailed description of the kinetics and thermodynamics of the interconversion process.

We could show that dependent on the structural constraints and the overall thermodynamic stability of the ground-state conformations, the refolding reaction either occurs via a pseudo-knotted or linear transition state. This initial structural description of transition states further facilitates the understanding of how chaperones can enhance the refolding reaction.

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## PS239

Fri, 11:55-12:30

### Leveraging the Achilles heel of high-gamma nuclei to observe low-gamma nuclei.

Abhinav Dubey<sup>1</sup>, Andras Boeszoermenyi<sup>1</sup>, Thibault Viennet<sup>1</sup>, Patrick Fischer<sup>1</sup>,  
Denitsa Radeva<sup>2</sup>, Helena Kovacs<sup>3</sup>, Vladimir Gelev<sup>2</sup>, Gerhard Wagner<sup>4</sup>, Ilya Kuprov<sup>5</sup>,  
Wolfgang Bermel<sup>3</sup>, Koh Takeuchi<sup>6</sup>, Haribabu Arthanari<sup>\*,7</sup>

<sup>1</sup>Dana Farber Cancer Institute, <sup>2</sup>Sofia University, <sup>3</sup>Bruker Biospin, <sup>4</sup>Harvard Medical School,

<sup>5</sup>University of Southampton, <sup>6</sup>AIST, <sup>7</sup>Harvard Medical School

Sensitivity and resolution have been the two important traits in NMR of biomolecules. With the advent of cryogenically cooled probed and non-uniform sampling methods, the battle of sensitivity and resolution has to be revisited. <sup>1</sup>H has long enjoyed the limelight due to its inherent sensitivity. The large gyromagnetic ratio of <sup>1</sup>H is its nemesis when dealing with high molecular weight systems since the dipolar contribution to relaxation is governed by the square of the gyromagnetic ratio. However, low gamma nuclei, though insensitive, have slower relaxation rates thus providing sharper resonances – a desired factor when dealing with a crowded spectrum. With reduced dipole-induced relaxation, the relaxation of the low gamma nuclei is often affected by chemical shift anisotropy (CSA). Here, we follow on the previously established TROSY effect which cancels part of the CSA induced relaxation of the low-gamma nuclei using the dipole of the high-gamma nuclei- and develop <sup>13</sup>C and <sup>15</sup>N detected experiments for large molecular weight systems. The architecture, unique advantages and the limitations of these experiments will be presented.

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**PS240**

Fri, 10:30-11:05

**High Throughput Hyperpolarization for Drug Screening**

Geoffrey Bodenhausen\*

*Ecole Normale Supérieure*

Many limitations of state-of-the-art drug screening by nuclear magnetic resonance (NMR) can be overcome by means of high-throughput hyperpolarization. There is an urgent need for innovative experimental screening techniques to identify new drugs as the resistance of « superbugs » against known drugs, e.g., against mycobacterium tuberculosis and other pathogens. Screening techniques must be capable of ranking promising drug candidates (“ligands”) according to their affinity for a protein, a nucleic acid, or a macromolecular complex (“targets”), in order to inhibit their function. Ligand-based NMR methods can monitor parameters such as chemical shifts, diffusion coefficients, dissociation constants  $K_D$ , and kinetic  $k_{on}$  and  $k_{off}$  rates. NMR is particularly powerful to identify weakly binding ligands, which are crucial for fragment-based drug discovery (FBDD). However, even when boosted by current Dynamic Nuclear Polarization (DNP) methods, NMR is exceedingly slow and cumbersome. Our team is working towards the transformation of DNP-enhanced NMR into a competitive method for drug screening by introducing several ground-breaking innovations: multiplexed hyperpolarisation, high-speed transfer of frozen droplets, in situ dissolution, multiplexed detection using a stack of microfluidic detection chambers, and improved contrast due to long-lived states (LLS) of nuclei such as  $^{19}\text{F}$  and, more surprisingly,  $^2\text{H}$  in deuterated heavy drugs.

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**PS241** Fri, 11:05-11:30

**Frequency-Chirped Millimeter-Wave Control of  $^{13}\text{C}$ -DNP in Diamond**  
Daphna Shimon\*, Chandrasekhar Ramanathan  
*Dartmouth College*

Electron and nuclear spins in diamond have long coherence and relaxation times at room temperature, making them a promising platform for applications such as biomedical and molecular imaging and nanoscale magnetic field sensing. While the optically-active nitrogen-vacancy (NV) defect has received a great deal of attention, the substitutional nitrogen (or P1) center also exhibits long coherence and relaxation times. These P1 centers are typically present at significantly larger concentrations (about an order magnitude larger) than NVs, allowing us to explore the role of P1-P1 interactions in mediating DNP. The system can, in principle, show DNP via the solid effect (SE), cross effect (CE) and Overhauser effect (OE) depending on the P1 concentration and the field.

Here, we show enhancement of natural abundance  $^{13}\text{C}$  nuclei found within the diamond, using the unpaired electron of the P1 center (concentration 110-130 ppm) in particles with a 15-25  $\mu\text{m}$  diameter, under static conditions at room temperature and 3.4 T. From the DNP spectrum we conclude that both the SE-DNP and OE-DNP mechanisms are active. The OE, in our case, results in negative enhancement, in contrast to previous results reporting positive OE enhancements. A negative OE implies that zero-quantum relaxation is more effective than double-quantum relaxation, likely due to strong anisotropic hyperfine interactions. We also explore the effect of frequency modulation (FM) of the DNP mechanism. Preliminary results suggest that the OE benefits from faster FM ( $>100$  kHz) whereas the SE does not. This suggests that we can control which DNP mechanism is effective using FM parameters such as frequency, amplitude and shape.

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**PS242**

Fri, 11:30-11:55

**Signal-improved real-time NMR spectroscopy of proteins by hyperpolarized water**Dennis Kurzbach<sup>\*,1</sup>, Gregory L Olsen<sup>2</sup><sup>1</sup>*University of Vienna*, <sup>2</sup>*Unvirsity of Vienna*

Hyperpolarized water produced by dissolution dynamic nuclear polarization (dDNP) has recently been shown to enable the detection of hyperpolarized spectra of proteins with up to 300-fold improvement in signal amplitudes. With this dDNP approach, novel insights can be gained into solvent accessible surfaces, ligand interactions, and complex protein geometries. Examples of applications to host-ligand systems including peptides and folded as well as intrinsically disordered proteins (IDPs) have demonstrated the broad applicability of the hyperpolarized water approach. [1-5]

In this contribution, we present recent efforts to combine dDNP with real-time NMR, aimed at tracking protein-ligand binding events and protein-solvent interactions at a sub-Hertz sampling rate. Two applications will be presented: (1) The use of hyperpolarized water to examine the kinetics underlying protein-ligand interactions. Here, non-equilibrium dynamics in the osteopontin-heparin host-ligand system were monitored in a dDNP experiment by simultaneous mixing of the protein with the ligand and hyperpolarized water. (2) A proof-of-concept for real-time protein dDNP at residue-resolution at hand of Ubiquitin in hyperpolarized water by a statistical analysis of time-series of 1D dDNP spectra.

**References:** [1] P. Kaderavek, F. Ferrage, G. Bodenhausen and D. Kurzbach, *Chem. Eur. J.*, 2018, 24, 13418-13423. [2] O. Szekely, G. L. Olsen, I. C. Felli and L. Frydman, *Anal Chem*, 2018, 90, 10, 6169-6177. [3] D. Kurzbach, E. Canet, A. G. Flamm, A. Jhajharia, E. M. Weber, R. Konrat and G. Bodenhausen, *Angew Chem Int Ed Engl*, 2017, 56, 389-392. [4] G. Olsen, E. Markhasin, O. Szekely, C. Bretschneider and L. Frydman, *J Magn Reson*, 2016, 264, 49-58. [5] G. L. Olsen, O. Szekely, B. Mateos, P. Kadeřávek, F. Ferrage, R. Konrat, R. Pierattelli, I. C. Felli, G. Bodenhausen, D. Kurzbach and L. Frydman, 2019, in preparation.

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**PS243**

Fri, 11:55-12:30

**Wither the Spin Diffusion Barrier**Kong Ooi Tan<sup>1</sup>, Michael Mardini<sup>1</sup>, Chen Yang<sup>1</sup>,  
Jan Henrik Ardenkjær-Larsen<sup>2</sup>, Robert G. Griffin<sup>\*,1</sup><sup>1</sup>MIT, <sup>2</sup>Technical University of Denmark

In the last few years microwave driven dynamic nuclear polarization (DNP) has become the method of choice to enhance signal intensities in a variety magic angle spinning (MAS) NMR experiments. In particular, because of the large signal enhancements, it is used to address a variety of important chemical, biological and physical questions that are otherwise inaccessible. Despite the success of DNP, there is not a detailed understanding of the manner in which the high polarization of the electron is transferred to the surrounding nuclei or where these nuclei are located relative to the polarizing agent in an amorphous glassy matrix. In a number of different papers the size of the “spin diffusion barrier” surrounding the paramagnetic center has been postulated to be as large 20-40 Å, and therefore to encompass a large number of spins. In this paper we perform an experimental and theoretical analysis of the recently rediscovered three-spin solid effect (TSSE) and show that it is exquisitely sensitive to the electron-nuclear distances. We exploit this feature to determine the size of the spin diffusion barrier surrounding the trityl radical in a glassy glycerol–water matrix and therefore the location of the protons involved in the initial transfer step. They are less than 6 Å from the electron and located on the perimeter of the trityl molecule. Thus, there is effectively not a spin diffusion barrier in the case of trityl in DNP juice. Furthermore, <sup>1</sup>H ENDOR experiments indicate that the electron polarization is first transferred to the intramolecular <sup>1</sup>H’s on the trityl radical, and then via spin diffusion to glycerol molecules in intimate contact with the trityl and subsequently to other <sup>1</sup>H’s in the solvent.

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**PS244**

Fri, 10:30-11:05

**Developments of NMR for Applications in Chemical Engineering and Medicine**Robin Dykstra<sup>1</sup>, Sergei Obruchkov<sup>2</sup>, Mark Hunter<sup>2</sup>,Paul Teal<sup>1</sup>, Yu-Chieh Tzeng<sup>3</sup>, Petrik Galvosas<sup>\*,4</sup><sup>1</sup>*School of Engineering and Computer Science, Victoria University of Wellington,*<sup>2</sup>*Robinson Research Institute, Victoria University of Wellington,* <sup>3</sup>*Department of Surgery & Anaesthesia, University of Otago,* <sup>4</sup>*MacDiamid Institute for Advanced Materials and Nanotechnology, School of Chemical and Physical Sciences, Victoria University Wellington*

To enable wider application of NMR/MRI technologies in science, engineering and medicine, approaches must be specialised, accessible, simple and affordable. In this context this lecture will give recent examples from our journey of pushing MR boundaries.

We will report on the parallel acquisition of  $q$ -space, thus enabling real time monitoring of averaged propagators [1]. We will also discuss Magnetic Resonance Pore Imaging (MRPI) at resolutions well beyond the limits of conventional MRI [2] and demonstrate how recent advances in Rheo-NMR enable further insight into complex fluids under shear [3].

With respect to medical applications the lecture will report on measuring averaged fractional anisotropy [4], discuss options for the measurement of bone-to-total-volume ratios [5], metabolic rates of cells under mechanical stress and developments towards sensors for blood oxygenation [6].

To expand NMR and MRI into non-conventional areas as highlighted above it is necessary to develop affordable electronics [7] which meets the requirements of purpose built MR systems. Furthermore, experimental protocols and data analysis are required to be robust, especially under conditions of low signal to noise ratios [8,9]. Together with appropriate magnet systems we see great potential of NMR and MRI to be used in process control, quality assurance, point of care sensors as well as in academic and industrial research.

**References:** [1] Kittler, W. et al., *Phys. Rev. E* **92** 023016 (2015). [2] Hertel, S. et al., *Phys. Rev. E* **92** 012808 (2015). [3] Galvosas, P. et al., *Magn. Reson. Chem.* Doi:10.1002/mrc.4861 (2019). [4] Zong, F. et al., *Magn. Reson. Chem.* **55** 498-507 (2017). [5] Brizi, L. et al., *Magn. Reson. Med.* **79** (2018). [6] Thomas, D., Victoria University of Wellington, Hdl:123456789/3. Web. (2018). [7] Ang, A. et al., Proceeding ISMRM-ESMRMB 16-21 June 2018 Paris, France. [8] Teal, P. et al., *Inverse Problems* **31** 045010 (2015). [9] Anjum, A. et al., *Magn. Res. Chem.* **56** 740-747 (2018).

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**PS245**

Fri, 11:05-11:30

## New developments in production of proton-hyperpolarized propane gas for MRI

Oleg G. Salnikov<sup>\*1</sup>, Kirill V. Kovtunov<sup>2</sup>, Nuwandi M. Ariyasingha<sup>3</sup>,  
Panayiotis Nikolaou<sup>4</sup>, Eduard Y. Chekmenev<sup>3</sup>, Igor V. Koptug<sup>2</sup>

<sup>1</sup>International Tomography Center SB RAS and Novosibirsk State University,

<sup>2</sup>International Tomography Center SB RAS, <sup>3</sup>Wayne State University,

<sup>4</sup>Vanderbilt University Institute of Imaging Science

Hyperpolarization allows one to increase the NMR sensitivity by several orders of magnitude. The main drivers behind the development of hyperpolarization techniques are their biomedical applications. For example, the inhalation of hyperpolarized noble gases, such as <sup>129</sup>Xe and <sup>3</sup>He, enables functional imaging of lung diseases. However, highly specialized <sup>129</sup>Xe and <sup>3</sup>He MR equipment and software is required which is not available on conventional clinical MRI scanners. Therefore, <sup>1</sup>H-hyperpolarized gases, e.g. propane, represent a promising alternative. Hyperpolarization of propane can be accomplished by pairwise addition of parahydrogen to propylene over heterogeneous catalyst.

Here, we present our recent results on hyperpolarization of propane gas. We developed propane polarizer that enables production of hyperpolarized propane on a clinical scale (production rate >0.3 L just in 2 s, that is more than an order of magnitude greater than that demonstrated previously). Importantly, high polarization levels (~1%) can be retained despite the increase in production rate, allowing stopped-flow slice-selective high-resolution 2D MRI visualization. It was demonstrated that at ~0.05 T magnetic field hyperpolarized propane occurs as a long-lived spin state, which lifetime  $T_{LLS}$  is ~3 times greater than  $T_1$ . The use of buffering gases leads to the increase of propane polarization despite slight  $T_{LLS}$  decrease. Cryocollection of hyperpolarized propane, which can be employed for buffering gas separation, increases  $T_{LLS}$  up to 14.7 s in the liquid state, which is higher than that of gaseous hyperpolarized propane at any pressure studied. We also explored feasibility of propane hyperpolarization via hydrogenation of cyclopropane with parahydrogen. <sup>1</sup>H polarization up to 2.4% was obtained, that is several times greater than that obtained with propylene as a precursor. The resulting NMR signal enhancement was sufficient for 2D MRI despite relatively low chemical conversion of cyclopropane substrate.

This work was supported by Russian Science Foundation (grant #17-73-20030) and DOD CDMRP W81XWH-15-1-0271.

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**PS246**

Fri, 11:30-11:55

### Ultra-high field MRI and MRS: Opportunities and Challenges from Anatomical Imaging and Metabolite Detection for Biological Specimens

Julia R. Krug<sup>\*,1</sup>, Remco van Schadewijk<sup>2</sup>, Frank J. Vergeldt<sup>3</sup>, Andrew G. Webb<sup>4</sup>, Huub J.M. de Groot<sup>2</sup>, A. Alia<sup>2</sup>, Luisa Ciobanu<sup>5</sup>, Henk Van As<sup>3</sup>, Aldrik H. Velders<sup>6</sup>

<sup>1</sup>Laboratory of BioNanoTechnology and Laboratory of Biophysics, Wageningen University & Research, <sup>2</sup>Solid-state NMR, Leiden University, <sup>3</sup>Laboratory of Biophysics, Wageningen University & Research, <sup>4</sup>C.J. Gorter Center for High Field MRI, Leiden University Medical Center, <sup>5</sup>UNIRS, Neurospin, Joliot, DRF, CEA, Université Paris-Saclay, <sup>6</sup>Laboratory of BioNanoTechnology, Wageningen University & Research

Magnetic Resonance Imaging at ultra-high field strengths (17-22 T) provides both opportunities and challenges for non-invasive imaging of biological specimens. As low sensitivity is the most common drawback of MRI applications, the most apparent opportunity of ultra-high field imaging manifests itself by an augmentation in the Signal-to-Noise ratio (SNR). To this end, using similar RF coils, we found that the SNR increased by a factor of 6 when going from 14.1 T to 22.3 T. This SNR increase can be used for faster imaging (a factor of 32), higher resolution imaging reaching  $(5.5 \mu\text{m})^3$  [1] for an acquisition time of 58 h 35 min (3D FLASH, field-of-view  $1.6 \times 1.1 \times 1.1 \text{ mm}^3$ ), and direct metabolite detection by localized spectroscopy (10 mM acetate in a voxel volume of 27 nL in 18 min). In addition to spectroscopy, the increased chemical shift dispersion offered by ultra-high magnetic fields also benefits chemical exchange techniques such as Chemical Exchange Saturation Transfer (CEST) imaging which allows indirect metabolite detection by making use of the exchange of labile metabolite protons with water protons. We show that CEST at 17 T outperforms CEST at 7 T not only in terms of sensitivity but also by better separating the contributions of individual metabolites (glutamate vs. glucose). Lastly, concerning the challenges of MRI at ultra-high field, the increase of susceptibility artifacts caused by air spaces and paramagnetic ions is detrimental to image quality. We will show such effects and discuss possible solutions by examples of in vivo root specimens of *M. truncatula* and electrode materials.

**References:** [1] Krug et al., High spatial and temporal resolutions with increasing  $B_0$  and decreasing transceiver coil dimensions, (manuscript in preparation).

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**PS247**

Fri, 11:55-12:30

## In vivo three-dimensional extracellular pH mapping of tumors using EPR

Hiroshi Hirata\*

*Hokkaido University*

Background and aim: Acidosis and low-oxygen status are hallmarks of solid tumors. Acidification in extracellular space in solid tumors reflects a shift of cellular metabolism for tumors. Thus, visualization of extracellular pH (pHe) is useful to understand the pathophysiological status of tumors. A method of three-dimensional (3D) pHe mapping of murine tumors using electron paramagnetic resonance (EPR) is introduced in this talk.

Methods: pHe was measured with a 750-MHz home-built continuous-wave EPR spectrometer/imager using a pH-sensitive nitroxyl radical probe (dR-SG) [1,2]. pHe maps were obtained by four-dimensional spectral-spatial EPR imaging. For the proof-of-concept experiment, solution samples, adjusted to 6.60, 6.80, and 7.00 pH units, were visualized. Murine squamous cell carcinoma (SCC VII) cells were implanted into the right hind legs of mice. Tumor-bearing mouse legs were monitored 5 and 8 days after the implantation. Also, tumor xenograft mouse models of human-derived pancreatic ductal adenocarcinoma cells (MIA PaCa-2, SU.86.86, and Hs766t) were measured when the tumor volumes reached approximately 1 cm<sup>3</sup>.

Results: 3D pH maps were reconstructed for three tubes containing different pH solutions. The pH resolution was achieved at 0.078 pH units, and the trueness of pH values was 0.026 pH units [3]. 3D pHe maps of SCC VII tumors on day 5 and 8 exhibited the progress of acidification. The tumor xenograft mouse models exhibited different levels of acidification and inhomogeneous spatial distribution of pHe in tumor tissues.

Conclusion: In vivo pHe mapping was demonstrated with tumor mouse models. The results suggest that the method of 3D pH mapping can be applied to future studies of tumor mouse models that involve tumor acidification.

**References:** [1] A. A. Bobko et al., *Magn. Reson. Med.* 67, 1827 (2012). [2] H. Sato-Akaba et al., *Anal. Chem.* 81, 7501 (2009). [3] D. A. Komarov et al., *Anal. Chem.* 90, 13938 (2018).

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**PS248**

Fri, 10:30-11:05

**Ultrasensitive beta-detected NMR at CERN: first results in physics and biology**

Magdalena Kowalska\*

UNIGE, CERN

Beta-detected NMR is up to 10 orders of magnitude more sensitive than conventional NMR, because it is based on the detection of beta-particles from hyperpolarized short-lived nuclei. Our project aims at applying it for the first time to liquid samples relevant in chemistry and biology, thus extending its use from nuclear structure and material science studies in solid environments.

Our experimental setup, built in 2016 and upgraded in 2017 and 2018, is located at the CERN-ISOLDE facility, where over 1000 different radioactive nuclei can be produced. We use optical pumping with lasers on isotopes of different metallic elements, resulting in nuclear polarizations up to 90%. The decrease in the anisotropic emission of beta radiation from such nuclei is then used to detect the NMR response, leading to the extreme sensitivity of beta-NMR.

First studies on  $^{26}\text{Na}$  in liquid samples were performed by us in 2017, which lead to much narrower beta-NMR resonances than seen previously in solid hosts. Thanks to the shimming and active stabilisation of our 1.2 T electromagnet at the 1 ppm level, in 2018 we could determine the magnetic moments of several short-lived Na nuclei with about 100 times improved precision. This provides a self-consistent set of nuclei to be used in beta-NMR studies in liquid samples, connected to the moment of stable  $^{23}\text{Na}$ . In 2018 we furthermore probed the interaction of Na cations with DNA G-quadruplex structures, present e.g. in telomeres. Present upgrades to the experimental setup should allow to apply this approach to isotopes of more chemical elements (e.g. K, Cu or Zn) in an even broader range of research topics, such as alkali-metal batteries.

This contribution will introduce the technique and describe the experimental setup, and will concentrate on the most recent results in physics and biology, followed by a short outlook.

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**PS249**

Fri, 11:05-11:30

## Direct magnetic field dependence of NMR shielding

Anu M. Kantola<sup>\*,1</sup>, Perttu Lantto<sup>1</sup>, Ivo Heinmaa<sup>2</sup>, Juha Vaara<sup>1</sup>, Jukka Jokisaari<sup>1</sup>

<sup>1</sup>University of Oulu, <sup>2</sup>National institute of chemical physics and biophysics, Estonia

Nuclear shielding is considered independent of the magnetic field strength when analysing NMR experiments. However, already in 1970, Ramsey proposed on theoretical grounds that this may not be valid for heavy nuclei. Here we present experimental evidence for the direct field dependence of shielding, using <sup>59</sup>Co shielding in Co(acac)<sub>3</sub> dissolved into chloroform as an example. This low-spin diamagnetic Co(III) complex features a very large and negative nuclear shielding constant of the central Co nucleus. We carry out variable temperature NMR experiments in four different field strengths ranging from 7.05 to 18.79 T. As there is a well-known sensitivity of the <sup>59</sup>Co NMR frequency to temperature, we introduce Xenon gas into the sample and use the known temperature dependence of the <sup>129</sup>Xe signal to calibrate the temperature. Signal from a Xenon gas sample is used as a frequency reference. The experiments result in temperature dependent magnetic field dependence in the order of -10<sup>-3</sup> ppm T<sup>-2</sup> for the <sup>59</sup>Co shielding constant, arising from the direct modification of the electron cloud of the complex by the field. First-principles non-linear response theory results in values ranging from -10<sup>-5</sup> to -10<sup>-3</sup>, in reasonable agreement with the experiment. Upon increasing field strengths available in contemporary NMR setups, the direct magnetic field dependence of NMR parameters becomes a factor to take into account in studies of materials and molecular structures. Furthermore, direct field-induced effects may in the future provide entirely new tools for materials characterisation.

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**PS250**

Fri, 11:30-11:55

**Towards single-shot readout of NV centers in diamond by  
low-temperature spin-to-charge conversion**

Dominik Irber<sup>1</sup>, Michael Kieschnick<sup>2</sup>, Fei Kong<sup>3</sup>, Fazhan Shi<sup>3</sup>, Jiangfeng Du<sup>3</sup>, Jan Meijer<sup>2</sup>,  
Friedemann Reinhard<sup>\*,1</sup>

<sup>1</sup>*TU München, Walter Schottky Institut*, <sup>2</sup>*Universität Leipzig*, <sup>3</sup>*USTC China*

We present our recent progress in implementing an improved readout scheme for the nitrogen-vacancy (NV) center's spin-state combining resonant excitation at low (4 Kelvin) temperature with spin-to-charge conversion. Resonant excitation exploits that the optical excitation spectrum at low temperature has sufficiently narrow linewidths[1,2] to selectively address the spin-sublevels. In combination with a second laser pulse, a spin-to-charge conversion[3,4] protocol can be implemented, where the NV center is spin-selectively excited and converted to different charge-states. These are more stable than the initial spin-state and can be read-out with single-shot fidelity, even by inefficient collection optics.

Compared to the state-of-the-art readout[5], this work promises to accelerate readout by a factor of up to 100. Besides, laser power in the optical regime can be reduced, which lowers the risk of photodamage for future sensing experiments with biological samples.

We expect our scheme to become a core enabling tool for single-molecule electron spin resonance detected by NV centers.

**References:** [1] A. Batalov, Physical Review Letters 102, 195506 (2009). [2] M.W. Doherty, New Journal of Physics 13, 025019 (2011). [3] B.J. Shields, Physical Review Letters 114, 136402 (2015). [4] X.-D. Chen, Physical Review A 7, 014008 (2017). [5] D.A. Hopper, Micromachines 9, 437 (2018).

This project has received support from DFG via projects SPP1601 and RE3606/3-1.

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**PS251** Fri, 11:55-12:30

**Magnetic Resonance Spectroscopy of A Single Molecule**  
Fazhan Shi\*  
*University of Science and Technology of China*

Magnetic resonance (MR) is one of the most important techniques for characterizing compositions, structure and dynamics of molecules. Over the past several years, quantum sensing with Nitrogen-Vacancy (NV) center has opened a new door for magnetic resonance spectroscopy of a single molecule. In my talk, I will mainly introduce several new experimental results on both of methods and biology applications. (I) Zero-field electron spin resonance (ESR) spectroscopy on nanoscale. We successfully measured the zero-field ESR spectrum of a few electron spins, by precisely tune the energy levels of NV centers to be resonant with the target spins, and directly resolved the hyperfine coupling constant. This work break the sensitivity limitation and open the door of practical applications of the zero-field ESR. (II) ESR spectroscopy of a single protein in poly-lysine and a single DNP duplex in aqueous solution. The work represents a step forward towards magnetic resonance investigation of biomolecules in their native environments at the single-molecule level. (III) We realized one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy of two coupled nuclear spins and resolved its structure.

**References:** [1] Fazhan Shi, et al., Single-DNA electron spin resonance spectroscopy in aqueous solutions, *Nature Methods* 15, 697 (2018). [2] Fei Kong, et al., Zero-field electron spin resonance spectroscopy on na-noscale, *Nature Communications* 9, 1563 (2018). [3] Fazhan Shi, et al., Single-protein spin resonance spectroscopy under ambient conditions, *Science* 347, 1135 (2015). [4] Fazhan Shi, et al., Sensing and atomic-scale structure analysis of single nuclear spin clusters in diamond, *Nature Physics*, 10, 21-25 (2014). [5] Tobias Staudacher, et al., Nuclear magnetic resonance spectroscopy on a (5nm)<sup>3</sup> volume of liquid and solid samples, *Science*, 339, 561 (2013). [6] Zhiping Yang, et al., Two-dimensional nanoscale nuclear magnetic resonance spectroscopy enhanced by artificial intelligence, *arXiv:1902.05676* (2019).

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**PS252**

Fri, 10:30-11:05

**Allosteric signaling pathways and energetics in the glucocorticoid receptor**Mikael Akke<sup>\*,1</sup>, Christian Köhler<sup>2</sup>, Ulrich Weininger<sup>1</sup>, Göran Carlström<sup>1</sup>,Tim Kaminski<sup>2</sup>, Anders Gunnarsson<sup>2</sup>, Ulla Karlsson<sup>2</sup>, Karl Edman<sup>2</sup><sup>1</sup>*Lund University*, <sup>2</sup>*AstraZeneca*

The glucocorticoid receptor (GR) binds steroid hormones, leading to structural rearrangements that drive DNA binding, recruitment of coregulator proteins, and ultimately gene regulation. Different receptor-ligand complexes have distinct interactions with coregulators, resulting in differential gene regulation. The allosteric mechanism within the ligand-binding domain (LBD) has remained unknown. We used a combination of NMR relaxation dispersion and surface plasmon resonance to delineate allosteric signaling pathways within the LBD and their energetics. CPMG dispersions map out a dynamic network of residues linking the ligand-binding pocket to the activation function-2 interface, where helix 12, a switch for transcriptional activation, undergoes ligand and coregulator dependent conformational exchange dynamics. We quantified the observed variation in coregulator affinity and helix 12 activation in terms of free energies of allostery between sites. The results pinpoint how differential activation of GR arises from gradual population shifts within a dynamic ensemble of conformations.

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**PS253**

Fri, 11:05-11:30

## Rheological NMR for the study of polymer dynamics

Benjamin Kohn<sup>1</sup>, Vincent Körber<sup>2</sup>, Enno Stündel<sup>1</sup>, Petrik Galvosas<sup>3</sup>, Ulrich Scheler<sup>\*,4</sup>

<sup>1</sup>Leibniz-Institut für Polymerforschung Dresden e.V., <sup>2</sup>Leibniz-Institut für Polymerforschung Dresden e.V., <sup>3</sup>School of Chemical and Physical Sciences, Victoria University of Wellington, <sup>4</sup>Leibniz-Institut für Polymerforschung Dresden e.V.

Rheo NMR has been applied to investigate the effect of external shear on the aggregation and on the chain dynamics of polymers. A Couette cell with the polymer melt or solution in the gap is applied. To get further insight, oscillating rotation in addition to continuous rotation has been applied.

An in-house built rheo NMR system using a servo motor, avoiding any vibrations has been used on a spectrometer with a microimaging accessory. For the oscillatory shear a crank mechanism has been introduced between the motor and the drive of the Couette rotor.

The combination of PFG NMR with imaging enables measuring the flow profiles. In particular for low-viscosity liquids like dilute solutions or lower molecular weight polymers flow pattern deviating from the expected linear velocity gradient are observed. Around the turning point in the oscillatory shear counterflow is observed which apparently leads temporarily to a shear rate larger than the gap averaged shear rate applied when the angular velocity is at its peak value. This effect strongly depends on the viscosity.

In particular the spin-spin relaxation time  $T_2$  measured via CPMG is sensitive to the polymer chain segment motion. The expected shear-induced order in polymer melts and solutions is detected for very low molecular weight (oligomers) only. For higher molecular weight the loss or rearrangement of entanglements leading to longer chain segments between the entanglements and thus enhanced polymer dynamics manifested in longer  $T_2$  is the dominating effect.

This has been observed for both continuous and oscillatory shear at large shear angles. Varying the angle in the oscillatory shear in addition enables to study the onset of this change of the polymer chain motion and thus the onset of the rearrangement of the entangled polymers.

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**PS254**

Fri, 11:30-11:55

**Molecular ordering and dynamics in anisotropic  
soft materials studied by low-resolution proton NMR**

Anna Naumova, Kay Saalwächter\*

*Martin-Luther-Univ. Halle-Wittenberg*

With the advent of site-specific isotope labeling and deuteration, the study of local dynamics in biological macromolecules, has reached levels of unprecedented accuracy. There are, however, many cases in e.g. soft materials science, where such strategies are not feasible. While classical carbon-13-based solid-state NMR techniques are often possible, they nevertheless suffer from low sensitivity at natural abundance. Often, proton low-resolution time-domain NMR is fully sufficient, in particular when dealing with materials consisting of identical repeat units. Specifically, multiple-quantum NMR has been established as the most quantitative approach to extract order parameters and correlation times and assess the dynamic heterogeneity in such systems [1]. This is despite of data analyses being restricted to rather simple theories approximating the multi-spin dipolar coupling situation in terms of a second moment. While the methodology can be considered mature for the case of isotropic samples, challenges arise for oriented samples. Here, we highlight a recent methodological advance concerning the order parameter analysis of oriented materials with uniaxial dynamics, tested on liquid crystals [2] and now applied to the investigation of local chain stretching in mechanically deformed swollen elastomers. The latter project extends our earlier work concerned with the molecular-level deformation of polymer chains in bulk elastomers [3].

**References:** [1] K. Saalwächter, in: G.A. Webb (ed.), *Modern Magnetic Resonance*, DOI 10.1007/978-3-319-28275-6\_59-2, Springer 2017. [2] A. Naumova, C. Tschierske, K. Saalwächter, *Solid State Nucl. Magn. Reson.* 2017, 82-83, 22. [3] M. Ott et al., *Macromolecules* 2014, 47, 7597.

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**PS255**

Fri, 11:55-12:30

## Extending the range of magnetic fields for high-resolution biomolecular NMR by orders of magnitude

Fabien Ferrage\*

*CNRS and Ecole Normale Supérieure*

Higher magnetic fields lead to higher sensitivity and higher resolution. Reaching higher fields is key to study biomolecular systems of increasing complexity. Yet, higher fields are not optimal for all applications of NMR, neither for all nuclei. For instance, the chemical shift anisotropies of carbon-13 nuclei in many chemical entities, or that of fluorine-19 lead to transverse relaxation rates incompatible with efficient NMR experiments of large biomolecules and assemblies at the highest magnetic fields. On the other hand, low magnetic fields provide rich information on molecular dynamics, as demonstrated by relaxometry, but are generally associated with sensitivity and resolution incompatible with site-specific studies of biomolecules. A dilemma for biomolecular NMR is: how can we benefit from the highest magnetic fields available while optimizing the field-dependent sensitivity, resolution and information of most NMR experiments?

The solution to this dilemma is to couple high-field NMR with low- or variable-field NMR in a single spectrometer. We use a sample shuttle to displace the NMR sample in the stray field of a high-field magnet to explore low magnetic fields in the course of an NMR experiment, while keeping polarization and detection at high field for sensitivity and resolution. In addition, the sample shuttle couples a magnetic center at 0.33 T with a magnetic center at 14.1 T in a two-field NMR spectrometer. This system allows us to perform pulse sequences, where each part is performed at the most optimal of the two fields. We will show a series of applications to the determination of site-specific protein dynamics on nanosecond timescales as well as a series of examples of two-field NMR experiments that provide more efficiency or information than the equivalent high-field-only experiment. Two-field NMR spectroscopy opens a route to boost the potential of high-resolution biomolecular NMR.

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## P001

## Low-resolution NMR investigations of polymer immobilization in nanocomposites

Mozhdeh Abbasi<sup>\*,1</sup>, Sol Mi Oh<sup>2</sup>, So Youn Kim<sup>2</sup>, Kay Saalwächter<sup>1</sup><sup>1</sup>Martin Luther University Halle-Wittenberg, Halle, Germany, <sup>2</sup>UNIST, Ulsan, South Korea

Adding nanosized fillers into a polymer matrix alters its mechanical properties from the behavior of the pure state [1]. We used static low-resolution <sup>1</sup>H NMR spectroscopy to study model nanocomposites consisting of poly(ethylene oxide), mixed with spherical silica nanoparticles. Attractive interactions lead to slowing down of segmental motion and formation of an interfacial layer around the nanoparticles. NMR studies distinguished the three different phases of polyethylene oxide chains dynamics based on different transverse relaxation behavior [2].

The relaxation times concluded for different phases in the nanocomposite sample are related to the strength of the dipolar coupling between proton spin pairs. The quasi-rigid phase with strong dipolar coupling but some fast and strongly anisotropic mobility shows fast  $T_2$  decay on the time scale of microseconds, an intermediate phase with residual dipolar couplings arise from increased chain motion and the mobile phase with fast segmental motions, which almost average out the dipolar couplings with a rather long  $T_2$  in the >ms range.

The study addresses the effects of polymer molecular weight, size and loading of the nanoparticles on the interfacial layer properties. We found a significant curvature effect, leading to a thicker bound polymer layer around a less curved surface, which also was reported by theoretical work [3]. We observed a thicker interfacial layer as molecular weight decreases which is in contrast to earlier theoretical predictions but confirms recent experimental findings [4].

We further assess the time scales of the motion in the two constrained phases via a dipole-refocused  $T_2$  by using the MSE and <sup>13</sup>C  $T_1$  measured under MAS condition.

**References:** [1] A. Mujtaba et. al. ACS, Macro. Lett. 2014, 3, 481. [2] K. Schaler et. al. Macromolecules 2013, 46, 7818. [3] S. Gong et.al. ACS Macro. Lett. 2014, 3, 773. [4] S. Cheng et. al. Phys. Rev. Lett. 2016, 116, 038302.

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P002

Pressure effects on the conformational equilibrium of the  $\beta_1$ -adrenergic receptor

Anne Grahl, Layara Abiko<sup>\*</sup>, Stephan Grzesiek  
*Universität Basel*

G protein-coupled receptors (GPCRs) are versatile chemical sensors, which transmit the signal of an extracellular binding event across the plasma membrane to the intracellular side, leading to the activation of downstream effector proteins.[1] This function is achieved via the modulation of a highly dynamical equilibrium of various conformational receptor states.[2,3]

Despite significant advances in structural and biochemical knowledge on GPCRs, precise atomic details of the receptor dynamics are lacking.

The variation of pressure may change chemical equilibria and kinetics providing information about volume differences between the various states of reactants.[4] Here we have probed the effect of pressure on the conformational equilibria of the  $^{15}\text{N}$ -valine-labeled  $\beta_1$ -adrenergic receptor ( $\beta_1\text{AR}$ ) in various ligand-bound states by solution NMR. The pressure response is ligand-dependent and provides mechanistic insights into GPCR activation.

**References:** [1] Alexander, S. P.; Christopoulos, A.; Davenport, A. P.; Kelly, E.; Marrion, N. V.; Peters, J. A.; Faccenda, E.; Harding, S. D.; Pawson, A. J.; Sharman, J. L.; Southan, C.; Davies, J. A. *British Journal of Pharmacology* 2017, 174, S17. [2] Nygaard, R.; Zou, Y.; Dror, R. O.; Mildorf, T. J.; Arlow, D. H.; Manglik, A.; Pan, A. C.; Liu, C. W.; Fung, J. J.; Bokoch, M. P.; Thian, F. S.; Kobilka, T. S.; Shaw, D. E.; Mueller, L.; Prosser, R. S.; Kobilka, B. K. *Cell* 2013, 152 (3), 532. [3] Isogai, S.; Deupi, X.; Opitz, C.; Heydenreich, F. M.; Tsai, C.-J.; Brueckner, F.; Schertler, G. F. X.; Veprintsev, D. B.; Grzesiek, S. *Nature* 2016, 530 (7589), 237. [4] Frauenfelder, H.; Alberding, N. A.; Ansari, A.; Braunstein, D.; Cowen, B. R.; Hong, M. K.; Iben, I. E. T.; Johnson, J. B.; Luck, S. J. *Phys. Chem.* 1990, 94 (3), 1024.

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Developing bioorthogonal polarization agents for in-cell DNP NMR spectroscopy

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<sup>1</sup>University of California, San Diego, <sup>2</sup>University of California

Solid-state dynamic nuclear polarization (DNP) NMR utilizes radical-bearing polarization agents to enhance the signal of nearby nuclei. Consequently, small sample sizes along with insensitive and higher-dimension experiments become feasible on realistic time scales. These sensitivity gains are particularly intriguing for applications to biological systems, where the molecule of interest is often a small volume fraction of the total sample. Here, we describe a generalizable bioorthogonal targeted DNP approach where the polarization agent is conjugated to proteins carrying a genetically incorporated unnatural amino acid. We demonstrate the ability to target recombinantly expressed proteins in cells and report the benefits of using localized polarization to achieve signal enhancements. We envision this strategy to propel structural biology research into cells with native molecular concentrations.

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### Towards A Unified Description of Intrinsically Disordered Protein Dynamics under Physiological Conditions using NMR Spectroscopy

Wiktor Adamski<sup>\*,1</sup>, Sigrid Milles<sup>1</sup>, Justine Magnat<sup>2</sup>, Damien Maurin<sup>1</sup>,

Malene Ringkjøbing Jensen<sup>1</sup>, Christophe Moreau<sup>2</sup>, Nicola Salvi<sup>1</sup>, Martin Blackledge<sup>1</sup>

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While intrinsically disordered proteins (IDPs) constitute about half of the human proteome, molecular models of their functional behavior *in vivo* remain elusive. As proteins of this class do not possess a stable, three-dimensional structure, but sample ensembles of diverse conformations on rather flat energy landscapes, their function is inherently linked to their dynamics occurring on multiple timescales. Recently, we used temperature-dependent <sup>15</sup>N spin relaxation to reveal the physical origin of IDPs backbone motions in the ps-ns range. In our model three dominant dynamic modes are relaxation active: fast librational motions, conformational sampling of backbone dihedral angles and slower chain-like segmental dynamics [1, 2]. Herein, we extend the temperature-dependent model-free analysis by systematically modifying viscosity and, consequently, <sup>15</sup>N spin relaxation rates, to probe the differential effect of molecular environment on the three identified dynamic modes. We carry out our study on two IDPs with remarkably different dynamic properties: the C-terminal domain of Sendai Virus nucleoprotein, which feature a transiently populated helical recognition motif, and the disordered domain of the cancer-associated mitogen-activated protein kinase kinase (MKK4), which is devoid of any secondary structure. Our results suggest that the effects of temperature and viscosity on dynamics can be combined to develop a unified description of the effect of molecular environment parameters on IDP dynamics. In fact, the proposed model is able to predict molecular environment-induced effects on dynamic modes probed by <sup>15</sup>N spin relaxation rates *in vitro* and *in vivo* with remarkable accuracy. Overall, we expect that the model will shed some light onto the complex dynamics of IDPs engaged in dynamic complexes in a diverse range of molecular environments.

**Literature:** [1] Abyzov A., et al., JACS 2016, 138, 6240 – 6251. [2] Salvi N., et al., Angew. Chem. Int. Ed. 2017, 56, 14020.

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A Low-Temperature Broadband NMR Probe for Multinuclear Cross-Polarization

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Dissolution dynamic nuclear polarization (D-DNP) is currently the subject of many new developments in view of boosting the sensitivity of nuclear magnetic resonance spectroscopy (NMR) and imaging (MRI). Most D-DNP probes are designed for one or two nuclei at most. The investigation of multiple nuclei, therefore, requires manufacturing a number of different costly probes. In addition, changing the probe is a time-consuming process since the system that works at low temperature (usually between 1.2 and 4.2 K) must be warmed up, thus increasing the likelihood of contamination. In this work, we describe a double resonance <sup>1</sup>H-X broadband D-DNP probe where the X channel covers a broad range of nuclei from <sup>15</sup>N to <sup>23</sup>Na (i.e., from 28.9 to 75.5 MHz at 6.7 T). This probe can be used not only to observe “direct” polarization build-up on both channels, but also to perform cross-polarization (CP) in the manner of Hartmann and Hahn between <sup>1</sup>H and any other X nucleus [1]. Unlike most conventional designs, the tuning and matching circuits are immersed in superfluid helium at temperatures down to 1.2 K. Intense radio-frequency (RF) fields with amplitudes on the order of 83 kHz can be applied simultaneously to two nuclei using RF amplifiers with powers below 100 W. The system can operate at temperatures in a wide range between 1.2 and 300 K. In this probe, a horizontal solenoidal coil is used to generate radio frequency (RF) pulses with a homogeneous  $B_1$  magnetic field, as evidenced by nutation experiments. Such a coil is compatible with recent techniques for the rapid ejection of frozen bullets.

**References:** [1] S. Jannin, A. Bornet, S. Colombo, G. Bodenhausen, Low-temperature cross polarization in view of enhancing dissolution Dynamic Nuclear Polarization in NMR, Chemical Physics Letters, 517 (2011) 234-236.

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**Preparation and binding studies with the START Domain of the Ceramide Transfer Protein (CERT) via Nuclear Magnetic Resonance (NMR)**

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<sup>1</sup>University of Potsdam, <sup>2</sup>Humboldt-Universität zu Berlin

Ceramide, an important component in the metabolism of sphingolipids, plays a significant role in proliferation and apoptosis of cells.<sup>1</sup> De novo synthesis of ceramide takes place at the cytosolic surface of the endoplasmic reticulum (ER), and then ceramide is transferred to the Golgi apparatus for conversion into sphingomyelin and glucosphingolipids, mainly by non-vesicular trafficking. Non-vesicular transport of ceramide is carried out by the ceramide transfer protein (CERT) that consists of peptidic motifs and multiple domains. [1] The C-terminal (StAR)-related lipid transfer (START) domain is the most important domain, given the fact that it is capable of extracting and accommodating ceramide in its deep hydrophobic cavity. CERT could be an attractive pharmacological target because of its involvement in common pathological processes such as Alzheimer's disease, infectious diseases and cancer.[2] A well-known antagonist of CERT is N-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl) dodecanamide (HPA-12);[1] however, there is limited structure-activity relationship (SAR) data available.

In this study, we aim to explore the interaction between CERT and HPA-12 and HPA-12 analogs to establish SAR of this compound class by nuclear magnetic resonance spectroscopy (NMR) in order to improve the inhibitory activity of these ligands for a potential drug design.

Herein, we used an optimized expression and purification protocol to prepare an isotopically labeled START domain for getting an idea about the suitability of the protein of interest in receptor-based NMR experiments. The labeled START domain's monomer in the presence and absence of the ligand yielded promising results in initial 1H-15N HSQC and TROSY NMR spectra. As a next step, sequence specific resonance assignments will be established in order to characterize the binding site and binding mode of HPA-12 and its derivatives.

**References:** [1] HANADA, Kentaro, et al. *Nature*, 2003, 426.6968: 803. [2] LIU, Jiawang, et al. *Future medicinal chemistry*, 2013, 5.12: 1405-1421.

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### Artefact-free broadband 2D NMR for separation of quadrupolar and paramagnetic shift interactions

Rihards Aleksis, José Carvalho<sup>2</sup>, Aleksander Jaworski<sup>3</sup>, Andrew J. Pell<sup>4</sup>

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Deuterium (<sup>2</sup>H) NMR is well-suited for probing the structure and dynamics of materials. The study of <sup>2</sup>H via NMR has gained popularity owing to the abundance of hydrogen in materials and the ease to substitute with <sup>1</sup>H and most importantly due to favorable NMR properties of <sup>2</sup>H. <sup>2</sup>H has spin  $I = 1$ , and has a quadrupole moment, the interaction of which with the electric-field gradient (EFG) affords invaluable information about the local hydrogen environment and any motional processes.

However, in paramagnetic materials, the combination of paramagnetic shift anisotropy (SA), quadrupolar broadening and inhomogeneous broadening due to bulk magnetic susceptibility (BMS) leads to featureless spectra, hence impeding extraction of individual NMR parameters. This issue has been addressed by introducing static 2D experiments which separate and correlate the shift (including isotropic shift, SA and BMS effects) and first-order quadrupole interactions. To date, two elegant experiments have been proposed for separation of the interactions which also provide information about the relative orientation between the two tensors, however, both methods, among other drawbacks, suffer from major artefacts in the quadrupolar dimension [1,2].

Here we present two new pulse sequences for separating the shift and quadrupolar interactions, which incorporate short high-power adiabatic pulses (SHAPs) [3]. The new experiments yield artefact free spectra and achieve greater excitation bandwidth. Application of the pulse sequences has provided unprecedented structural information on local hydride environments of deuterated forms of the oxyhydride ion conductor  $\text{BaTiO}_{3-x}\text{H}_y$ . These experimental data were of sufficient quality to provide insight into the local hydride structure of these materials, with the aid of quantum chemical/density-functional theory (DFT) calculations.

**References:** [1] Antonijevic, S., et al. J. Chem. Phys. 122, 044312, 2005. [2] Walder, B. J., et al. J. Chem. Phys. 142, 014201, 2015. [3] Aleksis, R., et al. Solid State Nucl. Magn. Reson., Just Accepted.

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P008

Mechanism of S-nitrosation of human thioredoxin by NMR

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Human thioredoxin (Trx) is a major player in signal transduction that involves the post-translational modification S-nitrosation (SNO). The mechanism for Trx disulfide reduction activity is well-known and it occurs through the redox-active cysteines, Cys32 and Cys35. S-nitrosation activity is a gain of function in mammals, which involves 3 redox-inactive cysteines, Cys62, Cys69 and Cys73, that are absent in basal eukaryotes and prokaryotes. We used NMR and several cysteine-mutants of Trx to describe the reactivity of each cysteine in the nitrosative site and propose a detailed site-specific mechanism for Trx S-nitrosation. S-nitroso glutathione (GSNO) is the S-nitrosated derivative of glutathione and is the natural down-stream signaling of nitric oxide (NO) in the cell. Trx receives NO from GSNO and transfers it to specific cellular partners, regulating many important biological processes. To measure the reactivity to each cysteine, we expressed Cys62, Cys69 and Cys73-only mutants. Cys73 and Cys69 were the most reactive to GSNO, whereas Cys62 formed the most stable nitrosated state. The CB and HB chemical shifts of each nitrosated state was informative of the local environment of the SNO group, informing that Cys62 is more prone to a nucleophilic attack that leads to S-transnitrosation than the other two cysteines. This was confirmed when we expressed the Cys62/Cys69-only mutant, which enabled us to describe the thermodynamic cycle for the Trx S-nitrosation. We globally fit the data, proposing a new kinetic/thermodynamic mechanism for the pathway for SNO in Trx. Cys62-SNO is the active form for transferring the SNO group to another cellular partner. We will also provide structural features of the nitrosated mutants.

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P009

A portable system for single-sided EPR Spectroscopy  
Belal Alnajjar, Jens Anders

**Abstract:** We present the design of a portable one-sided low cost system for EPR applications that uses a new shimming approach to achieve the required homogeneity. The proposed permanent magnet offers an open geometry enabling non-destructive EPR measurements on arbitrarily large objects. It has been designed, simulated and optimized using the finite element method. The system is based on four NdFeB magnetic blocks of size (50 x 40 x 30 mm<sup>3</sup>), mounted on an iron yoke. All four blocks are magnetically polarized normally to the iron yoke plane using two magnet groups with antiparallel orientation. This arrangement of magnetic blocks generates an external magnetic field parallel to the array surface. To improve the homogeneity of the magnetic field in the most sensitive volume, the air gaps between the magnetic blocks and the distance to the sensitive volume are optimized using a genetic algorithm. Moreover, instead of adding a pole piece or some additional magnetic shim units, the magnetic blocks themselves are used to improve the magnetic field homogeneity. In this approach, each main magnetic block is divided into smaller subunits that can be horizontally and vertically adjusted to provide a mechanical shimming. Here, to obtain a first order shim, the displacement of each block from the initial equidistant design has been optimized by applying a pattern search method. Using this approach, we were able to improve the magnetic field homogeneity by approximately two orders of magnitude. In addition to the single-sided permanent magnets, the proposed system also includes an EPR-on-a-chip detector that incorporates an array of oscillator-based EPR sensors with a sensitive volume of (1 x 1 x 0.4 mm<sup>3</sup>). The manufactured magnet achieves a homogeneity of 10<sup>-5</sup> over the sensitive region of the sensor.

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**P010**

**A comprehensive 2D NMR study on photoreceptor protein  
AnPixJ\_GAF2 as an example for red-green CBCRs**  
Susanne Altmayer, Chen Song, Wolfgang Gärtner, Jörg Matysik

Cyanobacteriochromes (CBCRs), proteins responsible for detection of light in cyanobacteria, are special not only because of their comparatively simple architecture, but also because they show an astonishing broad spectral variability. Like structurally related canonical phytochromes CBCRs use bilin molecules to absorb the light, but instead of the PAS-GAF-PHY tridomain which is necessary in phytochromes, for CBCRs a single GAF domain is sufficient for covalently binding the chromophore and maintaining the photochemistry. Phytochromes only absorb red and far-red light, but absorption spectra of CBCRs range from UV light to the far-red region.

The molecular explanation for this difference in absorption properties and the factors controlling electronic transition energies in bilin photoreceptors are still only marginally understood and there are several different models discussed. NMR is a well-suited technique to elucidate the regulating factors by giving information about the structure and the local electron density as well as on the charge distribution.

In our work we focus on the GAF2 domain of AnPixJ, a red-green photoreceptor from *Anabaena PCC7120*. AnPixJ\_GAF2 is one of the best studied CBCRs so far. Recently, our group succeeded in measuring 1D NMR spectra of AnPixJ\_GAF2, in-vitro assembled with <sup>13</sup>C-,<sup>15</sup>N-labelled Phycocyanobilin (PCB), in the resting state as well as in the active state and compared them to the corresponding spectra of phytochrome Cph1.[1,2,3] This project aims at pursuing 2D NMR measurements like <sup>13</sup>C-<sup>13</sup>C DARR and <sup>13</sup>C-<sup>1</sup>H MELODI measurements of resting and active state of a similarly labelled sample.

**References:** [1] Song C et al. (2015) J Phys Chem B. 30, 9688-9695. [2] Song C et al. (2015) Biochemistry. 54, 5839-5848. [3] Scarbath-Evers L et al. (2017) Phys Chem Chem Phys. 19, 13882-13894.

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## P011

## Speeding up the DNP acquisition of half-integer quadrupolar nuclei

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We have optimized the transfer of magnetization between  $^1\text{H}$  that can be utilized in indirect DNP (Dynamic Nuclear Polarization) experiments. Three different sequences for such a transfer exist presently: CPMAS, PRESTO (Phase-shifted Recoupling Effects a Smooth Transfer of Order) and D-RINEPT-SR4<sup>2</sup><sub>1</sub> (Dipolar-based Refocused-INEPT with SR4<sup>2</sup><sub>1</sub> recoupling). CPMAS is not a robust method due to the spin-locking of the quadrupole magnetization. PRESTO is inefficient for long distances and not robust to rf-field, CSA and offset. D-RINEPT-SR4<sup>2</sup><sub>1</sub> works well at ultra-fast MAS, but not at the moderate spinning speeds encountered with DNP ( $\nu_R = 10\text{--}15\text{ kHz}$ ). This small efficiency is mainly associated to the large losses related to  $^1\text{H}$ - $^1\text{H}$  interactions. We have introduced three changes in this D-RINEPT scheme that have scaled up by 16 its efficiency.

We show on  $\gamma$ -alumina that short distances  $^1\text{H}$  -  $^{27}\text{Al}$  DNP transfers obtained with this new sequence, are more efficient than with PRESTO and CPMAS. In the case of transfers with long-distances and/or low- $\gamma$  nuclei, this new sequence is much more efficient than PRESTO and direct DNP, and as example it multiplies by 3.9 the signal of  $^{17}\text{O}$  for Al-O-Al species of  $\gamma$ -alumina and by 5.5 that of  $^{95}\text{Mo}$  for  $\text{MoO}_3$  supported on  $\text{TiO}_2$ , which means a gain in time of 15 and 30 for same S/N, respectively. On this unlabeled sample, we have analyzed in details its surface structure from the point of view of  $^{17}\text{O}$ ,  $^{95}\text{Mo}$  and  $^{47,49}\text{Ti}$ .

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P012

CMOS integrated injection locked VCO arrays for optically hyperpolarized ESR detection in diamond

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Recently, the proposal and concept development of using chip-integrated voltage-controlled oscillator for electron spin detection has gained significant attention in the research community. Being at the same time highly sensitive and compact in size, these ESR-on-a-chip detectors are ideally suited for next generation portable ESR spectrometer [1]. Moreover, thanks to the fast electronic adjustability of both the resonant frequency of the embedded LC tank resonator and the frequency of oscillation the VCO-based approach allows for very fast rapid-scan [2] and even pulsed ESR experiment. Finally, the VCO-based detectors can be easily extended to arrays of injection locked arrays of VCOs, greatly improving the concentration sensitivity while maintaining a low complexity in the readout of the joint array frequency [3]. In the proposed talk, we extend the application range of the VCO-based approach to sensing the electron spin of NV-centers in diamond. In our experiments, we used optical pumping with laser powers up to 1 W to hyperpolarize the NV-centers and observed the growing ESR signal with increasing laser power. These preliminary results clearly show the potential of arrays of injection locked VCOs for dynamic nuclear polarization (DNP).

**References:** [1] J. Handwerker et al., “A 14 GHz battery-operated point-of-care ESR spectrometer based on a 0.13  $\mu\text{m}$  CMOS ASIC”, ISSCC 2016, pp. 476- 477. [2] A. Chu, et al., VCO-based ESR-on-a-chip as a tool for low-cost, high-sensitivity food quality control, BioCAS, 2017. [3] A. Chu et al., “An 8-channel 13 GHz ESR-on-a-Chip injection-locked vco-array achieving 200  $\mu\text{M}$ -concentration sensitivity,” ISSCC 2018, pp. 354-356.

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## P013

### Structural and Dynamic Studies of FKBP-12 from Microorganisms: A Biological Target for Drug Design

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Microbial infections remain a challenge for humankind with considerable impact on health care system of several countries due to its elevated mortality rate. The rising resistance and decrease of development of new agents has turned conventional therapy ineffective. In this way, the research of more effective drugs and targets for these diseases are essential. FKBP12 (peptidyl-prolyl cis-trans isomerase) enzyme was chosen as a target for such studies. Although human ortholog is already used as a target for compounds, this protein is also present in numerous disease-causing microorganisms, and differ by about 40% in their primary sequence compared to the first one. In this way, such enzymes are validated as biological targets. Initially, FKBP12 proteins from *Trypanosoma brucei* (TbFKBP12), *Trypanosoma cruzi* (TcFKBP12) and *Mycobacterium tuberculosis* (MtFKBP12) were selected. They were expressed in *E. coli* BL21(DE3), induced with IPTG and purified with steps of affinity and size-exclusion chromatography. The MtFKBP12 protein was isotopically labelled with <sup>15</sup>N and <sup>13</sup>C and had all experiments required for NMR assignment performed, besides NOESY spatial correlation experiments. Lists containing assigned NOEs, predicted dihedral constraints and hydrogen bonds were used as input of the structural calculation using Aria2.2/CNS1.2. Several calculation cycles were performed using standard simulated annealing protocols. Finally, the 20 best conformers were refined by dynamics in water. It was possible to observe that MtFKBP12 structure is similar with the other proteins of this family with a divergent N-terminal portion that shows little structural convergence. The backbone dynamics of MtFKBP12 were investigated measuring the relaxation parameters: <sup>15</sup>N R<sub>1</sub> and R<sub>2</sub> relaxation experiments and <sup>1</sup>H-<sup>15</sup>N heteronuclear NOE. Titration experiments with N-succinyl-AXPF- $\rho$ -nitroanilide (X: leucine or alanine) were performed with TbFKBP12 and MtFKBP12 proteins. Perspectives include structural and dynamics studies of the TcFKBP12 protein and identification of low affinity ligands that could subsequently become leading compounds for drug design.

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## P014

## Advanced NMR for Industrial Applications: Structure, Porosity, and Acidity

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The zeolites are well known for the decades for their excellent properties as adsorbents and catalysts. The large-scale application of these materials is ensured by petroleum chemistry as a part of fuel cracking catalysts (FCC) for gasoline production and hydrocracking catalysts (HCK) for diesel due to their thermal stability, strong acidity, and developed specific surface area. A main drawback of all zeolite materials is the mass-transfer restriction due to very small channel sizes (usually under 1.5 nm). To overcome these limitations and enhanced high temperature stability a set of postsynthesis treatments, such as steaming, dealumination and/or desilication, have been proved to be convenient and efficient.

In this work, we propose a generalized NMR approach for a comprehensive structural study of Y type zeolites by probing their porosity and acidity. Conventional <sup>29</sup>Si and <sup>27</sup>Al MAS NMR methods provide a structural overview on framework and extraframework Al species, and Si/Al ratio. Moreover, the surface OH groups can be probed without multistage complex pretreatment due to recently introduced dipolar filtering technique coupled with a spin echo [1]. The zeolite pore size distribution (PSD) and its porosity can be characterized by NMR cryoporometry [2], which is well adapted to cover large pore range from micropores (below 2 nm) to small macropores (up to 2 µm). The Brønsted acid sites, i.e. their strength and amount, are quantified by probe molecule <sup>31</sup>P NMR, which is complementary technique for routine temperature desorption of ammonia [3].

The generalized approach aids to provide some new clues in catalyst development by means of commercially available instruments and facilely implementable techniques. The discussed methods can be adapted to other porous materials that is advantageous for materials screening.

**References:** [1] Andreev and Livadaris, J Phys Chem C, 2017,121(26): p.14108. [2] Webber, Prog Nuc Magn Res Spec, 2010,56(1): p.78-93. [3] Lang et.al., ChemCatChem, 2016,8(12): p.2031-2036.

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P015

Observation of Intermolecular Transferred-NOE interactions in a 40 kDa Complex:  
MAP Kinase p38 $\alpha$  interactions with its recognition motif

Sabine Akabayov<sup>\*,1</sup>, Jacob Anglister<sup>\*,1</sup>, Suresh Kumar<sup>1</sup>,  
Naama Kessler<sup>1</sup>, Fred Naider<sup>2</sup>, Lewis Kay<sup>3</sup>

<sup>1</sup>Weizmann Institute, <sup>2</sup>City University of New York, <sup>3</sup>University of Toronto

NMR is a very powerful tool to study weak protein-ligand interactions exhibiting dissociation constants in the 1  $\mu$ M – 1 mM range. This weak binding is usually associated with fast ligand off-rates. One of the most widely used NMR technique to study such complexes is the measurement of chemical shift perturbation upon binding. This is being used to map the residues in the binding protein affected by ligand binding. However, this method is limited as it only provides information on which residues are possibly in the binding site but not on specific interactions with the ligand. It is our claim that transferred NOE measurements can be implemented to study intermolecular interactions for such complexes to obtain more informative structural data that can be used for the structure determination of these complexes.

Here we demonstrate the applicability of the intermolecular transferred NOE effect for studying intermolecular interactions between the MAP-Kinase p38 $\alpha$  and a kinase interaction motif (KIM) from the STEP phosphatase. The isotope-edited/isotope-filtered experiment was optimized to eliminate artifacts. At a 1:1 protein:peptide molar ratio the observed NOEs were weak and the signal-to-noise ratio was low. By increasing the molar ratio to 1:5 the signal-to-noise ratio improved by a factor of ~5 and many intermolecular NOEs could be clearly observed. Numerous interactions were observed between the methyl groups of Ile, Leu and Val of p38 $\alpha$  and protons of the STEP peptide. Additional intermolecular NOEs were detected using a uniformly <sup>13</sup>C/<sup>15</sup>N labeled p38 $\alpha$  sample and an unlabeled STEP KIM peptide, that together provide restraints to generate structures of the complex.

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## P016

Halogenated monoterpenes from South African Marine Algae *Plocamium* sp.

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South Africa's coastline, stretching more than 2500 km from the cold temperate coast west coast to the warm tropical environment of northern Kwazulu-Natal, has an exceptional environment with a high percentage of endemic species. SA's rich marine biodiversity therefore signifies the production of potent, biologically active, natural products (Davies-Coleman & Beukes, 2004). By 1995, 60% of the approved drugs and the new drug candidates for anti-cancer and anti-infective drugs were of natural origin (Grabley & Thiericke, 2000). These natural products have diverse structural scaffolds - opening new therapeutic approaches since they function as new probes. The supply of sufficient quantities for biological activity profiling, however, remains a major problem.

Algae are older than land plants and are a rich source of biologically active compounds (Stirk et al. 2003). Algae are classed into red, green and brown algae and the SA seaweed flora in the Indian Ocean is one of the most diverse regions in the world (Mattio et al. 2015). Chemical investigations of the red algae, e.g. *Plocamium*, have resulted in the isolation of many acyclic and cyclic halogenated monoterpenes containing multiple halogen atoms (Kladi et al. 2006). Incorporation of the halogens into the monoterpene skeleton is thought to be facilitated by a vanadium haloperoxidase enzyme (Vaillancourt et al. 2006). The main difficulties encountered in the structural elucidation of these monoterpenes (Mann et al. 2007; Afolayan et al. 2009), includes the stereochemical assignment of the halogenated chiral centres and the limited quantities isolated; the marine environment is protected, and supply is limited. Confirmation of these structures is often only accomplished through laborious synthetic means.

In this report, we study the NMR spectra of several polyhalogenated monoterpenes from South African red algae using a variety of NMR experiments to help determine any trends in the stereochemistry of the halogenated chiral centres.

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**P017**

**Reactivity of CO<sub>2</sub> with Aqueous Choline-Based Ionic Liquids and Boric Acid Probed by <sup>13</sup>C/<sup>15</sup>N Solid-State NMR Spectroscopy**

Andrei Filippov, Faiz Ullah Shah, Oleg Antzutkin\*

*Luleå University of Technology*

CO<sub>2</sub> absorption in a series of choline-based ionic liquids is investigated using solid-state <sup>13</sup>C and <sup>15</sup>N MAS NMR spectroscopy [1]. Natural abundance and <sup>13</sup>C enriched CO<sub>2</sub> gas was purged through 50 wt % aqueous solutions of alkyl(2-hydroxyethyl)ammonium threonine, [N1,1,n,2OH][Threo], (alkyl = butyl, pentyl and hexyl) and pentyl(2-hydroxyethyl)ammonium taurine [N1,1,5,2OH][Tau]. The process of CO<sub>2</sub> absorption results in precipitation of a solid sediment, which stays in equilibrium with the liquid phase. Upon degassing of the sample, the sediment is dissolved back into the IL-aqueous phase. Solid state <sup>13</sup>C and <sup>15</sup>N MAS NMR data suggest that the solid sediment is composed of neutral threonine (or taurine) in the zwitterionic forms and the liquid phase contained the products of reactions between the ionic liquids and CO<sub>2</sub> molecules. With admixing of boric acid to the reaction mixture a few additional reversible equilibria are detected. A plausible mechanism for formation of the solid sediments and the reaction products in liquid phases is suggested.

These findings are important for the scientific community working with aqueous ionic liquids as green solvents in various application. CO<sub>2</sub> can be readily absorbed by aqueous amino acid based ionic liquids from the atmosphere with reactions leading to formation of precipitated amino acids. Such systems may be useful in practical applications, in which absorption/desorption of CO<sub>2</sub> and recycling of ILs can easily be controlled by regulating of both pressure and temperature.

The financial support from the Swedish Foundation for Strategic Research (project No. EM16-003, 2018-2022) is acknowledged. The Kempe Foundation in memory of J. C. and Seth M. Kempe is acknowledged for the financial support (grant No. JCK-1707). Also the financial support from the Norrbotten Research Council (NoFo) (grant No. 17-103) is acknowledged for supporting this work.

**Reference:** [1] A. Filippov, O.N. Antzutkin, F.U. Shah, Journal of Molecular Liquids (2019) accepted for publication.

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P018

**Dynamics of the Membrane-anchored Autophagy-related Protein GABARAP**  
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The 117-residue GABA<sub>A</sub> receptor-associated protein (GABARAP) from *H. sapiens* is a versatile key regulator in autophagy. GABARAP is found as a soluble protein in the cytosol, as well as anchored to autophagosomal membranes during autophagosome formation and maturation. Anchoring to phospholipid membranes is achieved via enzymatic C-terminal lipid-conjugation of GABARAP, but the molecular mechanism how lipidation and oligomerization of GABARAP mediate autophagosomal development is still poorly understood. To gain insight, we investigated GABARAP with the membrane by NMR spectroscopy in concert with molecular dynamics (MD) simulations. As a membrane environment suitable for solution NMR spectroscopy, we prepared nanodisc-anchored GABARAP. To this end, the mutant GABARAP G116C/Δ117 was lipidated at the C-terminus using 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl) butyramide] (MPB-PE) and anchored to DMPC-nanodiscs with MSP1D1Δ5 scaffold proteins. Significant backbone amide chemical shift changes upon anchoring to the nanodisc are observed for amino acid residues at the C-terminal site of lipidation and in close spatial proximity (G116, Y115, G82), while the chemical shifts of the N-terminal residues are very similar to those of free GABARAP in solution. The backbone amide chemical shifts for K38 are also modulated as a consequence of direct interaction between this residue and the lipid membrane, as revealed by MD simulations. The pico- to nanosecond dynamics of the protein was determined experimentally from <sup>15</sup>N NMR relaxation spectroscopy of [U-<sup>2</sup>H, <sup>13</sup>C, <sup>15</sup>N] GABARAP anchored to DMPC-nanodiscs. Comparison with the dynamics of free GABARAP obtained in an integrated NMR, fluorescence, and MD study reported previously [1] corroborates the observation that membrane anchoring affects the C-terminus and the loop around G82 while leaving the N-terminal dynamics unaffected, thereby leaving the N-terminus free to associate with other membrane-anchored GABARAP molecules to mediate tethering of autophagosomal membranes, which will be studied in our future work.

**References:** [1] C. Möckel et al., J. Phys. Chem. B123, 1453-1480 (2019).

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P019

### A New Method for the Reliable Detection of C-13 Multiplets of Fluorine Containing Compounds

Dimitris Argyropoulos<sup>\*1</sup>, Sergey Golotvin<sup>2</sup>, Rostislav Pol<sup>3</sup>, Vladimir Mikhailenko<sup>3</sup>

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<sup>2</sup>Advanced Chemistry Development (ACD/Labs) Ltd,

<sup>3</sup>Advanced Chemistry Development (ACD/Labs)

In modern organic and medicinal chemistry, fluorine is commonly used to enhance the chemical properties of molecules in many desirable ways: it may delay the metabolism of the molecule, reduce the toxicity of aromatic groups, or increase the bioavailability. As a result, it is estimated that more than 20% of commercial pharmaceutical APIs and 30% of agrochemicals contain at least one fluorine atom.

In contrast to these benefits, the <sup>13</sup>C NMR spectra of fluorinated organic compounds are highly susceptible to interpretation errors. This is because <sup>13</sup>C spectra are commonly recorded using only <sup>1</sup>H broadband decoupling and the <sup>13</sup>C-<sup>19</sup>F couplings are still present. The <sup>13</sup>C-<sup>19</sup>F coupling constants can be very large (up to 250 Hz or more), which may result in multiplets severely overlapping with other peaks in the spectrum, amongst other problems. To mitigate this, it is possible to record <sup>13</sup>C spectra broadband decoupled from both <sup>1</sup>H and <sup>19</sup>F but this requires specialized NMR probes and decoupling techniques. Consequently, this approach is not considered practical for general, routine use.

Here we present an analysis method that reliably peak-picks and identifies multiplets in the <sup>13</sup>C spectra of organic compounds. This technique is based on accurately predicting the <sup>19</sup>F coupled <sup>13</sup>C spectrum of the proposed compound. Following prediction, we examine the regions of the experimental spectrum where the <sup>19</sup>F coupled carbons are expected in order to identify multiplets by peak position and the agreement in the predicted and observed coupling constants, in essence pattern-matching the experimental to the predicted spectrum. Provisions are taken if only part of a multiplet is observed. We show that regardless of whether the final results contain multiple, overlapping multiplets, the expected carbon resonances are reliably identified and assigned for each spectrum. Typical examples from common fluorine containing compounds are shown.

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## P020

**CARD domain of rat RIP2 kinase: production and structural insights**Sergey Goncharuk<sup>1</sup>, Liliya Artemieva<sup>\*,2</sup>, Konstantin Mineev<sup>1</sup>, Alexander Arseniev<sup>3</sup><sup>1</sup>*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow,**Russia, <sup>2</sup>Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow,**Russia, Moscow Institute of Physics and Technology, Dolgoprudnyi, Russia,*<sup>3</sup>*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia*

RIP2 is an intracellular adaptor protein that is essential for several reaction cascades. RIP2 is recruited when the bacterial fragments activate the innate immune pattern recognition receptors NOD2 and NOD1. That leads to NF- $\kappa$ B associated activation of proinflammatory cytokine production. Also, RIP2 involved in signal transduction by transmembrane neurotrophin receptor P75. That pathway is responsible for the regulation of neurons cell cycle.

Protein RIP2 has a two-domain structure: the N-terminal part possesses the catalytic kinase activity, while the C-terminal part is a caspase recruitment domain (CARD). CARD is essential for the interactions of RIP2 with its partner receptors, both NODs, and p75.

For the investigation of adaptor protein RIP2 functioning mechanism and protein-protein interactions, we focused on the structure determination of the CARD of rat RIP2 kinase. We established the clear and reproducible protocol of <sup>13</sup>C/<sup>15</sup>N-labeled RIP2CARD production in E.coli. Several expression constructs with N-terminal tags were tested, but all cases showed a sufficient amount of RIP2CARD in inclusion bodies. That led us to the development of the refolding protocol. The concentration of folded RIP2CARD was achieved as high as 10 mg/ml. We noticed peculiar ionic strength and pH-dependent behavior of the domain. The native state of the RIP2CARD was reached in the buffer with pH 4.2. At that pH level, RIP2CARD protein has a typical fold of protein from the death domain superfamily. Such an important role of pH and ionic strength on the protein behavior may be explained with a great amount of charged side chains. Comparison of rat RIP2CARD with human RIP2CARD and other proteins with common CARD fold several important differences. Conducted analysis of RIP2CARD behavior and structural specifics provides an opportunity to study further protein-protein interactions and rational drug design.

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P021

Decoherence Simulations of a Central Spin in Ordered Structures

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Some quantum computation schemes rely on having ordered structures of qubits with nano-scale positioning accuracy to perform correctly. One natural candidate for such a system of qubits is an ordered structure of electron spins in a solid-state sample. Such systems (e.g. NV<sup>-</sup> centers in diamond, and phosphorus doped <sup>28</sup>Si) exist in unordered forms, and have been shown to have favorable properties in terms of long coherence times. However, ordered systems of electron spins in solid state are, to date, unattainable. Therefore, the coherence properties of such ordered structures can not be probed experimentally, yet their behavior can be expected to be quite different than solid state qubits in unordered structures. To learn more about the decoherence properties of ordered spin structures, we turn to full spin Hamiltonian simulations of such systems, and investigate their dynamics. We first simulate unordered spin systems, and compare our simulation results with measurements to show that the simulation indeed captures real spin dynamics. We then move to simulations of ordered systems, and investigate their decoherence properties under various conditions.

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P022

**Ultrashort Broadband Cooperative Pulses for  
Multidimensional Biomolecular NMR Experiments**

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The continuous development of high-field NMR spectrometers greatly enhances the sensitivity and resolution of NMR experiments and enables application to ever more challenging and complex biomolecular systems. Next to technological hurdles of designing ultra-high-field magnets, spectroscopic challenges inevitable emerge. One critical aspect is the requirement for rf pulses with large bandwidths for excitation, refocusing, inversion and decoupling, while being constrained by an upper limit of applicable rf power. The rectangular pulse, which is the most frequently used pulse shape in NMR spectroscopy, has limited amplitude and phase properties at external magnetic fields largely exceeding 14 T. Here, we introduce a pair of numerically optimized broadband excitation pulses [1], which have been designed based on the cooperativity principle. We demonstrate by simulation and experiment, that these pulses, coined COOP pulses, cover an excitation bandwidth of 70 kHz at 100% amplitude with a maximum rf field of 10 kHz. In addition, the COOP pulses show small offset-dependent phase errors on the order of  $<1^\circ$  and are robust within  $\pm 5\%$  B1 inhomogeneity. After experimental verification of the optimized pulses, we successfully implemented the COOP pulses in several standard biomolecular NMR experiments. The novel COOP pulses overcome the current and future bandwidth problems at high magnetic fields, even beyond 28 T.

**References:** [1] S. Asami, W. Kallies, J.C. Günther, M. Stavropoulou, S.J. Glaser, M. Sattler, *Angew. Chem.* 130, 14706-14710 (2018).

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## P023

### Surprising, Rapid, Room-Temperature Lability of Zeolite Frameworks Revealed by $^{17}\text{O}$ Solid-State NMR Spectroscopy

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<sup>1</sup>University of St Andrews, <sup>2</sup>BP Chemicals Ltd, <sup>3</sup>BP Chemicals Ltd.

Zeolites are microporous aluminosilicates that have widespread industrial applications as heterogeneous catalysts, molecular sieves and ion exchange materials. The incorporation of trivalent aluminium into the tetrahedral silicate framework is charged balanced by the presence of organic or alkali metal cations or by the protonation of bridging oxygen sites. In the latter case, the resulting Brønsted acid sites are thought to give rise to many of the useful catalytic properties of zeolites.

Solid-state NMR spectroscopy is an excellent probe of the atomic-level environment, and provides information on the local structure, disorder, dynamics and chemical reactivity of zeolites. Despite making up approximately 2/3 of the zeolite framework, NMR spectroscopy of oxygen is rarely used in the characterisation of zeolites, owing to the very low natural abundance (0.037 %), moderate gyromagnetic ratio and quadrupolar ( $I = 5/2$ ) nature of  $^{17}\text{O}$ , the only NMR-active isotope. Furthermore, the high cost of isotopically-enriched reagents, and therefore the need for enrichment procedures that are cost-effective and atom-efficient, poses further challenges. Here, we demonstrate a novel, facile and room-temperature route to  $^{17}\text{O}$  enrichment of zeolites, which exploits the surprising lability and dynamic behaviour of the zeolite framework, even in the absence of acid. We demonstrate, using  $^{17}\text{O}$  MAS and MQMAS experiments, that  $^{17}\text{O}$  enrichment is possible for a range of zeolites (including mordenite (MOR), ferrierite (FER) and chabazite (CHA)), by creating a simple slurry of zeolite and small volumes (i.e., 50  $\mu\text{L}$ ) of  $\text{H}_2^{17}\text{O}(\text{l})$ . The facile enrichment of all O species in the framework (i.e., both Si-O-Al and Si-O-Si) occurs on a surprisingly rapid timescale and without any framework degradation (as demonstrated by  $^{29}\text{Si}$  and  $^{27}\text{Al}$  NMR), challenging the perceived stability of zeolite frameworks at room temperature, and raising interesting and important questions about their application in catalysis and ion exchange.

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P024

High-frequency EPR-ESE non-Kramers Tb<sup>3+</sup> centers in garnet crystals  
Roman Babunts\*, Yulia Uspenskaya, Elena Edinach, Alexander Gurin,  
Hike Asatryan, Andrey Badalyan, Nikolai Romanov, Pavel Baranov  
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Techniques of high-frequency electron paramagnetic resonance (EPR) and electron spin echo (ESE) were applied for investigation of non-Kramers Tb<sup>3+</sup> centers in single crystals of yttrium aluminum garnet, Y<sub>3</sub>Al<sub>5</sub>O<sub>12</sub> (YAG). The EPR-ESE techniques were created on the base of highly stable microwave bridges, quasisonant system for supplying microwave power to a sample and a closed-circle magneto-optical cryostat, placed on an optical table. It operates in the near-terahertz region (0.094 and 0.130 THz) both in continuous wave and ESE modes. An increase in the operating frequency of the spectrometer allows us to study spin systems with large initial splitting, which are not available for measurements in low frequency EPR range. Continuous wave and ESE-detected EPR spectra of non-Kramers Tb<sup>3+</sup> centers were recorded in YAG crystals at temperature range 1.5-40 K.

The EPR spectra of several types of Tb<sup>3+</sup> centers with close parameters were found, which fit a spin Hamiltonian  $H=g||u_B \cos\theta S_z + \Delta_x S_x + \Delta_y S_y + AS_z I_z$ . The lowest lying state is the MS= ±6 doublet with splitting of  $\Delta = (\Delta_x^2 + \Delta_y^2)^{1/2}$ . The most intensive signals have parameters  $g||=15.8\pm0.2$ ,  $g_{\text{perp}}=0$ ,  $\Delta_1=2.705\pm0.005\text{ cm}^{-1}$ ,  $A=0.197\pm0.005\text{ cm}^{-1}$ , ground state doublet splitting of other centers:  $\Delta_2=3.124\text{ cm}^{-1}$  and  $\Delta_3=3.857\text{ cm}^{-1}$ . The energy splitting  $\Delta_2$  is very close to the energy of 94 GHz microwave quanta and strong Echo signals were observed at zero magnetic field, coinciding with the hyperfine component for interaction with the Tb nucleus.

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## P025

## NMR of fullerenes and endofullerenes

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Endofullerenes are supramolecular complexes where one small (endohedral) atom/molecule is confined within a bigger, fullerene, molecule which acts as an enclosing cage.[1] Endofullerenes offer an ideal particle in a box system to observe quantum mechanical effects.

C<sub>60</sub> is an irregular truncated icosahedron, with two bond types in 2:1 ratio.[2] Ever since its discovery, the solution state <sup>13</sup>C NMR of C<sub>60</sub> has always been reported as a single peak. Which reflects its high degree of symmetry with all 60 carbon atoms magnetically equivalent. However, we have observed this is actually not the case. Upfield from the main C<sub>60</sub> peak there are two additional satellites in relative ratio 2:1. We have shown that these peaks arise from the <sup>13</sup>C-<sup>13</sup>C secondary isotope shift effect. This brings further proof to the structure of C<sub>60</sub> with just solution state NMR.

When C<sub>60</sub> is filled with an edohedral species, the C<sub>60</sub> <sup>13</sup>C NMR peak is shifted downfield depending on the species. We have shown this shift to be dependent on temperature. The shift seems to reflect the “pressure” a single atom/molecule exerts on its container (C<sub>60</sub>).

We show the NMR characterisation of CH<sub>4</sub>@C<sub>60</sub> in solution and solid state (MAS).[3] We expect to observe a quantum rotor induced polarization phenomena of endohedral <sup>13</sup>CH<sub>4</sub>, experiment to be done.

We also present a <sup>3</sup>He NMR study. The room temperature static solid state <sup>3</sup>He NMR spectrum of <sup>3</sup>He@C<sub>60</sub> contains one Lorentzian peak a few kHz wide. <sup>3</sup>He relaxation in the solid seems to be much faster than in solution. Relaxation and decoupling (MAS and/or static) experiments are planned, measuring from room temperature down to cryogenic liquid helium.

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Signal Pattern Plot: A simple tool for time-dependent metabolomics studies by <sup>1</sup>H NMR Spectroscopy

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The study of metabolic changes (metabolomics) is a rapidly growing field of research. Starting with the detection of potential disease markers in the 1970's, applications reach from toxicological and / or pharmacological studies to the field of food analysis. The metabolome is the last step in the so-called omics cascade where it reacts most sensitive to changes in genome, transcriptome, proteome or metabolome level of an organism. A major problem in food processing is the contamination or infestation with mold. Molds or their metabolites can lead to allergic reactions in the human organism. Furthermore, they can produce mycotoxins, which are toxic and carcinogenic. These mycotoxins are problematic because they are stable to heat and acids and they can remain stable even when processing the affected food. It is estimated that about 50% of all grains are contaminated with detectable mycotoxin concentrations. Their detection in food usually applies LC/MS and is dependent on the chemical complexity of targeted mycotoxins.

We used the metabolomics approach and associated advantages, e.g. small sample amount and high sample throughput, to detect significant changes in metabolite level due to mold formation. In this study hazelnuts were infected with eight different strains of fungus to observe changes in the metabolome over a period of two weeks. To visualize these changes we developed a signal pattern plot showing alteration (trend) of individual signals in the <sup>1</sup>H NMR spectrum over the observation time. The sign and intensity of alteration is color coded and allows simple interpretation of the signal pattern plot. Each species investigated generates an individual color pattern and therefore exhibits specific metabolic changes. Signals that change upon infection compared a to reference sample are easily identified and allow the assignment of chemical/biological markers.

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P027

**Probing the Solution Structure of the E. coli Multidrug Transporter MdfA using DEER Distance Measurements with Nitroxide and Gd(III) Spin Labels**  
Thorsten Bahrenberg<sup>\*,1</sup>, Eliane H. Yardeni<sup>1</sup>, Richard Stein<sup>2</sup>, Smriti Mishra<sup>2</sup>, Elia Zomot<sup>1</sup>, Bim Graham<sup>3</sup>, Kellie L. Tuck<sup>3</sup>, Thomas Huber<sup>3</sup>, Eitan Bibi<sup>1</sup>, Hassane S. Mchaourab<sup>2</sup>, Daniella Goldfarb<sup>1</sup>  
<sup>1</sup>Weizmann Institute of Science, <sup>2</sup>Vanderbilt University, <sup>3</sup>Monash University

Methodological and technological advances in EPR spectroscopy have enabled novel insight into the structural and dynamic aspects of spin-labeled integral membrane proteins. In addition to an extensive toolkit of EPR methods, multiple spin labels have been developed and utilized, among them Gd(III)-chelates which offer high sensitivity at high magnetic fields. Here, we applied a dual labeling approach in conjunction with Q-band (nitroxide) and W-band (Gd(III)) double electron-electron resonance (DEER) measurements to characterize the solution structure of the detergent-solubilized multidrug transporter MdfA from E. coli. Our results identify highly flexible regions of MdfA, which may play an important role in its functional dynamics. Comparison of distance distributions on the periplasm with those calculated using inward- and outward-facing crystal structures of MdfA show that, in detergent micelles, the protein adopts a predominantly outward-facing conformation, although more closed than the crystal structure. Parallel DEER measurements with the two types of labels led to similar distance distributions, demonstrating the feasibility of using W-band spectroscopy with a Gd(III) probe.

In a second step, we demonstrate the successful reconstitution of Gd(III)-labeled MdfA into Nanodiscs on a couple of double mutants. After successful reconstitution, the main distance between the spin labels diminishes for mutants labeled on the periplasm, but not on the cytoplasm. We assume that the conformation of MdfA might be dependent on its environment; however, a different relative orientation of the spin labels might as well be a possible explanation for this observation. Additionally, binding of a substrate induces a large conformational change in one mutant.

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## P028

**EPR spectroscopy of structural phase transition in  $\text{Mn}^{2+}$  doped  
 $[(\text{CH}_3)_2\text{NH}_2][\text{Cd}(\text{N}_3)_3]$  hybrid perovskite framework**

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Hybrid perovskite frameworks are an emerging family of materials with interesting dielectric, ferroelectric and magnetic properties which are useful in such applications as charge storage and optoelectronic devices. These porous frameworks are composed of metallic centers joined together by organic linkers. Each such pore confines a single molecular cation. The majority of such frameworks exhibit structural phase transitions followed by the cation ordering and framework deformation [1].

A powerful method to study structural changes in hybrid frameworks is EPR spectroscopy [2]. Here we employed CW X-band and Q-band EPR to investigate the structural phase transition in  $[(\text{CH}_3)_2\text{NH}_2][\text{Cd}(\text{N}_3)_3]$  (DMACd) azide framework doped with 0.05 mol% paramagnetic  $\text{Mn}^{2+}$  ions. The temperature dependent CW EPR spectra of DMACd: $\text{Mn}^{2+}$  powder show typical patterns of  $\text{Mn}^{2+}$  ions. Upon cooling, spectra exhibit a drastic change at the phase transition temperature of 178 K demonstrating that  $\text{Mn}^{2+}$  ions are susceptible to the transition. A sudden anomalous increase of EPR linewidth at 178 K followed by a maximum at 174 K indicates a first-order character of the phase transition.

To simulate the experimental CW EPR spectra of DMACd: $\text{Mn}^{2+}$ , the spin Hamiltonian with terms describing the electron Zeeman, hyperfine interactions and zero-field splitting was used. The determined value of the isotropic hyperfine constant matches the value of  $\text{Mn}^{2+}$  centers in  $\text{MnN}_6$  octahedra [3] confirming that these ions have successfully replaced  $\text{Cd}^{2+}$  centers. The temperature dependencies of the axial  $D$  and orthorhombic  $E$  zero-field splitting parameters, measuring the distortion of  $\text{MnN}_6$  octahedra, exhibit a sharp increase at 178 K, which confirms a strong first-order character of the structural phase transition.

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P029

### Conformational thermodynamics of the heterodimeric ABC exporter TmrAB observed with PELDOR

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Several human diseases result from malfunctions of ABC systems. Many ABC exporters contain asymmetric nucleotide-binding sites (NBSs) and some of them are inhibited by the transported substrate.[1] To actively transport diverse chemically substrates across biological membranes, ATP-binding cassette (ABC) transport complexes use the energy of ATP binding and subsequent hydrolysis. In this work, we investigated the heterodimeric ABC exporter TmrAB[2,3] from *Thermus thermophilus* using pulsed electron-electron double resonance (PELDOR/DEER) spectroscopy. In the presence of ATP, TmrAB exists in an equilibrium between inward- and outward-facing conformations.[3] This equilibrium is modulated by changing the ATP concentration and thereby differences between the two asymmetric NBSs can be observed during the transition from the inward- to outward-facing conformation. By determining the temperature dependence of this conformational equilibrium, we investigated the thermodynamics underlying the energy coupling during ATP-induced conformational changes in TmrAB. Our results demonstrate that ATP-binding alone drives the global conformational switching to the outward-facing state and allows the determination of the entropy and enthalpy changes for this step. With this knowledge, the Gibbs free energy of this ATP induced transition can be calculated. This work reveals for the first time the feasibility to determine the conformational thermodynamics of a large membrane protein complex. This method could be a general approach for similar proteins.

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P030

NMR investigation of the mechanism of substrate entrance  
in the active site of ribose 5-phosphate isomerase (Rpi)

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Tuberculosis is one of the deadliest diseases, affecting approximately 10 million people in 2017. This classifies tuberculosis as epidemic in several countries and lead to an increasing number of multidrug-resistant strains. Thereby, the development of new drugs is essential to effective treatments. The ribose 5-phosphate isomerase (Rpi) catalyzes the interconversion of D-ribulose-5-phosphate and D-ribose-5-phosphate, a precursor of nucleotides and cofactors. There are two families of Rpi, called type A and type B. The RpiA is present in most eukaryotes, while RpiB is found, almost exclusively, in prokaryotes and some basal eukaryotes and fungi. Thus, the enzyme RpiB is a good target for drugs, since it is essential to cell growth and there is no human homologue. The purpose of this project is a better understanding of the substrate uptake mechanism of RpiB of *Mycobacterium tuberculosis* (MtRpiB) by a combination of NMR, molecular dynamics simulation and enzymatic inhibition. The heterologous enzyme was expressed in *Escherichia coli* and purified from cell lysate in a two-step process of nickel affinity and gel filtration chromatography. <sup>2</sup>H,<sup>13</sup>C,<sup>15</sup>N-triple-labeled MtRpiB was produced, NMR triple resonance experiments were acquired and the MtRpiB backbone resonances were assigned. Next, some screening experiments were performed and ligand candidates were selected through saturation transfer difference (STD). The results indicated strong interaction with mononucleotides and a decrease in the affinity in di- and trinucleotides. The chemical shift perturbation showed that AMP binds deeply at the active site, while ATP more superficially. NMR-derived docking and molecular dynamics simulation suggested that binding occurs in two steps. We propose a flipping mechanism, which the phosphate is essential for interaction and the nitrogenous base can change its orientation from a buried state to a solvent-exposed state. The data obtained by NMR provides a basis for future drug design.

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P031

pH-induced structural changes in Alzheimer’s amyloid fibril Aβ(1-42)

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Alzheimer’s disease (AD) is associated with deposition of misfolded aggregates of Amyloid-β (Aβ). The Aβ(1-42) peptide is the most aggregation prone compound in senile plaques of AD patients and more neurotoxic compared to the shorter peptide Aβ(1-40). Recently, high-resolution 3D structures of different polymorphic Aβ(1-42) fibril types have been determined by solid-state NMR spectroscopy as well as cryo-EM [1-3].

The structure of Aβ(1-42) fibrils grown at low pH differs substantially from fibrils grown at neutral pH, in particular, the full N-terminus is part of the rigid fibril core and forms two extended β-strands [3].

In the present study, we investigate the stability of these fibrils at higher pH values and high salt concentrations using solid-state NMR spectroscopy. We observed changes of several resonances concerning both chemical shifts and intensities.

We found that our Aβ(1-42) fibrils are unperturbed up to pH values of 4, while at higher pH values alterations mainly for N-terminal residues as well as the extreme C-terminus are observed. While electrostatic interactions like salt bridges are partly perturbed at higher pH values, the overall fold of the fibrils does not undergo major changes.

**References:** [1] Wälti, M.A., et al., Atomic-resolution structure of a disease-relevant Aβ(1-42) amyloid fibril. *Proceedings of the National Academy of Sciences of the United States of America*, 2016. 113(34): p. E4976-E4984. [2] Colvin, M.T., et al., Atomic Resolution Structure of Monomorphic Aβ42 Amyloid Fibrils. *Journal of the American Chemical Society*, 2016. 138(30): p. 9663-9674. [3] Gremer, L., et al., Fibril structure of amyloid-β(1-42) by cryo-electron microscopy. 2017. 358(6359): p. 116-119.

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## P032

### Chromophore conformation in Photointermediates of Channelrhodopsin-2 by DNP-enhanced MAS NMR

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Channelrhodopsin-2 (ChR2) is a light-gated ion channel[1] which over the last years has attracted considerable interest due to its unparalleled role in optogenetic applications[2]. However, despite considerable efforts, it has remained elusive how molecular events during the photocycle including the retinal trans-cis isomerization and the de/re-protonation of the Schiff base are coupled to the channel opening mechanism. Recently, we have shown that in addition to the ground-state three photointermediates can be generated, trapped and then investigated using DNP-enhanced solid state NMR spectroscopy[3]: the early P1 K-like state and the slowly decaying late P4 and a third intermediate populated only under continuous illumination. After photoexcitation, the ground-state all-trans,15-anti conformation is changed to 13C-cis,15-anti. Although the chromophore conformation is crucial for the understanding of the channel mechanism, it is unknown for the later states. Specifically, it is unknown at which state of the photocycle the C13-cis conformation is converted back to the all-trans conformation. In principle the <sup>13</sup>C chemical shifts of the retinal provide such information. However, the protein environment in the later states also influences the chemical shifts and which might obscure the changes expected due to the conformation change of the chromophore.

Therefore, here we use the supercycled SR26 sequence [4] for exact distance measurements in the chromophore to unambiguously determine the conformation. To overcome sensitivity limitations and to trap the photointermediates at low temperatures all experiments were performed using DNP-enhanced MAS NMR in combination with illumination. A 60-fold sensitivity enhancement is achieved routinely. The presented data provides novel insight into the photoactive site of ChR2 during the photocycle.

**References:** [1] Nagel G, et al. (2003), PNAS 100(24):13940. [2] Zhang F, et al. (2011), Cell 147(7):1446. [3] Becker-Baldus J, et al. (2015), PNAS 112 (32):9896. [4] Kristiansen PE, et al (2004), Chem. Phys. Lett. 390, 1.

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P033

Local dynamics of a glass forming calcium rubidium nitrate melt probed by <sup>87</sup>Rb nuclear magnetic resonance

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The local dynamics of a supercooled calcium rubidium nitrate melt is studied by nuclear magnetic resonance (NMR) using the strongly quadrupole-perturbed <sup>87</sup>Rb nucleus [1]: The ion dynamics is probed in the range from milliseconds to nanoseconds by central-transition stimulated-echo techniques, line shape analyses, spin relaxation, and second-order dynamic shift effects. The low-temperature NMR line shapes agree with those predicted by the Czjzek model. The temperature dependent second-order dynamic frequency shift is described using the imaginary part of the spectral density. The correlation times measured with NMR are compared to time scales from electrical conductivity measurements with which they agree well.

**References:** [1] J. Beerwerth, S. P. Bierwirth, J. Adam, C. Gainaru, and R. Böhmer, “Local and global dynamics of the viscous ion conductors 2Ca(NO<sub>3</sub>)<sub>2</sub>-3KNO<sub>3</sub> and 2Ca(NO<sub>3</sub>)<sub>2</sub>-3RbNO<sub>3</sub> probed by <sup>87</sup>Rb nuclear magnetic resonance and shear rheology”, J. Chem. Phys. 150, 194503 (2019); <https://doi.org/10.1063/1.5093973>.

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## P034

# A modified CP-ENDOR sequence unmasks $^2\text{H}$ hyperfine tensors of an amino tyrosyl radical intermediate in *E. coli* RNR

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Electron-nuclear double resonance (ENDOR) spectroscopy is the method of choice for detecting magnetic nuclei in biomolecules which contain an unpaired electron spin is. However, due to its low gyromagnetic ratio, the detection of deuterium couplings is a particular challenge for ENDOR spectroscopy. The standard Mims ENDOR sequence, generally used for small couplings, suffers from line shape distortions. Yet, deuterium nuclei are among the most interesting targets for ENDOR spectroscopy. Hydrogen-bond environments of biomolecules can be investigated at the molecular scale by  $^2\text{H}$  ENDOR spectroscopy in combination with  $\text{H}_2\text{O}/\text{D}_2\text{O}$  buffer exchange. Thus, aiming at improved sensitivity and/or spectral resolution, alternatives to the conventional ENDOR sequences have been proposed.

We present a modified sequence of the recently introduced cross-polarization edited ENDOR sequence[1-4], named “without preparation pulse” (WOP) CP-ENDOR. The sequence is capable of detecting small hyperfine couplings between an electron spin and deuterium nuclei with high sensitivity and almost blind spot free ENDOR spectra.

The WOP CPENDOR sequence is used to disentangle hyperfine tensor features of the amino group of  $\text{ND}_2\text{Y}_{731}^\bullet$  in *E. coli* RNR which are usually distorted by Mims blind spots in the ENDOR spectrum recorded with the well-established Mims ENDOR sequence[5]. Our orientation-selective WOP CP-ENDOR investigations at 94 GHz/3.4 T allowed the establishment of the planarity of the functional group.

**References:** [1] Rizzato, R.; Kaminker, I.; Vega, S.; Bennati, M. *Mol Phys* **2013**, 111, 2809-2823. [2] Rizzato, R.; Bennati, M. *Phys Chem Chem Phys* **2014**, 16, 7681-7685. [3] Rizzato, R.; Bennati, M. *Chem Phys Chem* **2015**, 16, 3769-3773. [4] Bejenke, I.; Zeier, R.; Rizzato R., Glaser, S. J.; Bennati, M., to be submitted. [5] Nick, T. U.; Lee, W.; Koßmann, S.; Neese, F.; Stubbe, J.; Bennati, M., *JACS* **2015**, 137, 289-298.

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P035

Heme Biosynthetic Pathway Regulation Studied by  
NMR Spectroscopy and Computational Methods

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Sanz<sup>2</sup>, Francisco Javier Planes<sup>3</sup>, Oscar Millet<sup>2</sup>

<sup>1</sup>CIC bioGUNE / ATLAS Molecular Pharma, <sup>2</sup>CIC bioGUNE, <sup>3</sup>TECNUN

The Heme group is synthesized in a biosynthetic pathway by 8 enzymes. Defects on these enzymes lead to a family of diseases called Porphyrria. To understand the consequences of the alteration of this route, it is important to gain knowledge about its regulation and the changes in its metabolites. The development of a mathematical model of the route will provide us the capacity of predicting the effect of different mutations or other perturbations such as the inhibitor/modulator effect of possible therapeutic drugs.

By Nuclear Magnetic Resonance and High Performance Liquid Chromatography we have monitored different tandem reactions of the cytoplasmic enzymes involved in the heme synthesis in real time. We have assigned and measured the metabolites and porphyrins in each reaction step. The enzymatic turnover values for each enzyme have been estimated and implemented into a computational model. We have already performed *in vitro* enzymatic assays with the four cytosolic enzymes of the pathway. We have introduced intermediate metabolites in high concentrations as possible inhibitors of some enzymes. We have obtained different results on the quantification of metabolites depending on the metabolite introduced. The results suggest an enzymatic cross-talk regulation between the cytosolic proteins and their products.

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P036

FAST MAGIC-ANGLE SPINNING NMR UNVEILS STRUCTURAL  
DETAILS OF METAL SITES IN PARAMAGNETIC METALLOPROTEINS

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Vladimir Pelmeshnikov<sup>5</sup>, Martin Kaupp<sup>5</sup>, Leonardo Gonnelli<sup>6</sup>, Isabella C. Felli<sup>6</sup>,  
Roberta Pierattelli<sup>6</sup>, Lyndon Emsley<sup>1</sup>, Guido Pintacuda<sup>7</sup>

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Because of the hyperfine coupling between unpaired electrons and nuclear spins, NMR signals in paramagnetic systems often experience large shifts and possess short relaxation times, particularly in close proximity to the metal center, rendering observation of active sites in metalloproteins particularly challenging.

Here, we illustrate how, by adapting a set of NMR experiments originally developed for the study of complex non-biological paramagnetic materials, solid-state NMR can be used to detect and characterize signals from nuclei in the metal coordination sphere in paramagnetic metalloproteins. These experiments combine ultra-fast (60-100 kHz) magic-angle spinning, short high-powered adiabatic pulses (SHAPs), and short recoupling schemes.

The approach is applied to a benchmark <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N-labeled microcrystalline metalloprotein, the enzyme superoxide dismutase (SOD), which has two high-affinity binding sites for metal cations. In combination with first-principles paramagnetic NMR calculations, it is possible to detect and assign <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N signals from residues in the coordination sphere of the metal ions. Moreover, the obtained shifts are extremely sensitive to the structural details of the metal coordination sphere, and allow the determination of the structure of the protein metal site with very high precision.

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## P037

## NMR Using Solution Flow Circuit and Inductively-Coupled Microcoils

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Investigation of systems in evolution such as chemical reactions via NMR can be rendered comfortable if the intrinsic lack of sensitivity of this technique is circumvented and provided that quantitative data are obtained.

Here we present an integrated device based on a 3D-printed mini bubble-pump associated with fluidics and micro-detection that overcomes the sensitivity problems inherent to slow return of magnetization to equilibrium in liquid-state NMR. The use of a closed-loop circuit of the solution near the NMR magnetic center presents two main advantages: pre-polarization is achieved for the whole solution volume, this volume can be reduced to tens of microliters.

This device is installable into every commercial liquid probehead without modification; it is easily inserted from the top of the NMR magnet. A gas flow driven by a programmable syringe pump actuates a mini bubble-pump which leads to circulation of the liquid sample. A part of the solution circuit crosses the NMR detection region consisting of a micro-sole-noid inductively coupled to the coil of the commercial probehead. In order to optimize this coupling a rod fixed on the upper part of the insert and ended by a Vernier placed on top of the magnet enables angular positioning of the micro-coil.

The two resonance frequencies created by the coupling allow one to observe nuclei inaccessible with the host probe alone, or to study two different nuclei with the optimized detection allowed by the microcoil. To further increase the signal-to-noise ratio, this system can also be used to efficiently disperse gaseous species such as hyperpolarized xenon and parahydrogen to the solution.

The performances of this device, in particular with cryoprobes, will be presented.

**Acknowledgement.** Support from the French Ministry of Research (ANR 17-LCV2-0002-01 LabCom DESIR) is acknowledged.

**Reference:** G. Carret, T. Berthelot, P. Berthault, *Analytical Chemistry* 90 (2018) 11169–11173, DOI: 10.1021/acs.analchem.8b01775.

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P038

NMR in molecular recognition: the case of human galectin-1 and blood group antigens

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Glycans serve as recognition elements for glycan binding proteins at the surface of most cells and mediate key biological processes such as cell-cell adhesion, migration and other events critical for the development and function of complex multicellular organisms. At the heart of important living processes, protein-glycan interactions are also linked with several diseases, including infection, cancer or autoimmune disorders.

In this context, human galectin-1 (hGal-1), a b-galactoside binding lectin, is involved in a large variety of relevant biological processes such as inflammatory responses, differentiation trafficking, survival of immune cells and establishment and maintenance of T-cell tolerance and homeostasis in vivo [1]. Furthermore, hGal-1 overexpression in tumours correlates with a metastatic phenotype [2].

Considering its biological relevance and its growing importance as biomedical target, we have investigated the interaction of hGal-1 with naturally oligosaccharides containing LacNAc structures. In particular, we have studied the molecular recognition of blood group antigens (LacNAc and its 3'-O-b-Gal derivative, H type II, B type II and A type II) using Nuclear Magnetic Resonance (NMR) techniques. We have combined Saturation Transfer Difference (STD) NMR experiments with 15N-1H Heteronuclear Correlation Spectroscopy experiments to determine the binding features in these systems. In addition, to gain insights into the driving forces for the complex sugar-protein formation, we have also employed Isothermal Titration Calorimetry (ITC).

Overall, our results provide a comprehensive analysis from a molecular point of view of the glycan-lectin binding process to guide the design and development of galectin-based therapeutic tools.

**References:** [1] Camby, I.; Le Mercier, M.; Lefranc, F.; Kiss, R. Glycobiology. 2006, Volume 16, Issue 11, Pages 137R–157R. [2] Rodriguez, E.; Schettters, S.T.T.; van Kooyk, Y. Nature Reviews Immunology. 2018, Vol. 18, pp 204–211.

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P039

Human Telomeric G-quadruplex structures under oxidative stress

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<sup>2</sup>Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia, Slovenia

Telomeric regions are found at ends of chromosomes and protect the chromosome termini from deterioration or from fusion with neighbouring chromosomes. Due to their high G content, repetitive sequences in telomeres are able to fold into short four-stranded structures called G-quadruplexes. Telomere attrition is closely associated with cell aging and exposure to reactive oxygen species (ROS).

We systematically probed all 12 position of guanines in oligonucleotide d[T-2G3(T2AG3)3A] (hTel) by substitutions of 8-oxo-7,8-dihydroguanine (oxoG), which is the major product of ROS. G-quadruplex forming ability of modified oligonucleotides was evaluated through examination of NMR spectra [1]. A loss of G-quadruplex structure was observed for most oligonucleotides containing oxoG, but some positions in the hTel sequence can tolerate substitutions with oxoG. Due to oxoG's preference for the syn conformation, distinct responses were observed when replacing guanines with different glycosidic conformations. Accommodation of oxoG at sites in syn or anti in nonsubstituted hTel G-quadruplex requires a minor structural rearrangement or a major conformation shift, respectively. These G-quadruplex structures are still stable at physiological temperatures and should be considered detrimental in higher-order telomere structures.

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P040

Spin-orbit coupling and zero-field splitting in high-spin centers in solids

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The energy splitting of the spin states of high-spin ( $S > 1$ ) solid-state defects due to magnetic anisotropy is one of the key issues for their application in future quantum technologies. This splitting, addressable by electron paramagnetic resonance (EPR), sensitively depends on the intrinsic properties of the defect and appears in the absence of external fields, i.e. in the *zero-field*. For heavy-element systems, the dominant origin of this zero-field splitting (ZFS) is the spin-orbit coupling. We present a reliable theoretical framework for perturbative calculation of the spin-orbit ZFS within the plane-wave density functional theory (DFT) [1, 2]. We validate our approach against direct fully relativistic treatment with non-collinear spin polarization [3]. By considering a family of impurity-vacancy centers in diamond, we discuss the origin of the spin-orbit driven magnetic anisotropy of a defect in a light-element host. The reported theoretical results open up new avenues for a rational search and design of high-spin defects suitable for, e.g., quantum sensing and quantum information processing.

**References:** [1] P. Giannozzi, et al., J. Phys Cond. Matter 2017, 29, 465901. [2] P.E. Blöchl, Phys. Rev. B 1994, 50, 17953. [3] D. Ceresoli, U. Gerstmann, A.P. Seitsonen, and F. Mauri, Phys. Rev. B 2010, 81, 060409.

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P041

Structural investigation of the UCP3 regulatory element from human 3'-UTR by NMR-spectroscopy

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Regulatory elements in the UTRs of mRNA are involved in a multitude of biological events and can play an important role in post-transcriptional regulation.[1,2] Herein we investigated the regulatory element UCP3 from human 3'-UTR of mRNA coding for a mitochondrial membrane protein involved in fatty acid metabolism, obesity and insulin resistance.[3] In the Weigand lab it was shown, that UCP3 can be defined as a CDE (constitutive decay element) which is recognized by the protein Roquin. The regulatory sequence consists of two stem-loop structures connected by a linker. Utilizing NMR-spectroscopy and crystallography, we provide a structural basis for the understanding of the stem-loop conformation and its recognition by Roquin.

To gain structural information about the interaction with Roquin, we determined the three-dimensional structure of the 21nt CDE2 subdomain by NMR. Distance restraints for structure calculation were determined from <sup>1</sup>H,<sup>1</sup>H-NOESY spectra, which could be assigned unambiguously, reviewing the assignments in J-coupling driven NMR experiments like 3D-HCN and 3D-HCCH-TOCSY. Additionally dihedral restraints were determined, exploiting angular dependence of J-coupling, as well as cross-correlated relaxation rates.

Binding of CDE2 to Roquin was not only observed in ITC but also in protein targeted <sup>15</sup>N-correlation experiments. We performed crystallization experiments and were able to determine a crystal structure of the CDE2-Roquin complex. Additionally the CDE1 domain as well as the full CDE were investigated for Roquin binding with <sup>15</sup>N-correlation experiments.

**References:** [1] F. Mignone, C. Gissi, S. Liuni and G. Pesole (2002) Untranslated regions of mRNAs. *Genome Biol.*, 3. [2] L.W. Barrett, S. Fletcher and S.D. Wilton (2012) Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. *Cell. Mol. Life Sci.*, 69, 3613–3634. [3] F. Bouillaud, M. Alves-guerra and D. Ricquier (2016) Biochimica et Biophysica Acta UCPs, at the interface between bioenergetics and metabolism. *BBA - Mol. Cell Res.*, 1863, 2443–2456.

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P042

Expression, purification and NMR study of the 124-residues C-terminal polypeptide of Arkadia

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Arkadia is a RING E3 ubiquitin ligase that positively regulates TGF- $\beta$  signaling pathway by mediating degradation of the negative regulators Smad6 and Smad7 and the nuclear co-repressors Ski and Skil (SnoN)[1,2]. The domains that are required for the substrate recognition and ubiquitin ligase activity are located in the highly conserved area of the C-terminal 100 amino acids. This region is composed by the NRG and TIER amino acid segments, as well as a RING domain which comprises two Zinc(II) ions in a cross-brace topology. NRG and TIER are required for substrate recognition while the RING domain is required for the ubiquitin ligase activity[3].

In the present study the polypeptide that comprises the 124-residues C-terminal polypeptide of Arkadia, including the NRG and TIER segments along with the RING domain was cloned, expressed in Escherichia coli cells and subsequently purified through affinity chromatography for NMR structure, dynamics and interaction studies. Moreover, the E3 ligase ubiquitin activity of the 124-residues C-terminal polypeptide was monitored in vitro through auto-ubiquitination experiments.

**References:** [1] Levy L, Howell M, Das D, Harkin S, Episkopou V, Hill KS, 2007. Molecular and Cellular Biology 27(17): 6068-6083. [2] Nagano Y, Mavrakis KJ, Lee KL, Fujii T, Koinuma D, Sase H, Yuki K, Isogaya K, Saitoh M, Imamura T, Episkopou V, Miyazono K, Miyazawa K, 2007. The journal of Biological Chemistry 282(28): 20492-20501. [3] Sharma V, Antonacopoulou AG, Tanaka S, Panoutsopoulos AA, Bravou V, Kalofonos HP, Episkopou V, 2011. Cancer Research 71: 6438-6449.

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## P043

**Per aspera ad astra: paramagnetic NMR in solution via the solid state**

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<sup>1</sup>High-field NMR center Lyon (FRE2034 - CNRS/UCBL/ENS de Lyon), <sup>2</sup>High-field NMR center Lyon (FRE2034 - CNRS/UCBL/ENS de Lyon); IFP Energies nouvelles, <sup>3</sup>IFP Energies nouvelles

Paramagnetic NMR is a direct probe of electronic structure in many coordination compounds and organometallic systems. Unpaired electrons alter the NMR spectrum of the surrounding nuclei causing paramagnetic shifts and relaxation enhancements which report in a direct way on the structure of the compounds and configuration of the chemically active molecular orbitals. Such effects can nowadays be accurately predicted.[1]

However, in solution, i.e. the phase where the chemical activity of these molecules occurs, interpretation of paramagnetic NMR effects poses a problem, since i) molecular motion can blur the effects expected on the basis of single-crystal X-ray structures, and ii) the short coherence lifetimes induced by the paramagnetic centers hinder the acquisition of multidimensional correlations necessary for resolving and assigning each individual nuclear resonance.

We present here a strategy for circumventing these issues and achieving the NMR characterisation of small paramagnetic complexes in solution, via the acquisition and the interpretation of solid-state paramagnetic NMR spectra of their microcrystalline powders. Resonances are first resolved in 2D heteronuclear (<sup>1</sup>H, <sup>13</sup>C) and homonuclear (<sup>1</sup>H, <sup>1</sup>H) correlations acquired under fast (≥ 60 kHz) magic-angle spinning (MAS) with tailored broadband dipolar-based sequences,[2] then assigned with the help of ab-initio/DFT calculations[3] and NMR relaxation analysis. The solid-state assignment is then applied to 1D solution state NMR spectra, where shift differences are rationalized by solvent and crystal packing effects. The strategy is illustrated on a series of industrially relevant catalysts based on high-spin iron(II) complexes bearing amino and/or phosphine ligands.

**References:** [1] A. J. Pell et al., *Prog. Nucl. Magn. Reson. Spectrosc.*, **2019**, 111, 1–271. [2] a) A. J. Pell et al., *Prog. Nucl. Magn. Reson. Spectrosc.*, **2015**, 84–85, 33–72.; b) G. Kervern, et al. *J. Am. Chem. Soc.*, **2006**, 128, 13545–13552. [3] J. Vaara et al., *J. Chem. Theory Comput.*, **2015**, 11, 4840–4849.

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Double monoubiquitination modifies the molecular recognition properties of p15PAF

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1CIC bioGUNE, 2ITQB NOVA, 3Universidad de Granada, 4Université Montpellier, 5University of Leicester

The Proliferating Cell Nuclear Antigen-associated factor p15PAF (hereafter named p15) is a 12 kDa nuclear protein that regulates DNA repair during DNA replication (1). The p15 gene is overexpressed in several types of human cancer and its function is regulated by double monoubiquitination at lysines K15 and K24. Upon UV stress p15 is degraded facilitating the recruitment of the trans-lesion synthesis polymerase  $\Theta$  to PCNA at stalled replisomes. We have previously reported that p15 is an intrinsically disordered protein (IDP) which partially folds upon binding to PCNA and independently contacts DNA through its N-terminal tail. Here we present an NMR conformational characterization of p15 monoubiquitinated at both K15 and K24 via a disulfide bridge mimicking the isopeptide bond. Doubly monoubiquitinated p15 (dmUbp15) is monomeric, intrinsically disordered, binds to PCNA as non-ubiquitinated p15, but interacts with DNA with reduced affinity. These results are consistent with a model in which p15 binding slows down the sliding of PCNA along the DNA, which proceeds faster when p15 is ubiquitinated. Our SAXS-derived conformational ensemble of doubly monoubiquitinated p15 shows that the ubiquitin moieties form a transient dimer due to the high local effective concentration, suggesting that it is a binding target of DNA methyl transferase Dnmt1, as confirmed by calorimetry.

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**Towards structure determination of oligomeric Bcl-2 protein complexes using spectrally distinguishable spin probes and DEER**  
stephanie Bleicken\*,1, Markus Teucher2, Christina Elsner, Enrica Bordignon2  
*<sup>1</sup>stephanie, <sup>2</sup>Ruhr University Bochum*

We are interested in structure determination of the active form of the human- proapoptotic Bcl-2 protein Bax, which is crucial for the regulation and execution of apoptosis. In healthy cells Bax is mainly monomeric, and cytosolic, while activation leads to membrane-insertion into the mitochondrial outer membrane and apoptosis. Structure determination of active Bax has proven to be difficult due to its membrane-embedded nature, its engagement in homo- or hetero-oligomers, its inhomogeneous oligomer size, and the complicated multistep transitions during its activation. However, we were able to propose a 3D model of active Bax based on DEER spectroscopy in liposomes and isolated mitochondria. During those studies we faced the challenge that with even only one spin probe per monomer, Bax oligomerization created complex systems with multiple intra- and inter- monomeric spin distances that were difficult to disentangle. We reduced the complexity by using doubly-labeled proteins and spin diluted them with unlabeled Bax to retrieve intra-monomer distances, but our structural modeling would largely benefit from the assignment of intra- and inter-dimer distances. Towards this end and to further study hetero-complexes with other Bcl-2 proteins (determined by fluorescent techniques), we established a new protein system with spectrally distinguishable gadolinium and nitroxide spin probes. Therefore, we tested the structural and functional compatibility of different labels with different Bax variants, which showed that not all combinations are compatible and produce active spin-labeled protein. Moreover, we tested the biocompatibility of sterically protected nitroxide labels in human and bacterial cells. We succeeded to produce first active Bax homo- and hetero-complexes that contain spectrally distinguishable spin probes, to measure DEER on them and to assign complex spin-systems that could not be disentangled before. We believe this success marks the beginning of an exciting journey to explore the overwhelming complexity of Bcl-2 protein complexes via EPR.

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**P046****Fragment Screening of RNA and Proteins using Orthogonal Mixtures:  
Exploring a new Target Area for Drug Discovery**Marcel Blommers\*  
*Saverna Therapeutics*

Among the translated proteins are few validated drug targets. A recent estimate, based on currently approved drug substances, listed 218 validated protein targets. The choice of non-coding RNA as a drug target could be an alternative approach to efficiently control biomolecular signaling pathways. Currently, clinical trials are underway with oligonucleotides and small molecule drug candidates targeting RNA, demonstrating the great potential and feasibility of selecting RNA targets. Advances in drug discovery for RNA targets will be discussed and illustrated using several examples.

To efficiently explore the hitherto unknown chemical space for RNA ligands, we have developed fragment-based screening protocols in which we have screened orthogonal mixtures. This screening approach does not require individual compound spectra to decrypt primary hits. Furthermore, a cooperative ligand binding of two compounds in a mixture can be detected in the primary screen. The approach saved us a lot of effort in sample preparation and measuring time, without compromising on quality. The method is particularly suitable for screening in an academic environment when a new fragment library is used only for a few drug targets.

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P047

**Determination of functionally important proline conformations in proteins**  
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Intrinsically disordered proteins (IDPs) are enriched in “disorder-promoting” residues such as proline. In solution Xaa-Pro bonds can undergo a cis-trans isomerization. Functional studies show the biological relevance of the different types of conformation.[1] The *cis*-isomer is less frequent (5-15 %) due to steric hindrances and effects also the proline surrounding residues. Arising minor peaks can be detected with 5-15 % intensity.

Our goal is to characterize and sequentially connect the occurring minor signals, where the proline conformation determination plays a decisive role. The most commonly used method is based on C $\beta$ , C $\gamma$  chemical shift difference. In this context, we focus on novel N and/or C $\alpha$ ,H $\alpha$  resolved measurements with sufficient signal intensity for proline conformation elucidation.

- In the present study we used two selective NMR experiments:
- 3D HCACBN, in which the Pro sidechain carbon chemical shifts are detected via H $\alpha$ /H $\delta$  observation
  - 3D Pro\_CBCG\_CAHA that is based on a HCCH-TOCSY experiment and correlates Pro C $\beta$  and C $\gamma$  with the corresponding C $\alpha$  and H $\alpha$ .
- In both cases the sensitivity and resolution were further enhanced by applying real-time homodecoupling (BASEREX).[2]

Both measurements were tested on the 62 residues long transactivation domain of the tumor suppressor p53, containing 10 prolines. Due to the Pro cis-trans isomerization more than 20 minor peaks are detected.

We show that in the major component, all prolines favor the *trans* conformation, while in the minor conformers most prolines adapt a *cis* conformation. However, the minor of the biologically relevant P47 and in two minor forms of Pro-Pro motif one proline remain in *trans*. This characterization suggests structural differences: increased rigidity and turn-type propensity for the minor conformers – a feature that can be the basis for biological function explanations.

**References:** [1] Weiwad et al. 2004, *J Mol Biol* 339, 635-646. [2] Haller et al. 2019, *J Magn Reson* 302, 64-71.

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## P048

Enantiodiscriminative self-organization of  $\beta$ -peptides - structure elucidationDóra Bogdán<sup>\*1</sup>, Tamás Gáti<sup>2</sup>, István Mándity<sup>1</sup><sup>1</sup> *Semmelweis University, Department of Organic Chemistry; 2 Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, <sup>2</sup>Servier Research Institute of Medicinal Chemistry (SRIMC)*

$\beta$ -peptides are important peptidomimetic compounds. Here the formation of ordered backbone conformation of new pentamer  $\beta$ -peptides was studied by NMR (<sup>1</sup>H, ROESY, TOCSY, COSY NMR techniques) and ECD spectroscopy. The investigated pentamers include trans-[R,R]-2-aminocyclohexanecarboxylic acid (trans-[R,R]-ACHC) building blocks interrupted by different elements in the middle of the chain in order to study the effect of new structural elements on conformation. Trans-[R,R]-ACHC residues support H14 helix formation. The following  $\beta$ -amino acids were used as 3rd building blocks in the peptides:  $\beta$ -alanine, Z-dehydro- $\beta$ -alanine and two different types of bicyclic elements, as diexo-3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid (diexo-ABHEC) enantiomers or diexo-3-amino-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid (diexo-AOBHEC) enantiomers. In case of the flexible  $\beta$ -alanine building block, [1 R,2 R,3 S,4 S]-diexo-ABHEC and [1 R,2 S,3 R,4 S]-diexo-AOBHEC residues stable helices were discovered. Characteristic NOE-interactions for H14 helices were found and amide NH-ND exchange occurred over 10 hours. In ECD spectra intensive maximum and minimum bands were shown at 215 and 195 nm, which are also typical for H14 helices. Differences were found for [1 S,2 S,3 R,4 R]-diexo-ABHEC, [1 S,2 R,3 S,4 R]-diexo-AOBHEC and Z- $\beta$ -dehydroalanine as 3rd unit, stable self-organization could not be observed. In three peptides stable H14 helix was formed, in further three peptides less stable structures were identified. In case of bicyclic elements an effect was found on folding regarding the configuration. The O-atom in this residue has no effect on self-organization. A double bond in the middle unit (Z-dehydro- $\beta$ -alanine) rules out the helix formation.

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## P049

### Relaxation optimized two-field NMR spectroscopy: theoretical study of $^{13}\text{C}$ - $^{19}\text{F}$ correlations in aromatic groups of large biomolecular systems.

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Fluorine-19 seems to be an ideal target for site-specific studies of biomolecules: it has a gyromagnetic just below the one of proton, 100% natural abundance, a large chemical shift dispersion and offers a background-free signal in biomolecules. However, its large chemical shift anisotropy (CSA) leads to transverse relaxation rates too high for standard multi-dimensional NMR (transverse relaxation rates of about 1000 s<sup>-1</sup> for a global tumbling correlation time of 100 ns at 600 MHz). The CSA contribution to the relaxation rate scales quadratically with the magnetic field, leading to fluorine-19 transverse relaxation rates that are lower by more than two orders of magnitude for large proteins at low magnetic fields of around 1 T. Unfortunately, low-field NMR suffers from low sensitivity and resolution. Here, we show that two-field NMR spectroscopy can be used to take advantage of favorable relaxation properties at low field while preserving high-field sensitivity and resolution [1]. During the course of an experiment, the sample is shuttled between two vastly different magnetic fields where coherence pathways are controlled by two distinct probes. We describe a two-field two-dimensional NMR pulse sequence for the study of aromatic  $^{13}\text{C}$ - $^{19}\text{F}$  groups with the fluorine chemical shift evolution at low-field (down to 1 T) and high-field detection of the carbon TROSY component. Our calculations show that sensitivity can be boosted by up to two orders of magnitude with an enhanced resolution in the fluorine-19 dimension compared with a single-(high) field TROSY experiment [2]. We expect our theoretical results to be transposable to other nuclei with large CSAs such as carbonyl carbon-13 of peptide bonds. These simulations demonstrate the high potential of two-field NMR spectroscopy for the investigation of challenging systems.

**References:** [1] Cousin S.F et al., Phys. Chem. Chem. Phys., 18, 33187, (2016). [2] Boeszoermenyi A, et al., Nat. Methods, 16, 333 (2019).

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P050

**Substrate-protein interactions in the ABC-exporter MsbA by solid state NMR**

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The inner membrane protein MsbA of gram-negative bacteria conveys the translocation of its substrate Lipid A across the membrane, by ATP hydrolysis. Lipid A is the anchoring molecule for the LPS-molecules, which cover the surface of gram-negative bacteria and can trigger the innate immune response. Besides Lipid A, MsbA can also transport various dyes and drugs across the membrane, which makes this system an interesting target to study multidrug resistance[1].

For the transport mechanism, a 'trap and flip' model was proposed, in which the phosphate groups and glucosamines of Lipid A are coordinated by a ring of hydrophilic amino acids inside the binding pocket[2]. Extensive NMR studies in our lab[3] show that Lipid A can be copurified with MsbA under softer solubilization conditions, allowing for interaction studies in a more native liposome environment[4]. Based on this work MsbA-Lipid A interactions are probed during the catalytic cycle by MAS-NMR. Especially signal enhancement due to dynamic nuclear polarization in combination with trapping reagents, which can mimic single ATP states during hydrolysis, enables studying weak interactions between Lipid A and MsbA during the transport cycle. This will help to establish an overview of changing Lipid A positions and orientations, caused by ATP-hydrolysis-induced conformational changes, leading to a better understanding of the transport mechanism.

**References:** [1] Eckford (2008): Functional characterization of Escherichia coli MsbA: interaction with nucleotides and substrates. J. Biol. Chem. 283 (19): 12840–12850. [2] Mi (2017): Structural basis of MsbA-mediated lipopolysaccharide transport. Nature 549 (7671): 233–237. [3] Kaur (2016): Coupled ATPase-adenylate kinase activity in ABC transporters. Nat. Commun. 7: 1864. [4] Kaur (2015): The ABC exporter MsbA probed by solid state NMR – challenges and opportunities. Biological chemistry 396 (9-10): 1135–1149. [5] Kaur (2018): Unexplored Nucleotide Binding Modes for the ABC Exporter MsbA. J. Am. Chem. Soc. 140 (43): 14112–14125.

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## P051

### Combining NMR and EPR analysis for structural determination of multi-domains proteins

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Nuclear magnetic resonance (NMR) spectroscopy is a very powerful tool of investigation in structural biology, providing structural and dynamic information at atomic resolution on a protein either alone or in its interaction with small molecules or with other proteins/nucleic acids [1-2].

Electron Paramagnetic Resonance (EPR) spectroscopy well complements NMR data providing information useful to establish the presence of interactions between different parts of a single polypeptide or between two partner proteins even when the interaction is loose or transient and thus likely to escape NMR detection [3]. Furthermore, EPR has less severe limitation than NMR concerning molecular size and concentration of the samples and can provide information useful to complement data obtained with other biophysical techniques on various macromolecules, including membrane proteins and large proteins interacting with lipids. Double Electron-Electron Resonance (DEER) spectroscopy, in particular, permits to establish the distance between paramagnetic tags grafted on specific positions of a polypeptide. These data can thus be used as conformational restrains in conjunction with NMR data or in molecular dynamics simulations to provide high-resolution models of a protein in solution [4].

We will present results on the combination of NMR and EPR spectroscopies to characterize the structure of multi-domains proteins and to expand the knowledge about their physiological role and also on their implication in several pathologies.

**References:** [1] K. Wuthrich, *Science* (1989), 243, 45-50. [2] L. Banci *et al.*, *Prog. Nucl. Magn. Reson. Spectrosc.* (2010), 56, 244-267. [3] W.L. Hubbell *et al.*, *Met. Enzymol.* (2015), 564, 59-100. [4] G. Jeschke, *Ann. Rev. Phys. Chem.* (2012), 63, 419-446.

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## P052

## Structural insights into DNAzyme-mediated catalysis

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In addition to enzymes and ribozymes, synthetic catalytic deoxyribozymes (DNAzymes) can accelerate a broad range of reactions. However, the structural basis of how DNAzymes work remains mainly unclear. Among other applications, DNAzymes are particularly attractive as therapeutic agents due to their stability, low production costs and intrinsic mode of action which does not rely on additional molecules.

We designed a RNA-cleaving 10-23 DNAzyme to specifically reduce mRNA levels of Prion protein (PrP), which is a key player in several neurodegenerative disorders. However, although the DNAzyme is highly active *in vitro*, no activity could be detected *in situ*. To overcome this generally known limitation of DNAzymes, a profound knowledge of the catalysis is needed to guide structure-based engineering of improved DNAzyme variants.

In our study, we obtain the first high-resolution structural insights of the arguably most famous 10-23 DNAzyme (33 nucleotides) in the pre-catalytic complex with a stabilized RNA target (19 nucleotides). To obtain reliable structural information we used a combination of NOE full relaxation matrix analysis, residual dipolar coupling (RDC), paramagnetic relaxation enhancement (PRE) as well as novel <sup>19</sup>F labeling and acquisition strategies. We demonstrate that selective <sup>19</sup>F labeling provides a good alternative to cost-intensive isotope labeling, especially for DNA systems. Using an integrative approach the structure calculations were furthermore supported by small-angle X-ray scattering (SAXS) data and molecular dynamic (MD) simulations. We also determined the Mg<sup>2+</sup> binding sites using Mn<sup>2+</sup>-induced PRE effects and NOE contacts to the magnesium hydrate mimic Co(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup>. Complementary, we established fluorescence-based assays which allow us to record a high number of kinetic data providing a link between our structural findings and DNAzyme function.

Our study resulted in the first DNAzyme structure solved by solution NMR spectroscopy providing novel mechanistic insights into 10-23 DNAzyme-mediated catalysis.

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## P053

### NMR-based fragment placement using paramagnetic relaxation enhancement from a soluble spin label

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Fragment-based drug discovery is an essential strategy in the development of new clinical candidates. Both NMR and crystallography-based fragment screening can be applied to detect binding. A challenge in early-stage drug discovery is to acquire enough structural information on fragment placement in order to drive compound development. Crystallography may provide such information. However, achieving crystallisation and diffraction with weakly binding fragments is challenging. Also, unless high resolutions are obtained, fragment position and pose may be uncertain. The high concentrations required for crystallographic fragment screening may also lead to non-specific binding. Paramagnetic NMR is an alternative approach and can provide orientation information between a paramagnetic centre and a bound ligand.[1]

Here, we use a small, soluble, hydrophobic nitroxyl to paramagnetically probe the protein surface of our model system, IMP-13 metallo- $\beta$ -lactamase. Metallo- $\beta$ -lactamases are a significant challenge in antibiotic resistance, showing activity against all major classes of  $\beta$ -lactam antibiotics by hydrolysing the  $\beta$ -lactam ring.[2] Development of drugs against these enzymes is important in the fight against antibiotic resistance. We demonstrate that this compound can target the IMP-13 active site, through protein-detected PREs (paramagnetic relaxation enhancements). PREs between our soluble paramagnetic nitroxyl and novel fragments can be used for compound selection and may be used to position these ligands relative to the paramagnetic compound. This can provide important structural information early in fragment-based drug discovery programmes, without the requirement for protein modification e.g. incorporating a bound tag.

**References:** [1] W. Jahnke, L. B. Perez, C. G. Paris, A. Strauss, G. Fendrich, and C. M. Nalin, *J. Am. Chem. Soc.*, 2000, 122, 7394-7395. [2] T. Palzkill, *Ann. N. Y. Acad. Sci.*, 2013, 1277, 91-104.

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## P054

### Monitoring lipid metabolism in microalgae by high-field and benchtop NMR spectroscopy

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Microalgae are increasingly studied in both academy and industry owing to their broad applicative potential. Indeed, they are able to create biomass with significant productivities compared to traditional crops. Besides, several microalgae species present the ability to produce lipids thanks to a metabolic shift provoked by nitrogen starvation conditions. In order to better understand this phenomena and find the best way to produce lipids, biological and process research works are carried out by a substantial research community.

This work presents the development of tailored liquid-state NMR spectroscopy methods which provide complementary information on microalgal lipids. On the one hand, a high-field spectrometer (700 MHz) is used for the precise quantitative analysis of lipid extracts from microalgae. The available information and analytical specifications are assessed for three different pulse sequences: the classical <sup>1</sup>H and <sup>13</sup>C 1D NMR as well as the Quick QUIPU HSQC[1] an intrinsically quantitative 2D pulse sequence.

On the other hand, a benchtop spectrometer (43 MHz) is used for the non-invasive monitoring of microalgae in their cultivation medium[2]. Thanks to the implementation of a gradient coil in the hardware, the water peak can be efficiently removed with the WATERGATE-5 pulse sequence[3]. The main peak from in vivo lipids (1.2 ppm) is then observable and can be related to the total lipids. This statement was applied on the benchtop spectrometer coupled to a photobioreactor, an automated device for microalgae cultivation. For the very first time, the real-time non-invasive and in vivo access to lipid production kinetics can be obtained with this NMR hyphenated apparatus.

**References:** [1] Farjon *et al.*, *Anal. Chem.*, **2018**, 90, 1845–1851. [2] Bouillaud *et al.*, *Magn. Reson. Chem.*, **2018**. [3] Gouilleux *et al.*, *Magn. Reson. Chem.*, **2017**, 55, 91–98.

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P055

Sensitivity enhancement using Optimal Control  
optimized pulses and sample space extension

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Solid-state NMR analysis of biological samples has continuously been hampered by a lack of sensitivity. Especially complex multidimensional experiments suffer from a low signal-to-noise ratio as a consequence of low coherence transfer efficiencies. One reason for less than optimal magnetization transfers is the RF inhomogeneity of solenoids commonly used for excitation and detection in MAS solid-state NMR probes. Critically, the RF field, as produced by a solenoid, is experienced differently by a given spin packet at different times of one rotor period. We present an application of our refinement of Optimal Control methodology that, taking these RF variations into account, offers increased signal intensities for experiments relying on cross polarization coherence transfers. The contact pulse pairs are robust to various experimental factors and can furthermore be adjusted to work at a range of MAS frequencies by changing their duration to maintain rotor synchronicity.

In order to be able to use these new pulses to their full potential, though, the whole volume that becomes available for coherence transfers has to be actually filled with sample. To this end, we are designing a new type of sealing plug for Bruker's 1.3mm rotor system that prevents water loss in biological samples while still maximizing the amount of sample, thus increasing the detected signal.

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P056

A definition of the metabolic syndrome based on NMR-metabolomics of urine samples.

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Dolores Corella<sup>2</sup>, Nieves Embade<sup>1</sup>, José M. Mato<sup>1</sup>, Oscar Millet<sup>1</sup>

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Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that generate an increased risk in the onset of cardiovascular disease (CVD), liver disease, type 2 diabetes and chronic kidney disease. According to the World Health Organization (WHO), MetS is defined as the presence of insulin resistance and other two of the following risk factors: obesity (waist circumference or BMI > 30 kg/m<sup>2</sup>), hyperlipidaemia, hypertension or microalbuminuria. Yet, the relative weight of each of the individual factors in the complex syndrome, the effect of alternative molecular definitions of MetS and the putative role of neglected symptomatology (i. e. NASH) are not clearly understood. Moreover, due to the increasing number of people affected by MetS the identification of new diagnostic tools and the determination of new biomarkers for the early diagnosis and prognosis of this disorder becomes strategic.

We have used NMR-based metabolomics of urine to provide a quantitative metabolic fingerprint of the metabolic syndrome. Specifically, using a single, automated and standardized NMR-based analysis, we analysed urine samples that combined samples from a working population in the Basque Country (9000 volunteers) and from a specific study (OBENUTIC, > 500 patients). According to sample metadata, volunteers were classified into the 16 profiles that define a continuous symptomatic transition from the absence of all the risk factors (SuperHealthy) up to all the subtypes that canonically define the MetS condition. Multivariate analysis of median spectra profiles showed clear differences between the different subtypes emphasizing the significant weight of diabetes in the MetS metabolic definition. Together with univariate analysis, both targeted and untargeted, relevant metabolites involved in MetS were identified and this combination of biomarkers may be used as a measure of the progression toward pathogenic MetS states. Further research on specific biomarkers will likely reveal new relationships between MetS and other conditions.

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P057

Understanding the role of the protein quality control machinery  
in neurodegenerative diseases.

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University of Gothenburg

Molecular chaperones are essential for maintaining a functional proteome in the cells. Nevertheless, central functional aspects of chaperones are still not well understood at the atomic level, including how chaperones recognize their clients, and in which conformational states clients are bound.

We utilized mechanistic insights derived from bacterial chaperone:client complexes to investigate the functional role of chaperones in Parkinson's disease. Parkinson's is one of the most common neurodegenerative disorders, pathologically manifested by intracellular accumulation of aggregates of the intrinsically disordered protein  $\alpha$ -Synuclein. Systematic investigations on an array of chaperones identified a general chaperone interaction motive at the  $\alpha$ -Synuclein amino-terminus. The dominant role of chaperone interactions for cytosolic  $\alpha$ -Synuclein was validated with in-cell mass-spectrometry and NMR spectroscopy and the functional basis for the effects of several known post-translational modifications of  $\alpha$ -Synuclein could thus be reconstituted *in vitro*. The data reveal how molecular chaperones control the state and function of  $\alpha$ -Synuclein *in vivo* and how the disturbance of these interactions leads to progress of pathologically relevant  $\alpha$ -Synuclein-states ultimately leading to the aggregates observed within Lewy bodies.

Recently we have extended our studies also to different proteases and I will also present initial data indicating their importance in neurodegenerative diseases.

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P058

**Broad timescale conformational dynamics: Application of geometric approximation and adiabatic relaxation dispersion**

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Relaxation dispersion techniques are powerful tools to quantitatively characterize the chemical (or conformational) exchange across biologically relevant timescales [Palmer et al. Meth. Enzymol. 2001 & 2019]. Recently, a new type of data analysis, geometric approximation methodology [Chao & Byrd, JACS 2016], has been developed to decipher the complex experimental data associated with the adiabatic relaxation dispersion experiment [Mangia et al. JACS 2010], thus providing the assessment of a very broad range of timescales. The advantages of geometric approximation can also be applied to conventional CPMG experiments, including the use of different exchange models and related problems [Chao & Byrd, JMR 2017, Emerg. Topics Life Sci., 2018].

The original adiabatic relaxation dispersion experiment focused on protein backbone (15NH) dynamics. We have recently extended these methods to methyl groups in the new methyl-geoHARD experiment, combining geometric approximation, adiabatic relaxation dispersion techniques, and methyl TROSY effects [Tugarinov et al. JACS 2003]. Methyl-geoHARD can explore broad timescale conformational dynamics (ranging from 150 sec-1 to 100,000 sec-1) in the hydrophobic cores of large protein complexes.

We illustrate the detection and quantification of a broad distribution of collective motions and local motions of methyl groups within a moderately large enzyme (tauc = 24 ns). The method is quantitatively validated by comparison with the conventional SQ-CPMG [Lundstrom et al., JBNMR 2007] and other experimental data, for sites where the dynamics are within the timescale of both experiments. The technique is developed and optimized to address the large off-resonance effects at ultra-high magnetic fields (> 1GHz) and for large and complex biological systems (up to tauc = 50 ns). Overall, the potentials of geometric approximation methodology enable the analysis of complex relaxation phenomena and simplify the experiments to gain or retain sensitivity in challenging, large molecular weight proteins.

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P059

Ligand-induced domain liberation in a dimeric  
110 kDa enzyme monitored by NMR spectroscopy

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Two- or multicomponent systems allow bacteria to turn various environmental stimuli into specific cellular responses including but not limited to development, cell division, metabolism and even antibiotic resistance. A typical two-component system consists of a sensory histidine kinase, which autophosphorylates a histidine in response to the input signal, and a transcription factor termed response regulator that responds to kinase-mediated phosphorylation of a conserved aspartate on its receiver domain. Here, we study the more complex case of a multicomponent system or phosphorelay initiating from the hybrid histidine kinase ShkA that controls stalk biogenesis during cell cycle progression in *Caulobacter crescentus* [1]. The vital question how this phosphorelay system is controlled on the cellular level remained unanswered.

ShkA is a non-canonical hybrid histidine kinase as it is cytosolic, lacks any N-terminal input domains and contains two instead of one C-terminal receiver domains. We identified the input signal for ShkA activity as the second messenger cyclic-di-GMP (cdG). The crystal structure of ShkA in complex with AMPPNP showed compact domain arrangement that could not explain the enzyme activity. To get insight into the mechanism of cdG mediated relief of the locked state, we employed methyl-NMR spectroscopy on the dimeric 110 kDa enzyme of specifically isoleucine labeled full-length ShkA and its isolated Rec1 and Rec2 domains. NMR binding experiments indicated that cdG binds to the degenerated Rec1 domain, competing with a tethered domain linker and thereby liberating the Rec2 domain from the protein core. Spin relaxation experiments confirmed quantitatively the allosteric coupling of ligand binding and domain liberation that allows the protein to undergo the large domain motions necessary for phosphoryl transfer. Our study thus uncovered a novel mechanism of kinase activation by a second messenger.

Reference: [1] Biondi *et al.*, Mol. Microbiol. (2006) 59,386-401.

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P060

A high voltage CMOS NMR-on-a-chip transceiver for portable spectroscopy applications

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NMR spectroscopy is a nondestructive characterization technique that provides detailed information on molecular structures with incomparable specificity. With NMR, it is possible to observe chemical reactions, detect chemical substances, and to determine concentrations.

Commercial NMR devices are usually bulky, expensive and power hungry. To change this situation, our approach uses integrated circuit (IC) technology to monolithically integrate the entire NMR transceiver into a small application specific integrated circuit (ASIC) with a size of only a few square millimeters. As an addition to the state-of-the-art, the proposed NMR-on-a-chip transceiver is realized in a high-voltage CMOS technology, featuring supply voltages up to 20 V. Thereby, large output currents beyond 1 A can be generated on chip that are suitable to produce large B<sub>1</sub>-fields in conventional, millimeter-sized NMR coils without additional external driving blocks. With its very low manufacturing costs, the presented implementation presents an important step towards miniaturized and affordable NMR systems.

The ASIC integrates a conventional low intermediate frequency (low-IF) receiver together with an H-Bridge-based power amplifier and a PLL-based frequency synthesizer. The receiver, which consists of a low noise amplifier (LNA), a quadrature down conversion mixer and baseband amplifiers, achieves an input referred voltage noise around 1.5 / nV√Hz. The on-chip H-bridge in the transmit path, which operates from a 20 V supply, can deliver up to 1.4 A into coils with inductances between 10 nH and 500 nH. The integrated PLL frequency synthesizer is designed with an adjustable prescaler and frequency dividers to support the frequency range from 4 MHz to 100 MHz, covering all relevant Larmor frequencies of today's and future benchtop and portable NMR systems.

Overall, with its small form factor, low manufacturing costs and excellent TX and RX performance, the proposed NMR-on-a-chip transceivers presents an important step towards future high-performance, yet affordable benchtop and portable NMR systems.

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P061

Influence of noise level in training samples on  
deep learning reconstruction of MOLED-4 T2 mapping  
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*Xiamen University*

Quantitative magnetic resonance imaging (qMRI) has become one of the hotspots in the field of clinical diagnosis and scientific research. qMRI can be used for direct comparison of results across subjects and sites because it removes some confounding effects, such as experimental conditions, RF coils, equipment setups and so on. However, traditional qMRI usually takes long acquisition time and is very sensitive to subject motion since it depends on a series of images with different contrast weighting information.

Recently, we proposed a new imaging acceleration method called multiple overlapping-echo detachment (MOLED-4) planar imaging. MOLED-4 accelerates MRI acquisition by synchronously acquiring four echo signals, overlapped in the k-space, with different  $T_2$  weighting. A deep neural network model was designed to reconstruct  $T_2$  mapping from these overlapped signals. However, the accuracy of reconstructed  $T_2$  mapping is closely related to the noise level of training samples. To explore how noise level affects reconstruction accuracy, herein we designed six groups of training samples, which were randomly generated by simulation software, with noise level from 4 db to 104 db in an increment of 20 db, and trained the neural network with these samples. The networks trained by different groups of samples were then used to reconstruct human brain images experimentally acquired.

The results show that the texture of human brain will become blurred when the level of noise added to the training samples is too high, while the texture will become cluttered when the noise level is too low. A noise level that matches the experimental noise level yields most accurate  $T_2$  mapping.

Acknowledgement

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P062

Gadolinium effect at high magnetic field DNP: 70% <sup>13</sup>C polarization of [U-<sup>13</sup>C,U-<sup>2</sup>H] glucose using trityl

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<sup>1</sup>DTU, Health Tech, <sup>2</sup>Paul Scherrer Institute

The undisputed usefulness in biomedical applications, the high carbon polarization (up to 70%) and the long relaxation time constant after dissolution have made neat [1-<sup>13</sup>C]pyruvic acid (PA) plus trityl the most studied dissolution DNP sample.

Therefore, sometimes the literature is lacking detailed studies for different trityl-based samples, limiting the potential of other biologically interesting substrates. An example is the so-called “gadolinium effect”: admixture of trace amounts of Gd<sup>3+</sup> based compounds to the preparation can double the PA carbon polarization at 3.35 T, but has essentially no effect at higher field.[1,2]

Herein, we show that the trityl ESR properties, crucial for an efficient DNP process, are sample composition dependent. Working at 6.7 T with a substrate generally applicable mixture such as water:glycerol plus trityl, addition of Gd<sup>3+</sup> lead to a dramatic increase of [U-<sup>13</sup>C,U-<sup>2</sup>H]glucose polarization from 37±4% to 69±3%. This is the highest value reported to date and comparable to what can be achieved for pyruvic acid.[3] Moreover, performing ESR measurements at DNP conditions,using a home-built longitudinal detection (LOD) probe, we provide experimental evidence that gadolinium doping not only shortens the trityl electron spin-lattice relaxation time, but also modifies the radical g-tensor, leading to a considerable narrowing of the ESR spectrum linewidth. In the framework of spin temperature theory both effects, within the right boundaries, can justify an increase of the DNP enhancement.

The present study is of great interest for the hyperpolarization community because of the wide range of applications that highly polarized glucose can enable, and the insight into the DNP mechanism.[4]

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P063

**<sup>129</sup>Xe gas hyperpolarized via sublimation DNP at 6.7 T and 1.1 K using a reusable purpose built fluid path**

**Andrea Capozzi<sup>\*,1</sup>, Jan Henrik Ardenkjaer-Larsen<sup>2</sup>, Jean-Noel Hyacinthe<sup>3</sup>**

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<sup>3</sup>*Geneva School of Health Sciences, HES-SO, Switzerland*

<sup>129</sup>Xe is classically hyperpolarized using the well-established spin exchange optical pumping (SEOP) method. At optimal conditions and using <sup>129</sup>Xe enriched gas, SEOP can provide up to 3.6L/h of xenon hyperpolarized to 30%, enabling routine clinical <sup>129</sup>Xe MRI [1]. Besides SEOP, sublimation-DNP was proposed as an alternative technique to produce hyperpolarized xenon gas. Polarization levels of 5-7% were achieved with the promising advantage of using a generic dissolution DNP polarizer [2]. Although a deeper understanding of the xenon sample DNP matrix yielded higher polarization (i.e. 30% at optimal conditions using TEMPO free radicals at 5 T) [3], the throughput remains limited and the sample preparation cumbersome and hard to control [4]. Indeed, to increase the substrate concentration and thus the gas volume after extraction, the xenon is admixed to the radical doped glassing solvent when in the liquid state by mechanical stirring and repeated temperature cycles. In the present study, using a purpose build reusable fluid path (FP), we present a new approach for DNP xenon sample preparation. Admixture of xenon to the radical doped glassing solvent takes place directly in the sample cup. The latter is sonicated in a bath of melting ethanol (-114.1°C) until saturation of the solution. The cup can be connected leak-tight to the FP and loaded into the polarizer. As polarizing agent, we investigated the efficiency of the Finland trityl at 6.7 T and 1.1 K combining DNP and LOD-ESR measurements. The surprisingly short trityl radical T<sub>1e</sub>, due to the high magnetic field and presence of xenon in the matrix, required microwave frequency modulation to achieve a good DNP performance of 20% polarization in 2.5h.

**References:** [1] Norquay et al (2018), PRL, 121. [2] Comment et al. (2010), PRL, 105. [3] Capozzi et al. (2015), JPCCC, 119. [4] Kuzma et al, (2012), JCP, 137.

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## P064

**Investigating local environments of lanthanide doped  
yttrium aluminum garnet using solid-state NMR spectroscopy**

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Lanthanide-doped yttrium aluminum garnet (YAG) is a widely used class of materials in solid-state lasers as well as other optical applications. The analysis of the dilute substituent species requires powerful state of the art experimental techniques that focus on the local structures and how they interact with the YAG host lattice [1]. <sup>27</sup>Al solid-state Nuclear Magnetic Resonance (NMR) is a well-established tool for studying these atomic environments via the chemical shifts and the quadrupolar coupling parameters. The presence of the unpaired electrons of the lanthanide ions affects the NMR parameters of the observed nucleus which in turn provides an indirect probe to the electronic structure [2,3]. However, the paramagnetic shift anisotropy and inhomogeneous broadening due to bulk magnetic susceptibility can obscure the distinctive spectral features of the quadrupolar lineshapes, thus complicating their analysis, particularly in overlapping sites [3]. In this work, we investigate the effect of the substitution of a small percentage of Y<sup>3+</sup> with different lanthanide ions (Nd<sup>3+</sup>, Eu<sup>3+</sup>, Tb<sup>3+</sup> and Yb<sup>3+</sup>) using a combined high-resolution fast magic angle spinning (MAS) and two-dimensional satellite transition MAS spectroscopy (ST-MAS) [4]. We were able to identify paramagnetically shifted resonances that correspond to local environments with one and two lanthanide ions in the first coordination shell. We also demonstrate that we can separate the chemical/paramagnetic shift interactions from the rank 4 contribution of the second order quadrupolar interaction in the ST-MAS spectrum, which allowed the separation of overlapping sites and the extraction of quadrupolar parameters of the bulk and shifted sites.

**References:** [1] George, Nathan C., et al. Chem. Mater. 2013, 25, 3979-3995. [2] McCarty, Ryan J., et al. Solid State Nuclear Magnetic Resonance 79 (2016) 11–22. [3] Pell, A., et al. Prog. Nucl. Magn. Reson. Spectr. 111 (2019) 1–271. [4] Gan, Zhehong J. Am. Chem. Soc. 2000, 122, 3242-3243.

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3D printed Magnetic Tunnel for Hyperpolarization Transfer

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Christophe Pages<sup>2</sup>, Laurent Esteffe<sup>1</sup>, Jonas Milani<sup>3</sup>, Basile Vuichoud<sup>1</sup>,  
Quentin Chappuis<sup>1</sup>, Olivier Cala<sup>1</sup>, Sami Jannin<sup>1</sup>

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CNRS, CRMN FRE 2034, <sup>2</sup>ISA – Service Prototypes, <sup>3</sup>DNP Instrumentation

An unexpected great challenge in dDNP [1] has recently emerged with the use of ultra-shielded DNP magnets; the preservation of hyperpolarization during transfer from one ultra-shielded magnet to the other in ultra-low field regions, as magnetization not only relaxes but can be wiped-out in a non-adiabatic process.

We have recently proposed the design of a fluid path for dDNP [2] based on a micro gear pump (HNP Mikrosysteme mzt-11508X1) that could drive hyperpolarized samples at pressure exceeding 3 MPa, resulting in measured transfer times as short as 0.6 s over 5-meter-long distances.

However, transferring hyperpolarized solution at such speed through ultra-low field regions exacerbated this non-adiabatic polarization losses to the extent that enhancement factors of only two orders of magnitude were initially observed (when four orders of magnitude were expected).

To address this issue, we designed a simple 3D-printed magnetic tunnel [3, 4], based on basic blocks (straight, 45°, 135°, etc.) that can be assembled at wish, providing a safe near to 1 tesla magnetic field along the transfer. We then coupled this 3D-printed tunnel to our dissolution and transfer system and finally overcame the hurdles of hyperpolarization transfer through ultra-low fields.

We will discuss the process of non-adiabatic destruction of hyperpolarization and we will describe the design and performances of our full dissolution, transfer and injection system. We believe many laboratories may benefit from such a widely compatible system that 1) can be coupled to 5 mm bore magnets, 2) can accommodate any laboratory geometry, and 3) is highly resilient to any ultra-low fields environments.

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P066

Aggregation of Beta-Amyloid Peptides via Chemically Modulated Pathway

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The aggregation of beta-amyloid peptides is closely associated with Alzheimer’s disease. We have used different chemical devices such as liposomes and reverse micelles to encapsulate the 40-residue beta-amyloid peptides so that the spatial confinement could lead to the formation of nonfibrillar aggregates of beta-amyloid peptides. These on-pathway beta-sheet intermediates were used to seed the fibrillization of the monomer peptides. Solid-state NMR spectroscopy, particularly the ultra-fast MAS spectrum acquired at a spinning frequency of 140 kHz, revealed that the resultant proto-fibrils or fibrils have a more uniform structure than those formed in bulk solution, suggesting that the phenomenon of structural polymorphism commonly observed in beta amyloid fibrils may be largely due to multiple nucleation events.

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P067

Dynamic Nuclear Polarization Breaking out of the spin diffusion barrier?

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Dynamic nuclear polarization (DNP) has proved to be a powerful and versatile means to overcome the intrinsically low sensitivity of NMR[1]. A great challenge for DNP at present is to achieve high spin polarization in shorter times, which requires understanding spin polarization transfers but also spin diffusion dynamics[2].

Spin diffusion is indeed of major importance nearby the free electrons, precisely where DNP is supposed to be most efficient, but unfortunately paramagnetic shifts are said to prevent nuclei from sharing polarization with the rest of the sample. As a consequence, the so-called diffusion barrier is ubiquitous in the literature on DNP and MAS-DNP[3].

Using simple DNP and <sup>1</sup>H pulsed NMR experiments, we have witnessed the existence of an invisible polarization spin reservoir that is able to replenish significant polarization to the observable proton spin reservoir, after full saturation and switching off the microwaves.

A two-reservoir model can efficiently describe the experimental data and allows determination of the polarization exchange rate between the visible and hidden reservoirs, as well as their intrinsic relaxation and build-up rates. Relaxation were found to be significantly lower at 1.3 K than 3.8 K, as expected. Intriguingly, the exchange rate was also found to have a similar dependence, suggesting a phonon mediated mechanism.

Several possible explanations are proposed to account for the existence of this hidden reservoir: <sup>1</sup>H Zeeman order nearby electrons, 1H-electron dipolar order and <sup>2</sup>H Zeeman order cross-relaxing to <sup>1</sup>H. The relevance of these possible contributions will be discussed, and supported by further experiments.

We believe this finding will shed light on one of the most important, and misunderstood, fundamental mechanism of DNP.

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Structural transition of aqueous Melittin in presence of Trifluoroethanol:  
A NMR relaxometry and DNP Study

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Chandrakumar Narayanan<sup>2</sup>, Samanwita Pal<sup>1</sup>

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Investigation of structural transition of proteins/peptides is a major area of research unraveling their structure, dynamics and functions. In the present study we aim to understand the solvation dynamics of a model peptide Melittin (MLT) with potential antimicrobial and anticancer property. Over last decades, studies related to structural transition exhibited by MLT in solution with varying physical conditions such as temperature, pH and concentration have gained considerable attention. However, the effect of solvent on such structural dynamics are yet to be explored cogently. In this context we present a set of experiments based on NMR relaxometry and Dynamic Nuclear Polarization (DNP) studies to decipher the effect of trifluoroethanol (TFE) as a cosolvent for aqueous MLT. The choice of experiments performed at low field at one hand enabled us to overcome the strong contribution of 19F CSA at high field while the ODNP condition allowed probing molecular dynamics of the order of 10-100 picoseconds timescale regime present in MLT solution.

In accordance with the literature, Circular Dichroism (CD) experiments performed for aqueous MLT solution with TFE as cosolvent at two different physiological conditions demonstrated monomer-tetramer transition. Further, MLT structural transitions were characterized by observing the changes in the dynamical properties of TFE by employing 19F relaxation and diffusion measurements at 11.7 T. A series of 19F relaxation experiments were also performed at low magnetic field of 0.35 T in presence and absence of a polarizing agent TEMPOL. The steady state ODNP experiments allowed us to probe the solvation dynamics of TFE around a melittin with the aid of the change in TFE diffusion coefficient (D) that was extracted using TFE correlation time. The decay in average D (TFE and TEMPOL) in presence of Melittin as a function of TFE concentration could be attributed to the gradual tetramer to monomer transition.

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P069

An accurate detection method of human blood glucose by low field nuclear magnetic resonance

Yi Chen, Qing Yang, Rongsheng Lu, Zhonghua Ni, Hong Yi

This article is committed to developing a portable method for accurate detection of human blood glucose by low field nuclear magnetic resonance technology. A portable NMR apparatus (magnetic field frequency: 26 MHz) was constructed by manufacturing a miniature NMR probe for micro sample detection and integrated with a lightweight permanent magnet and an electronic control system.

In order to improve the detection sensitivity of human blood glucose by the present NMR apparatus, serum samples of different glucose concentrations were pre-reacted with a small amount of glucose oxidase and ferrous ions for 1 hour before nuclear magnetic measurement. Hydrogen peroxide produced by the catalytic decomposition of glucose in serum can oxidize ferrous ions, and the significant ferromagnetic transition between ferrous iron and ferric iron can be accurately captured by NMR lattice relaxation detection. The triplet pulse sequence (TPS) is used to measure the lattice relaxation, which saves more than one order of magnitude of measurement time compared to the commonly used IR pulse sequence.

The experimental results detected by our NMR apparatus show that when the blood glucose concentration of different samples is from 4 to 20mM, the T1 time gap between tested sample and blank groups is accordingly from 50 to 400ms, and there is a good linear relationship between blood glucose concentration and T1 time. Therefore, a mathematical model regarding serum glucose concentration and LF-NMR detection results can be obtained by experiments. In conclusion, the proposed portable low field NMR method can be an effectively used tool for human blood glucose accurate detection.

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**P070****Optically-generated Overhauser Dynamic Nuclear Polarization**Daniel Cheney\*, Christopher Wedge*University of Huddersfield*

Overhauser Dynamic Nuclear Polarization (DNP) is a powerful technique for improving the signal-to-noise ratio of liquid state NMR. It usually uses microwaves to saturate the EPR transitions in radicals, which, owing to cross-relaxation, results in polarization transfer to nuclear spins. An alternative approach has recently been demonstrated, using visible light to generate triplet states of a dye, which is quenched to produce electron spin polarization via the radical-triplet pair mechanism (RTPM). The main advantage of this approach lies in its potential ability to enhance signals beyond that which is possible in microwave-driven DNP.

One of the most significant limitations of Overhauser DNP is that polarization transfer to dipolar-coupled protons becomes less efficient at high magnetic fields, where most modern NMR is carried out due to its improved resolution and chemical shift dispersion. A number of groups have attempted to counter this by generating polarization at low fields, then transferring the sample to high fields for detection. In our work, we attempt to generate light-induced DNP of water protons, using the nitroxide radical TEMPO, and the xanthene dye rose bengal, in the fringe field of a 1 T benchtop NMR magnet. This is rapidly transferred to the detection field via peristaltic pumping. With the aid of numerical simulations, extensive studies have been carried out to optimize the radical and dye concentrations, the polarization field, the sample geometry, and the degree of solvent deuteration.

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## P071

**EPR studies of neuroglobin immobilized within mesoporous materials**

H.Y. Vincent Ching<sup>\*1</sup>, Zainab Hafideddine<sup>2</sup>, Stefano Loreto<sup>3</sup>, Pegie Cool<sup>3</sup>,  
Vera Meynen<sup>3</sup>, Sylvia Dewilde<sup>4</sup>, Sabine Van Doorslaer<sup>1</sup>

<sup>1</sup>Department of Physics, University of Antwerp, <sup>2</sup>Departments of Physics &  
Department of Biomedical Sciences, University of Antwerp, <sup>3</sup>Department of Chemistry,  
University of Antwerp, <sup>4</sup>Department of Biomedical Sciences, University of Antwerp

Proteins are increasingly being used in numerous technological applications such as biosensors, biofuel cells and biocatalysts. Often the immobilization of a protein in a synthetic matrix is essential.[1,2] Porous matrices have warranted much attention due numerous factors, such as their high surface areas, meaning improved protein loading and high number of active sites; the protection of the protein in the pores from possible bacterial degradation; and/or for the controlled release of the proteins.

Nevertheless, the incorporation of a protein in a porous material is nontrivial. In fact, the preparation of such protein loaded materials is often limited by the size compatibility between the pore and the protein, the surface properties (charge and hydrophobicity) and experimental parameters such as pH and ionic strength.[3,4] Moreover, the quest for a suitable protein-porous matrix combination not only require the synthesis of matrices with tailored textural properties, but also the development of appropriate methodologies that can be used to determine the effect of the synthetic matrix on the structure and stability of the protein.

Recently, we have been interested in the incorporation of human neuroglobin (NGB) into mesoporous silica (SBA-15) and titania with potential application in biosensing. Using EPR, we have monitored conformational changes to the NGB heme-pocket upon incorporation and subsequent manipulations. We have also performed site-directed mutagenesis and spin labeling, and have compared the mobility and structure of free and immobilized NGB using EPR. These results will be presented.

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P072

NMR Study of DNA Binding Domain of Transcription Factor MEIS1  
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Transcription factors are proteins that bind specific sites or elements in regulatory regions of DNA, known as promoters or enhancers, where they control the transcription or expression of target genes. Homeobox genes, of which the most well-characterized category is represented by the HOX genes, play a crucial role in normal development. MEIS1 (myeloid ecotropic viral insertion site 1) is a viral integration site in murine myeloid leukemia cells. This gene encodes a homeobox protein belonging to the TALE ('three amino acid loop extension') family of homeodomain-containing proteins. TALE proteins are distinguished by the presence of three extra amino acids in the loop binding the first to the second alpha helix of the homeodomain. The highly conserved DNA-binding TALE proteins define the family and is responsible for specific recognition of a common sequence motif, [5'- TGACA- 3']. Further DNA-binding specificity within TALE family members is determined by adjacent DNA sequences and through the binding of additional transcriptional partners.

We performed <sup>1</sup>H/<sup>15</sup>N HSQC experiment and imino proton NMR experiments between MEIS1 and consensus MEIS1 target DNA (msDNA). The exchange rate constants of the imino protons for the wild type and mutant were measured by using water magnetization experiment. We compared to the binding affinity of MEIS1 for both wild type and mutant and characterized its target DNA recognition.

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P073

**GGauss/s Rapid frequency scan Electron Spin Resonance enabled  
by phase-locked voltage-controlled oscillator**

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Rapid-scan (RS) electron-spin resonance (ESR) is a technique to boost the signal-to-noise ratio (SNR) in ESR experiments by allowing the use of larger B1 magnitudes without saturating the sample. State-of-the-art rapid-scan setups [1] use fast field sweeps produced by modulation coils in combination with relatively low-Q resonators to achieve a large detection bandwidth. Due to effects of the coil inductance and coil heating, the maximum scan rate is limited and the state-of-the-art reported to date using this approach is about 10 MGauss/s [1]. With such scan rates, rapid-passage effects were observed for samples with relatively long relaxation times in the  $\mu$ s range [1]. For samples with shorter relaxation times, the required scan rates are significantly higher.

This poster discusses the advantages of using the recently proposed voltage-controlled oscillator (VCO) [2] EPR-on-a-chip approach that enables scan rate in the GGauss/s range, thereby greatly increasing the application range of the RS ESR technique. In the proposed poster, we will mostly focus on the possibility of embedding the VCO into closed loop controls for the oscillator phase and amplitude. Here, a precise control of both the phase and the amplitude of oscillation are crucial for a faithful reconstruction of the continuous-wave ESR spectra from the RS data.

Together with a theoretical discussion of closed-loop VCO based ESR experiments, in the proposed poster we will show measured RS data from an array of injection-locked VCOs that is phase-locked to an external reference with a phase-locked loop (PLL) bandwidth exceeding 40 MHz, enabling closed-loop scan rates exceeding 1 GHz/ $\mu$ s.

**References:** [1] J. Möser et al, Using rapid-scan EPR to improve the detection limit of quantitative EPR by more than one order of magnitude, JMR 281. [2] A. Chu et al., An 8-channel 13 GHz ESR-on-a-Chip injection-locked vco-array achieving 200  $\mu$ M-concentration sensitivity, ISSCC 2018.

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## P074

## Amplitude-Modulated High-Power XiX Decoupling

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Phase-alternating decoupling (XiX) [1,2] is one of the simplest and efficient high-power decoupling methods in solid-state NMR for faster MAS frequencies. It consists of two pulses of equal length but with a phase difference of  $180^\circ$ . We present variations of this heteronuclear decoupling sequences with improved performance based on numerical calculations of the second-order cross terms [3]. By changing either the amplitude or the length of one of the two pulses, the effective nutation angle of the basic two-pulse element of XiX is changed from 0 to  $180^\circ$ . Such a modification leads to narrower lines compared to the standard high-power XiX scheme. We show experimentally that the two options to implement the effective  $180^\circ$  flip angle, either by amplitude or by pulse-length variation, lead to similar results and significantly longer  $T_2'$  times compared to standard XiX. For both sequences the optimum parameters can be calculated from experimental settings with simple analytical expressions, resulting in a decoupling scheme, which is easy to set up.

**References:** [1] P. Tekely, P. Palmas, D. Canet, Effect of Proton Spin Exchange on the Residual  $^{13}\text{C}$  MAS NMR Linewidths. Phase-Modulated Irradiation for Efficient Heteronuclear Decoupling in Rapidly Rotating Solids, J. Magn. Reson. Ser. A. 107 (1994) 129–133. [2] A. Detken, E.H. Hardy, M. Ernst, B.H. Meier, Simple and efficient decoupling in magic-angle spinning solid-state NMR: the XiX scheme, Chem. Phys. Lett. 356 (2002) 298–304. [3] K.O. Tan, V. Agarwal, B.H. Meier, M. Ernst, A generalized theoretical framework for the description of spin decoupling in solid-state MAS NMR: Offset effect on decoupling performance, J. Chem. Phys. 145 (2016) 094201. doi:10.1063/1.4961909.

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P075

In-situ NMR highlights structural change during apple heating

Sylvie Clerjon\*, Alexandre Leca, Catherine Renard, Jean-Marie Bonny, Amidou Traore  
INRA

Microstructure and texture evolution of foods during cooking is difficult to characterize, because conventional techniques cannot analyze the internal structure, while preserving product’s integrity. For apple, one of the most processed fruits, understanding thermal degradations in essential. Such phenomena was thus approached with *in-situ* quantitative NMR during the cooking process.

Five Golden Delicious apples were sampled in four 10 mm long and 5 mm diameter cylinders. Each of the twenty samples was sealed in parafilm. The thermal treatments were performed *in situ* at 9.4 T using a 5-mm diameter microimaging RF coil. A controlled hot air flow cooked the sample from 20 to 60°C, 2°C-step. After 6 min for stabilization at each temperature plateau, T2 measurement were performed with a CPMG pulse sequence ( $\tau=125\mu\text{s}$ ,  $\text{TR}=5\text{s}$ , 256 echoes, acquisition duration=2min30s).

All the echo decay curves were analyzed using non-negative least squares algorithm [1]. An insight to the evolution of the internal structure of the sample was gained by studying the resulting T2 distribution.

The T2 distributions displayed two distinct patterns before and after 53°C. Indeed, the T2 of vacuolar, cytoplasmic and cell wall compartments evolved little before 53°C with a marked shift in the T2 value of the vacuolar fraction at 53°C (from 270 ms to 200 ms) along with the disappearance of the cytoplasmic fraction. This critical temperature is in accordance with firmness changes measured on samples cooked at various temperature.

This study points out the powerful of multiexponential T2 analysis in resolving subcellular changes during apple *in situ* cooking at high magnetic field. It provides longitudinal information about structure change, probably attributable to the membrane degradation [2], on a same sample during the whole process.

References: [1] Istratov, A. A. and O. F. Vyvenko, Rev. Sci. Instrum. (1999). [2] Gonzalez, M. E., et al, J. Food Sci. (2010).

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P076

Quantum mechanical MRI simulations: solving the matrix dimension problem

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We propose a solution to the matrix dimension problem in quantum mechanical simulations of MRI experiments on complex molecules. This problem is very old; it arises when Kronecker products of spin operators and spatial dynamics generators are taken – the resulting matrices are far too large. However, spin and spatial operators individually have manageable dimensions, and the action by their Kronecker products on any vector may be computed without opening those products; this procedure is now implemented in *Spinach*.

MRI simulations of complex metabolites in 3D with diffusion, flow, kinetics, and quantum mechanical treatment of spin relaxation, are now possible, as well as simulations of spatially distributed ultrafast, pure shift, diffusion, and flow driven NMR experiments. This level of generality hinges on:

1. The ability to treat classical degrees of freedom (diffusion, hydrodynamics, radiofrequency and microwave phases, stochastic tumbling, etc.) at the same conceptual level as spin degrees of freedom [1].
2. The ability to survive enormous Kronecker products. In realistic systems (ten spins in 3D), the direct products of spin and spatial dynamics generators have the dimension in excess of  $10^{12}$  [2].
3. Code parallelisation over cluster architectures, including the possibility of using a GPU on each node of the cluster.

This report is about solving all of this, and on where the dark art of simulating a time-domain magnetic resonance experiment stands at the moment. Two recent innovations are the abandonment of Liouville equation in favour of Fokker-Planck equation [1] as the core formalism, and the use of tensor structured objects that never open Kronecker products [2].

**References:** [1] I. Kuprov, “Fokker-Planck formalism in magnetic resonance simulations”, *Journal of Magnetic Resonance*, 2016, 270, 124-135, and references therein. [2] A.J. Allami, M.G. Concilio, P. Lally, I. Kuprov, “Quantum mechanical MRI simulations: solving the matrix dimension problem”, *Science Advances*, in press.

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## P077

Strategies for  $^1\text{H}$ -detected dynamic nuclear polarization magic-angle spinning NMR.Maria Concistre<sup>\*1</sup>, Subhradip Paul<sup>2</sup>, Philip Williamson<sup>1</sup><sup>1</sup>University of Southampton, <sup>2</sup>University of Nottingham

Over the last two decades there have been remarkable advances in solid-state NMR (SSNMR) experiments for the characterization of protein structure and function. However still at this time, some applications of SSNMR are complicated, impaired or made impossible by the intrinsic low sensitivity of the technique. Moreover, most protein studies in solid-state NMR have so far relied on labelling and detection of nuclei with low gyromagnetic ratios including  $^{13}\text{C}$  and  $^{15}\text{N}$ , a technique which is time consuming, costly and not always feasible.

To overcome these limitations, advances are being made in two, potentially complementary, areas: dynamic nuclear polarization (DNP) and proton-detect fast-MAS NMR. Extending these approaches to large systems, complex biomaterials and unlabeled samples require a combination of these two methodologies. This is the ultimate aim of this work but such combination is not as trivial as it may sound. On the one hand, proton detection in solids is notably difficult since the density of proton within biomaterials results in a strong network of homonuclear dipolar couplings which significantly compromise resolution. On the other hand, MAS-DNP is typically run at 100K mostly using radicals exploiting the cross-effect and despite improvements in technology, the spinning speeds available on DNP systems still lag behind those of conventional fast-MAS probes for  $^1\text{H}$  detection. On a positive note, the development of new radicals for use at high magnetic fields and under fast-MAS is an area of active research.

In this contribution we address some of these challenges and the advances done towards  $^1\text{H}$ -detected DNP-enhanced MAS. Working on a model sample made by the protein GB3 expressed in different labelled varieties and degree of deuteration, we demonstrate that proton-detected MAS-DNP may be used to study biomaterials in 'widely' available commercial instruments.

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P078

### Introducing specificity in solid-state DNP NMR of RNA

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*University of Rostock*

Dynamic nuclear polarization (DNP) provides a significant sensitivity gain for solid-state NMR under magic-angle spinning (MAS). However, both resolution and selectivity are of high importance as well, and may be compromised under DNP conditions. Particularly, this applies towards biomolecular applications where inhomogeneous line broadening under cryogenic conditions often prevents single-site resolution which is required for the unique assignment of spectroscopic resonances to specific building blocks in biopolymers.

Ribonucleic acid (RNA) poses an extraordinary challenge, as rather long oligomers are constituent of only four different nucleotide building blocks but may adopt a wide range of different conformations and interaction motifs. Furthermore, the ribose as the subunit with the largest degree of structural flexibility, however, is chemically equivalent in every monomer. Therefore, spectral overlap under DNP conditions typically hinders the direct extraction of geometrical constraints for uniformly isotope labeled samples.

In this presentation, several routes towards achieving single-site resolution and/or spectral selectivity will be discussed. First, simple isotope labeling schemes utilizing the unique ability of RNA to spontaneously hybridize from individual strands can be used to build the RNA complex in a modular manner. This significantly reduces the number of isotope-labeled nucleotides (thus increasing the chance to obtain single-site resolution) as well as enables orthogonal labeling (e.g.,  $^{13}\text{C}/^{15}\text{N}$ ) for specific observation of inter-strand as well as inter-stem contacts. This can be achieved with efficient in-vitro transcription without need for highly expensive solid-phase synthesis. Second, utilization of a specifically bound paramagnetic metal-ion polarizing agent may enable the extraction of direct electron-nucleotide distances in combination with the aforementioned isotope-labeling schemes by direct metal DNP. Finally, the introduction of methyl groups by binding of either small ligands or proteins allows for specific spectroscopy by SCREAM-DNP (Specific Cross Relaxation Enhancement by Active Motions under DNP) using a direct  $^1\text{H}$ - $^{13}\text{C}$  cross-relaxation transfer between ligand and RNA.

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P079

Generating, Storing and Transporting Hyperpolarization with  
HYPOP (Hyperpolarizing Porous Polymers)

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Chemistry, Catalysis, Polymers and Processes

Fifteen years after its invention, dissolution dynamic nuclear polarization (dDNP)[1] has become one method of choice to enhance signals in both MRI and liquid state NMR. Despite exciting prospects, this method remains restricted to few research groups as it needs in principle to be performed at the point of use, with specialized equipment and personnel. However, a series of recent advances have successfully demonstrated that dDNP could potentially be performed remotely, i.e. without the need of a polarizer on-site.[2]

In this work, we present a new generation of polarizing matrices termed HYperPolarizing Polymers (HYPOP). [3] These matrices offer the possibility of physically separating the molecules of interest from the polarizing agents on hundreds of nanometer scales by immobilizing the polarizing agents inside the protonated porous polymer walls. This porous material can be hyperpolarized and impregnated with solutions of interest. Polarization is transferred to the target nuclear spin through <sup>1</sup>H-<sup>1</sup>H spin diffusion followed by <sup>1</sup>H-<sup>13</sup>C cross polarization.

In this context, we show that HYPOP can polarise metabolites mixtures in solution as well as neat pyruvic acid. Here we will show

- 1) how significant amounts of carbon-13 polarization (P(<sup>13</sup>C) > 20%) can be generated using cross polarization on carbon-13 containing compounds embed in the matrix
- 2) that these exhibit polarization storage lifetimes of several hours at 4.2 K, offering a new route to transportable hyperpolarized molecules
- 3) that the extraction of the molecules of interest out of the HYPOP can be done while retaining a significant part of the hyperpolarization.

References: [1] Ardenkjaer-Larsen, J. H. et al. Proc. Natl. Acad. Sci. 100, 10158–10163 (2003). [2] Ji, X. et al.. Nat. Commun. 8, Article number: 13975 (2017). [3] El Darai, T. et. al. In preparation.

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## P080

On the  $^{91}\text{Zr}$  chemical shielding scale

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The  $^{91}\text{Zr}$  SSNMR has become a useful tool for structural studies of crystalline systems, for example zirconocenes and zirconium-containing metal-organic frameworks, despite of a low natural abundance (11.2%) and receptivity ( $1.07\text{E}-3$  relative to  $^1\text{H}$ ), and a quite high quadrupole moment ( $-0.176\text{E}-28\text{ m}^2$ ), of the  $^{91}\text{Zr}(I=5/2)$  nucleus. However, the  $^{91}\text{Zr}$  chemical shielding scale has not yet been established, even though several standards are being used in  $^{91}\text{Zr}$  SSNMR investigations. This issue has been addressed by carefully selecting an extended set of compounds with precisely determined structures and with reliably measured  $^{91}\text{Zr}$  SSNMR chemical shifts,  $\delta$ , and performing the plane-waves density functional theory (PW DFT) based calculations of the corresponding chemical shielding values,  $\sigma$ , while covering almost 500 ppm in the region of typical bonding environments of zirconium atoms. The level of agreement between PW DFT computations (carried out using several functionals and schemes for a treatment of the relativistic correction) and experiment has been established. Based on this, for an immediate estimation of the chemical shift from a GIPAW-RPBE-ZORA calculation of the chemical shielding, it is proposed to use

$$\delta = -\sigma + 1876 \text{ ppm}$$

with  $\pm 25$  ppm of a tight (95%) confidence band of the intercept (it should be noted that the slope is fixed at  $-1.0$  in this simple model). In addition, an accuracy of PW DFT predictions of the  $^{91}\text{Zr}$  quadrupolar and the chemical shift anisotropy parameters has been evaluated. These results are crucial for the NMR crystallography approach to zirconium compounds in the condensed phase. Specifically, the local environment of zirconium sites in ring-opening polymerization catalysts has been accurately characterized by a combination of advanced  $^{91}\text{Zr}$  SSNMR techniques (WURST-QCPMG with VOCS), PW DFT calculations, and X-ray diffraction analysis.

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P081

### Hyperpolarized singlet state after geminal hydrogenation with para-hydrogen preserved by spin-locking

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It has been demonstrated that hyperpolarization using PHIP can be effectively achieved using a ruthenium-based *trans*-hydrogenation catalyst in contrast to typical *cis*-hydrogenation reaction [1,2]. This led to a development towards hyperpolarized metabolites such as fumarate, using pairwise *trans*-hydrogenation of an unsaturated precursor [3]. Aside of the desired reaction to form fumarate, the ruthenium-based catalyst also forms a geminal hydrogenation (gem-hydrogenation) product [4], where the reaction with parahydrogen populates the singlet state of the geminal pair of protons. Therefore, to investigate the possibly long-lived singlet state of the gem-hydrogenation product, the Sarkar pulse sequence was employed [5]. The populated singlet spin order was maintained by a spin locking RF-field of variable duration at different magnetic fields.

The initial singlet state lifetime measurements were done on a degassed sample made via *trans*-hydrogenation reaction of disodium acetylenedicarboxylate using [RuCp<sup>\*</sup>(MeCN)3] PF6 as acting catalyst. Further experiments were involved performing the same reaction with para-enriched hydrogen at 333 K during active spin-locking.

The singlet state lifetime measurements indicated a long-lived state at different magnetic fields. Moreover, these findings were followed by a successful attempt to use spin-locking to store hyperpolarization of the gem-hydrogenation product for the order of minutes. Herewith, spin locking was proven to be an effective method to preserve molecules' CH2 group singlet state spin order, which is of interest for possible applications e.g. as MRI contrast agent [6].

**References:** [1] D. Schleyer et al., New J. Chem., 25, 3 (2001). [2] K. Radkowski et al., Angew. Chem, 52, 1 (2013). [3] B. Ripka et al, Chem. Commun., 54, 12246 (2018). [4] M. Leutzsch et al, Angew. Chem., 54, 42 (2015). [5] R. Sarkar et al, J. Am. Chem. Soc. 129, 2 (2007). [6] K.M. Brindle et al, PNAS, 66, 2 (2011).

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## P082

**An experimental access to the microwave saturation factor at 9.4 Tesla in liquid state**Danhua Dai, Vasył Denysenkov, Thomas F. Prisner<sup>2</sup><sup>2</sup>*Institute of Physical and Theoretical Chemistry and Center for Biomolecular Resonance*

Liquid state (Overhauser) Dynamic Nuclear Polarization (DNP) experiments have been performed at high magnetic fields (9.4 T) on aqueous solution of <sup>14</sup>N-TEMPOL nitroxide radicals. To determine the saturation factor of the electron spin, the <sup>1</sup>H paramagnetic shift as a function of the microwave power was measured as previously reported [1]. We could show that the paramagnetic shift depends on the <sup>14</sup>N-TEMPOL radical concentration. The suppressed paramagnetic shift can be determined by measuring the NMR line shift as function of the applied microwave power, which allows a quantitative determination of the DNP saturation factor.

**Reference:** [1] P. Neugebauer, J. G. Krummenacker, V. P. Denysenkov, G. Parigi, C. Luchinat and T. F. Prisner, Phys. Chem. Chem. Phys., 15, 6049 (2013).

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59Co Internal Field Nuclear Magnetic Resonance:  
A complimentary tool to identify phase composition of cobalt nanoparticles  
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<sup>1</sup>Bangalore University, <sup>2</sup>Indian Institute of Science

It is known that cobalt exhibits polymorphism: it exists in both hcp and fcc phases. Synthesis method and other thermodynamic conditions are known to play a role in determining the phase composition of cobalt nanoparticles. In this work, we have studied the phase composition of the cobalt nanoparticles synthesized using two different solvents (water) and ethanol. XRD measurements confirm the existence of fcc phase in commercial cobalt nanoparticles (Co@A), co-existence of fcc and hcp phases in Co@B (water as a solvent), while the existence of the hcp phase in Co@C (ethanol as a solvent). We have determined the phase composition of cobalt nanoparticles using 59Co Internal Field Nuclear Magnetic Resonance (IFNMR) technique. We have used a homemade IFNMR spectrometer operating in the frequency range 10-230 MHz. Our studies reveal that the Co@A has fcc as a major phase with minor quantity hcp phase. Co@B exhibits approximately equal amount of fcc and hcp phase while Co@C exhibits hcp as a major phase with minor fcc phase. SEM micrograph studies confirm the cobalt particles to have spherical shape in the fcc phase. The cobalt particles exhibit both spherical and dendrite morphology confirming the co-existence of fcc and hcp phases, while the sample with pure hcp phase exhibit the dendrite morphology. Our studies also throw light on understanding the effect of solvent in the phase formation of the cobalt nanoparticles.

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**Accurately determined solution structure of small molecule drugs  
reveals link between solution and solid-state conformational behaviour**

Hugh Dannatt\*, Martin Watson, Zoltan Takacs, Charles Blundell

*C4X Discovery*

Accurately determined conformations of biological agents are valuable assets in drug discovery. By enabling rational modifications, knowledge of the conformational envelope of biological ligands may enable the design of increased binding affinity, reduced off-target effects, or the discovery of an entirely new chemical series through “scaffold hopping”. The use of NMR to study the range of conformations adopted by molecules in solution (their “dynamic 3D-structure”) is becoming more widespread, however the success of this approach can be undermined by a sparsity of <sup>1</sup>H nuclei, poor signal dispersion, or exchange processes.

The fenamates are a group of small molecules (<300 Da) that have biological activity as non-steroidal anti-inflammatory drugs, and have been used to understand crystal nucleation, growth and polymorphism. Their structure is such that that no conformationally-dependent <sup>1</sup>H-<sup>1</sup>H scalar couplings and very few distance-defining NOE measurements can be made. Signal dispersion is also poor, deepening the challenge that these molecules present. Although there was no single set of measurements that permitted the determination of their solution conformational behaviour, the dynamic 3D-structures of two very similar members of the group – mefenamic acid and flufenamic acid – were accurately determined by the synergistic combination of orthogonal NMR parameters.

The results reveal clearly measurable differences in the conformational envelopes of the two molecules, which are in agreement with their conformational behaviour as studied by small molecule and protein crystallography. Not only does this further demonstrate our ability to precisely determine molecular shape for use in rational design, these results provide direct experimental evidence for a link between behaviour in different phases, which has implications on the interpretation and use of data collected by different experimental techniques, and may further our understanding of self-assembly and nucleation processes.

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P085

Multi-nuclear NMR detectors - a comprehensive study

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One of the most powerful features of nuclear magnetic resonance spectroscopy is the ability to address almost each element of the periodic table. Building up on this feature, addressing multiple nuclei simultaneously represents another source of information that NMR is capable to deliver, contributing to elucidate the structure and dynamics of a molecular system with atomic resolution. At the same time, this also represents a source of technical challenges in the field of multi-nuclei detection hardware development.

It is the purpose of this contribution [1] to comprehensively present the ensemble of technical challenges related to NMR detection techniques where single coil detectors are used. We cover different RF circuit topologies where multiple frequencies are resonant with a single coil, i.e., trap and tank circuits, as well as the broadband detection schemes, i.e., delay line, transmission line, non-tuned, non-matched coils. We analyse the building blocks of these complicated circuits and investigate in detail the performance of these methods by pondering the challenges and the opportunities.

This work also aims at providing a unitary basis to discuss the RF engineering challenges associated with addressing multiple frequencies and at the same at bridging the “understanding” gap among different groups of NMR practitioners.

Reference: [1] H. Davoodi , M. Jouda, J. G. Korvink, N. MacKinnon, and V. Badilita, *Broadband and multi-resonant sensors for NMR*, Progress in Nuclear Magnetic Resonance Spectroscopy (2019).

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**P086**

### Introducing Dynamics to NMR Crystallography of Aluminophosphate Zeolitic Frameworks

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*University of St Andrews*

Over the last decade, the use of periodic density functional theory (DFT) calculations to aid the assignment and interpretation of NMR spectra of complex solids has become almost mainstream. This new link between bulk crystallographic structure and local NMR interactions via the electron density has given birth to the field of NMR crystallography.

Here, we investigate the NMR crystallography of  $^{31}\text{P}$  NMR spectra of aluminophosphate zeolites (AlPOs), which are of interest as adsorbents, catalyst supports and catalysts, as well as having unusual negative thermal expansion (NTE) behaviour.  $^{31}\text{P}$  NMR spectroscopy is a valuable technique for characterising the local structure of AlPOs, capable of providing information on the number of crystallographic P sites, their relative populations, and the positions of any dopant atoms in the framework. Assigning  $^{31}\text{P}$  NMR spectra may, however, require multinuclear NMR experiments and/or DFT calculations, which can be time-consuming, computationally costly, and challenging in cases involving disorder or dynamics.

We demonstrate that the DFT-level  $^{31}\text{P}$  chemical shift ( $\delta_{\text{iso}}$ ) can be predicted reasonably accurately (to within  $\sim 1.1$  ppm) using a simple structure-spectrum relationship based on the mean P-O bond lengths and P-O-Al bond angles. We automate this prediction process using the DIStortion COde (DISCO), which reduces the computational cost to only  $\sim 3$  ms per P atom. We then apply these accurate, low-cost predictions of  $^{31}\text{P}$   $\delta_{\text{iso}}$  to various situations including the presence of disordered guest molecules within the pores or disordered framework-bound anions (where the structure is insufficiently complete to allow DFT calculations), and to the outputs of molecular dynamics (MD) calculations (where the sheer volume of structures generated makes DFT calculations prohibitively costly). The use of MD calculations allows us to investigate NTE, with DISCO providing a link between this unusual and interesting bulk phenomenon and the underlying local structural changes.

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## Self-organized Gold Nanoreceptors on Silica Nanoparticles for NMR Chemosensing

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## Abstract

Nanoparticle-assisted NMR chemosensing exploits the recognition abilities of gold nanoparticles in solution to detect and identify specific classes of molecules in multi-analyte mixtures. The nanoparticle-analyte recognition event is exploited to induce a selective magnetization transfer via the nuclear Overhauser effect from the nanoparticle's monolayer to the interacting analytes only. The big advantage of NMR chemosensing over conventional chemosensors is the possibility to recover the complete NMR spectrum of selected (classes of) molecules, thus allowing the immediate identification of false positives.

In this context, we propose an improvement of the current technique which lowers the detection limit down to 10  $\mu\text{M}$  on standard instrumentation. The method can be applied in all cases where charged monolayer-protected gold nanoparticles are used to sense organic ions in solution, and it is based on the electrostatic adhesion of the nanoreceptors onto larger silica nanoparticles of opposite charge. This arrangement allows to enhance the overall rotational correlation time of the receptor and improve the spin-spin dipolar interactions responsible for the magnetization transfer, without however affecting the affinity constant of the binding equilibrium.

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## P088

## Design of paramagnetic tags for NMR Spectroscopy:

## A new strategy to yield fast, selective and irreversible tagging of protein

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Paramagnetic restraints have been used in Biomolecular NMR for the last three decades in order to elucidate and refine structures but also to characterize protein/ligand interactions[1]. Pseudo Contact Shifts (PCS) are the most commonly used restraints, consisting in measuring a shift between a paramagnetic species and a diamagnetic reference, whose magnitude depends on the distance from the paramagnetic centre. A common technique to generate such restraints consists in the attachment of Lanthanides ions to the protein via a Lanthanide-Binding-Tag (LBT).

In order to design such LBTs, it is important to consider the efficiency and stability of the conjugation, the geometry of the complex (conformational exchanges and coordination) and the chemical inertness of the ligand. Here we describe a photocatalyzed thiol-ene reaction for the cysteine-selective paramagnetic-tagging of proteins[2]. We designed three LBTs with vinylpyridine moieties which were attached to model protein GB1 and medically relevant tcPex14[3] in a fast and irreversible fashion. One of those vinyl-pyridine containing ligand is a cross-bridged cyclam, introduced here as a new class of highly rigid and inert tags for NMR. Those three ligands yielded medium to large tensors with different Lanthanides and were carefully characterised via NMR and Relaxometry. The influence of the coordination geometry on the tags' properties was studied. We believe those findings are relevant for the future development of Lanthanide-Binding-Tags.

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Open microwave resonator with enlarged flow rate for MRI DNP applications

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Recently, Magnetic Resonance Imaging (MRI) has strongly advanced its performance by Dynamic Nuclear Polarization (DNP) methods. One of them is a continuous flow of hyperpolarized substrate produced by a DNP polarizer placed directly inside the bore of a 1.5 T MRI scanner [1,2]. Here we present a new open Fabry-Perot resonance structure with a 4-fold increased flow rate of hyperpolarized substrate in comparison to the cylindrical TE013 microwave cavity used so far. The Fabry-Perot resonator geometry optimized for enlarged aqueous sample volume was calculated by using the quasi-optical theory, and justified the design results by simulating the resonator with CST Microwave Studio. The resonator performance in comparison to the TE013 cylindrical cavity was tested by accomplishing MRI experiments on a blood-vessel phantom. The increased flow rate of this new resonance structure will allow an improved contrast for MRI applications with animals.

Acknowledgements:

The work was supported by the German Research Society (DFG) and by the Institute of Applied Physics of the Russian Academy of Sciences under project No. 0035-2019-0001.

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## P090

## Structural dynamics of fluorinated proteins using dissolution-DNP

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Dissolution Dynamic Nuclear Polarisation (d-DNP) helps achieving large NMR signals in solutions by polarizing nuclear spins using microwave irradiation, followed by a rapid dissolution with hot solvent. The d-DNP setup at UoN is based on a dual iso-centre magnet[1] with its top section (3.4 T) used for polarizing the sample at low temperatures (~1.8 K), and the bottom section (9.4 T) used for solution NMR measurements at room temperature. The system was optimized to achieve 300 ms dead time during which the sample is dissolved and transferred into an NMR tube. Many protein folding, unfolding and aggregation events develop on a similar timescale. As a model we use the refolding process of hen egg-white lysozyme in which <sup>19</sup>F-containing labels were introduced. Such <sup>19</sup>F-labelling helps studying specific protein sites without interference from naturally occurring signals. Furthermore, the <sup>19</sup>F chemical shifts are rather sensitive to the chemical environment, making them rather good probes for structural changes.

Maximum nuclear polarization was achieved with 40 mM water soluble TEMPOL as a polarizing agent in the glassy partially deuterated water/DMSO at the optimal microwave frequency of 94.05 GHz. Hen egg-white lysozyme was chemically modified to covalently attach trifluoroacetyl groups (CF<sub>3</sub>CO-) to six surface exposed amines of lysine residues. The protein was dissolved in a matrix consisting of 60/30/10 v:v DMSO-d<sub>6</sub>/D<sub>2</sub>O/H<sub>2</sub>O with 40 mM TEMPOL polarizing agent and 100 mM 5-fluorouracil, presence of which is shown to improve the enhancements of lysozyme. 900 ms after the dissolution, the NMR signals were acquired using a 900 pulse. The signal enhancements for <sup>19</sup>F-nuclei of lysozyme and 5-fluorouracil were ~300 and ~1000 respectively, which show overall suitability of the approach.

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Probing protein to drug-candidate interactions in the membrane by DNP-enhanced solid-state NMR

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$\alpha$ -synuclein aggregates are a hallmark of Parkinson's Disease (PD). Modulation and understanding of the toxic species is, therefore, a key goal in disease treatment. The small molecule anle138b, a 3,5-diphenyl-pyrazole derivative, has shown efficacy in PD animal models.[1] Here we report the interaction of anle138b embedded in phospholipid membranes, with  $\alpha$ -synuclein aggregates. We used DNP-enhanced solid-state NMR to probe the nature of this interaction implementing different labeling strategies. 100K temperatures for DNP allow us to study the oligomers of the protein in association with relevant low concentrations of anle138b. Cross-peaks between anle138b and the protein are shown in 2D-TEDOR and -hNHHc spectra, identifying a backbone interaction and pointing to a binding location in the protein structure, which is also backed by a computational study[2].

**References:** [1] Wagner, J., et al., Acta Neuropathologica, 2013. 125(6): p. 795-813. [2] Matthes, D., et al., ACS Chemical Neuroscience, 2017. 8(12): p. 2791-2808.

Abstract with a figure (pdf file):  
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**Green solvents from the NMR perspective: structure and dynamics of Ionic Liquids and Deep Eutectic Solvents**

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Alberto Mannu, Andrea Mele  
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Ionic liquids (ILs) and Deep Eutectic Solvents (DESS) are two strongly related classes of materials of great interest in green chemistry and with enormous potential in several technological and industrial applications.

ILs are well-known low melting salts with unique physicochemical features. To tune properties and structural organization of the final materials, there is nowadays a growing interest in combining two or more ILs. However, while significant progress has been made in our understanding of pure ILs, mixtures of ILs are still not well characterized.

Recently the green nature of ILs has been criticized and the scientific community moved progressively towards DESSs as economic and environmentally benign alternative. These low transition temperature mixtures consist of a hydrogen bond donor salt and a hydrogen bond acceptor, which self-associate to form a eutectic phase characterized by an unusual low melting point. The properties of DESSs may be enhanced by adding a suitable third component, such as water or the macrocyclic oligosaccharide  $\beta$ -cyclodextrin ( $\beta$ CD). Both these additives act as competitors for hydrogen bond within the DES network, thus modulating the physico-chemical properties of the system.

In the race to exploit IL mixtures and DESSs in innovative and popular applications, the study of the materials at the molecular level has received less attention. Yet structure, molecular interactions and motions are responsible for their unique properties. It is then imperative to investigate structural and dynamic properties of ILs and DESSs, and how they change in complex mixtures. To this end, NMR spectroscopy is a technique of choice. Here we use  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{19}\text{F}$  NMR relaxation and diffusion measurements, together with 2D homonuclear ROESY and heteronuclear HOESY correlation experiments, to study mixtures of 1-alkyl-3-methylimidazolium-based ILs as well as choline chloride-based DESSs under progressive addition of water and in the presence or absence of  $\beta$ CD.

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pH-Dependent Protonation of Phl p 6 Studied by NMR and cpH-aMD  
Valentin Dietrich, Florian Hofer, Anna S. Kamenik, Klaus R. Liedl, Martin Tollinger

We use state-of-the-art NMR experiments to measure apparent pKa values in the native protein environment and employ a cutting-edge combination of enhanced sampling and constant pH MD simulations to rationalize strong pKa shifts. Phl p 6 serves as an ideal model system for both methods due to its high number of titratable residues despite its comparably small size. Combining microscopic structural details from MD simulations and macroscopic ensemble averages from NMR shifts leads to a comprehensive view on pH dependencies of protonation states. Overall, we find striking agreement between simulation-based pKa predictions and experiment. However, our analyses also suggest fundamental differences in the underlying structural origin of the observed pKa shifts. The NMR data reveal a weak relation between pKa shifts and close contacts to charged residues, where the strongest influence derives from the dipolar character of alpha helices. Accelerated constant pH MD simulation on the other hand suggests immediate proximity of opposite charges, followed by vicinity of equal charges as major driving forces for pKa shifts.

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NMR study of structural injection grouts for cultural heritage restoration

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Historical and cultural-heritage buildings were mainly built of stone or a mixture of stone and brick, using lime-based mortars. Because of poor quality of the used mortar and the presence of voids in the walls, the load bearing capacity of such walls is mostly insufficient and the buildings deteriorate in the course of time. An efficient technique for the repair and/or strengthening of the historical buildings is by grout injection, where grouts made of materials compatible with the existing ones should be used. Incompatible materials may cause further deterioration or damage of the structure. This situation has happened in Europe after World War II, when many historical buildings were repaired with cement mortars that were incompatible with the existing lime-based ones in terms of chemical, physical and mechanical properties. In this work, we present a design and properties of combined lime-cement structural injection grouts containing 70 % of hydrated lime and 30 % of cement as a binder, using white Portland cement of different strength classes. We have monitored the time evolution of the grout setting process during hydration from early to late stages by proton NMR spectroscopy, using the method of inverse Laplace processing of the spin-lattice relaxation data. The so obtained “NMR hydration curves” of the grouts were compared to pure cement pastes. The NMR hydration curves reveal that the time evolution of the grout setting process is significantly slowed down (by more than 10 days) relative to the cement pastes. We have also determined the compressive strengths of the set grouts, which are in the range 1.5 – 2.5 MPa, appearing as efficient materials for the repair of cultural-heritage buildings.

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Light-Controlled Lipid Bilayers and Their Effect onto  
Membrane Proteins Observed by MAS-NMR

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Magic angle spinning nuclear magnetic resonance spectroscopy (MAS-NMR) is a prevailing technique in case of structural/dynamic studies of membrane proteins directly in the native liposome environment. Here we demonstrate light-based toolboxes for MAS-NMR by which some intrinsic aspects of the lipid environment, such as membrane lateral pressure, can be changed and its effect onto membrane proteins can be tracked. Liposomes containing the light-switchable azo-PC have been prepared and their properties have been characterized by MAS-NMR. Conditions for efficient photoconversion have been established revealing significant changes in the bulk lipid order parameters. The effects of these changes in the lipid bilayer onto the embedded membrane protein diacylglycerol kinase are described based on multidimensional <sup>13</sup>C-<sup>15</sup>N correlation spectra. In *Escherichia coli*, DgkA is responsible for the phosphorylation of diacylglycerol to phosphatidic acid at the expense of ATP. Probing its response towards alterations of the mechanical bilayer properties is essential for understanding its functional mechanism. In addition, we will demonstrate how to maintain a pH gradient across a lipid bilayer under MAS-NMR conditions using light-driven proton pumps.

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Real-time, in situ peptide-liposome formulation release kinetics monitored by NMR spectroscopy

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The encapsulation of active ingredients in liposomes is used in several domains such as food, pharmaceutical and cosmetic industries. Liposomes are drug delivery systems (DDS) used both for lipophilic cargos and for hydrophilic cargos. Such encapsulation allows a controlled release of active ingredients over time [1].

To increase the efficiency of peptide drugs, we aim at designing optimized liposomes by playing both on peptide structure and liposome composition. Potential parameters to be improved are peptide-liposome interactions, encapsulation efficiency and release kinetics. To reach this goal, we explored the potentiality of Nuclear Magnetic Resonance (NMR) spectroscopy to characterize peptides, liposomes and their behavior during release. The active ingredients used in this study are apelin-derived peptides used in cardiovascular diseases for their role in cardiovascular homeostasis regulation [2].

Liposome and peptide structures as well as their interactions were characterized by <sup>1</sup>H, <sup>31</sup>P NMR and cryo-EM. We also used Diffusion Ordered Spectroscopy (DOSY) to discriminate between the inner and the outer space of liposomes. This method allows determining the translational diffusion coefficient (D) of molecules that reports on their apparent size. Significant differences were observed for D of free and encapsulated peptides, due to the difference of molar mass between peptide and liposome, allowing distinguishing encapsulated and released peptides. We then investigated the potentiality of DOSY for peptide incorporation and real-time release quantification. Moreover, <sup>1</sup>H NMR spectra were used to monitor and quantify peptide release kinetics by spectral integration.

To conclude, we showed that liquid-state NMR can be used to follow the release kinetics of peptides in liposomal formulation in-situ and in real-time without perturbing the process. This approach could certainly be extended to other active ingredients and DDS used for pharmaceutical and cosmetic applications.

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### Assignment of a Fully Protonated Dimeric Protein with >100 kHz MAS NMR at High Fields

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The restrictive limits of molecular tumbling have made the study of large proteins and protein complexes a subject ideally suited to the strengths of magic-angle-spinning (MAS) solid-state NMR. Protons form a dense network of strong dipolar interactions and their shifts are sensitive reporters of protein structure and interactions. Higher MAS rates and highly deuterated samples have allowed for higher resolution and sensitivity by allowing <sup>1</sup>H detection of samples previously limited by their strong dipolar interactions. Recent advances have made MAS rates ranging from 100-111 kHz accessible and removed the need for deuteration and re-protonation protocols that are often problematic for the expression of many proteins. We demonstrate here the ability of ultra-high speed MAS NMR to shed light on the structure and dynamics of large proteins and their complexes and present here an application of these strategies at high magnetic fields (≥800 MHz) making steps towards the full resonance assignment of the fully protonated β<sub>2</sub> clamp.

The dimeric β<sub>2</sub> clamp from *E. Coli* DNA polymerase III forms a large (2x40.6 kDa) complex that is crucial to bacterial DNA replication. β<sub>2</sub> is one sub-unit of the greater *E. Coli* replisome, which contains almost 30 protein subunits that control the concerted leading- and lagging-strand DNA synthesis at replication forks during chromosomal DNA synthesis. The β<sub>2</sub> clamp is of particular interest as it forms a hub of protein-protein interactions essential for the function of at least 11 proteins involved in DNA replication and repair.

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Slow ring flips in protein GB1 studied by aromatic  $^{13}\text{C}$  relaxation dispersion methods

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MLU Halle-Wittenberg

Ring flips of phenylalanine and tyrosine are a hallmark of protein dynamics. They report on transient breathing motions of proteins. In addition, flip rates also depend on stabilizing interactions in the ground state, like aromatic stacking or cation- $\pi$  interaction. So far, experimental studies of ring flips have almost exclusively been performed on aromatic rings without stabilizing interactions [1]. Here we investigate ring flip dynamics of protein GB1 at pH 7.0 using  $^{13}\text{C}$  aromatic relaxation dispersion methods for various temperatures (between 10 °C and 40 °C) and pressures (0.1 up to 100 MPa). We found that all four residues of the cluster, Y3, F30, Y45 and F52, display slow ring flips [2]. Interestingly, F52, the central residue of the cluster, which makes aromatic contacts with all three others, is flipping significantly faster, while the other rings are flipping with the same rates in the range of errors. Determined activation enthalpies and activation volumes are in the same range of other reported ring flips of single aromatic rings. There is no correlation of the number of aromatic stacking interactions to the activation enthalpy, and no correlation of the ring's extent of burying to the activation volume. Because of these findings, we speculate that F52 might undergo concerted ring flips with each of the other rings.

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## Unraveling the Solid-State Aggregation Behavior of Supramolecular Polymers

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Supramolecular polymerization of  $\pi$ -conjugated scaffolds and molecules is an approach to obtain a manifold of functional materials, e.g. for organic electronic devices. Understanding the aggregation behavior of supramolecular polymers is thus a premise to control the forming products and their function. Contrary to regular polymers, supramolecular polymers are mainly stabilized by noncovalent interactions, e.g.  $\pi$ - $\pi$ -stacking, hydrogen bonds, and van der Waals interactions. The combination of multiple noncovalent interactions may further lead to cooperative or non-cooperative aggregation.

Although the aggregation process can be investigated by liquid-state NMR, the structures of the resulting supramolecular polymers are inaccessible as a result of the strong dipolar broadening caused by the aggregation even in solution. Additionally, the stabilizing motif for the aggregates is of high interest since multiple noncovalent interactions are present in the sample. Both issues can be addressed by solid-state NMR.

Herein, we present the aggregation behavior and solid-state structures of two different  $\pi$ -conjugated Pt(II) complexes. These oligophenylethylene (OPE)-based Pt(II) complexes are substituted symmetrically with chiral alkyl side chains and unsymmetrically with a triethyleneglycol and dodecyloxy side chain, respectively. For both complexes two different aggregates are formed and the cooperative aggregating species is only accessible by solid-state NMR. We obtained the solid-state structure by a combined approach of 1D experiments (<sup>1</sup>H MAS and <sup>13</sup>C{<sup>1</sup>H} CP/MAS NMR) and 2D homonuclear and heteronuclear correlation experiments (<sup>1</sup>H-<sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H}) supported by DFT calculations. We could on this basis identify Pt(II)- $\pi$  interactions,  $\pi$ - $\pi$ -stacking and the unconventional N-H...O-alkyl hydrogen bond as stabilizing motif. The techniques used here represent a general solid-state NMR and DFT strategy for obtaining structural constraints for  $\pi$ - $\pi$  conjugated systems.

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## P100

## Structural Investigation of Tetramethylrhodamine Binding Aptamers

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RNA aptamers - artificial RNA molecules with high affinity and specificity to a target ligand - are versatile tools in the fields of biotechnology and medical biology. They also present ideal model systems to delineate general insights about RNA-ligand interactions such as the correlation between structural complexity and ligand affinity and specificity. A series of RNA aptamers binding to the fluorescent xanthene dye tetramethylrhodamine (TMR) have been reported recently.[1] These aptamers vary in structural complexity and affinity. We have investigated the binding properties, the fluorescence characteristics and the structure of several of these TMR aptamers. The structure of one aptamer of higher structural complexity, TMR-3, was investigated further by NMR spectroscopy. The aptamer of 54 nt length was predicted to form into two stem-loops and a closing stem connected by a three-way junction of unknown topology. In order to assign the resonances of this sizeable RNA, we combined conventional techniques with a site specific labeling approach. The high resolution structure reveals a large gain in structure upon ligand binding from an open three-way junction to a tight arrangement with ligand-mediated coaxial stacking between two of the aptamers stems. The binding features of TMR-3 to TMR are both common and different from the binding mode of other aptamers with affinity for ligands carrying planar aromatic ring systems such as the malachite green aptamer which binds to the tetramethylrhodamine related dye malachite green or the flavin mononucleotide aptamer.

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Investigating the structure of recombinant nucleocapsids  
from Dengue virus by solid-state NMR

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Dengue is the most widely spread mosquito-borne viral disease affecting the human population. It is estimated that about 390-million dengue infection occur each year, of which 96 million shown signs of severity. Dengue virus (DENV) is a single-stranded positive-sense RNA virus featuring an icosahedral, ordered envelope and a nucleocapsid of unknown structure.

The structure of the Dengue viral particle has been solved by cryo-EM1, revealing the molecular organization of the envelope. However, the capsid made from core proteins and viral RNA remains undetected in this context. The core protein structure could only be solved in its dimeric form using NMR in solution2, and only sparse information is available about the assembled capsid. It is unknown today if Dengue virus nucleoprotein complexes display highly ordered nucleocapsids, or consist in amorphous packs of the viral genome3.

We here investigate the structure of the DENV nucleoprotein complex by combining recombinant sample preparation methods with solid-state NMR. We present the first solid-state NMR spectra of DENV-Core reassembled in presence of the dengue viral RNA. 2D-13C-13C-DARR NMR spectra show a well-structured protein with comparable quality as those recorded on the hepatitis B virus capsid protein 4, 5. We report the 13C and 15N assignments of DENV-Core in the nucleoprotein complex using 2D and 3D solid-state NMR experiments and compare our assignment with the solution NMR assignment of DENV-Core dimer. These results are a first step towards a structural characterization of the dengue nucleoprotein complex.

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P102

**Accurate  $^1\text{H}$ - $^{14}\text{N}$  distance measurements by phase-modulated RESPDOR at ultra-fast MAS**

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The combination of a phase-modulated (PM) saturation pulse and symmetry-based dipolar recoupling into a rotational-echo saturation-pulse double-resonance (RESPDOR) sequence has been employed to measure  $^1\text{H}$ - $^{14}\text{N}$  distances. Such a measurement is challenging owing to the quadrupolar interaction of  $^{14}\text{N}$  nucleus and the intense  $^1\text{H}$ - $^1\text{H}$  homonuclear dipolar interactions. Thanks to the recent advances in probe technology, the homonuclear dipolar interaction can be sufficiently suppressed at a fast MAS frequency ( $\Omega_{\text{R}} \geq 60$  kHz). PM pulse is robust to large variations of parameters on quadrupolar spins, but it has not been demonstrated under very fast MAS conditions. On the other hand, the RESPDOR sequence is applicable to such condition when it employs symmetry-based pulses during the recoupling period, but a prior knowledge on the system is required. In this article, we demonstrated the PM-RESPDOR combination for providing accurate  $^1\text{H}$ - $^{14}\text{N}$  distances at a very fast MAS frequency of 70 kHz on two samples, namely L-tyrosine.HCl and N-acetyl-L-alanine. This sequence, supported by simulations and experiments, has shown its feasibility at  $\Omega_{\text{R}} = 70$  kHz as well as the robustness to the  $^{14}\text{N}$  quadrupolar interaction. It is applicable to a wide range of  $^1\text{H}$ - $^{14}\text{N}$  dipolar coupling constants when a radio frequency field on the  $^{14}\text{N}$  channel is approximately 80 kHz or more, while the PM pulse length lasts 10 rotor periods. For the first time, multiple  $^1\text{H}$ - $^{14}\text{N}$  heteronuclear dipolar couplings, thus multiple quantitative distances, are simultaneously and reliably extracted by fitting the experimental build-up curves to the simulated ones. These determined distances are in excellent agreement with those derived from diffraction techniques.

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## P103

**Studies of metal-ions interactions with ultra-sensitive beta-detected NMR**

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In the case of alkali metal cations, NMR suffers from low sensitivity, which is due to small abundance of the NMR-active isotope and a low degree of polarization related to small values of the magnetic moments, and spin above ½ leading to large quadrupolar interactions.

Herein, we present  $\beta$ -detected NMR, allowing for the signal detection with ten orders of magnitude higher sensitivity than in conventional NMR. The motivation behind this interdisciplinary project is to combine the enhancement of nuclear spin polarization via Optical Pumping (OP) with ultrasensitive resonance detection using  $\beta$ -particles emitted by the radioactive nuclei. Atoms of the selected radioisotope of alkali metal (e.g. <sup>26</sup>Na) are pre-shaped into a beam, polarized and implanted into the liquid state host. The synchronous application of continuous wave RF allows to induce a continuous change in the spin polarization, observed as time-integrated anisotropy of  $\beta$ -decay.

The main advantage of the  $\beta$ -NMR measurements, beside the ultrahigh sensitivity, is the online character of the experiment. The recorded chemical shifts and longitudinal relaxation times are a direct representation of the kinetics happening in the liquid system within the life time of the selected radioisotope. It allows for the real-time investigation of the processes such as structural rearrangements or protein folding catalysed by the alkali metal cations.

First liquid state measurements with  $\beta$ -NMR technique at ISOLDE, CERN, were performed in 2017, after the commissioning of the experimental setup in 2016. At the end of 2018 the Chemical Shift and T1 relaxation time were measured for the first biological sample representing the <sup>26</sup>Na-promoted formation of G-quadruplex structures. Results will be presented in comparison with high vacuum conventional NMR spectra, showing influence of the pressure change and possible water contamination on the solution-alkali cation interaction.

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P104

Dosimetric Properties of Some Asthma Drugs: An EPR Study  
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Selçuk University

The purpose of the present work to investigate the dosimetric properties of various asthma drugs using Electron Paramagnetic Resonance (EPR) Spectroscopy. Since asthma drugs are widely used, it would be valuable to determine dosimetric properties to be used as emergency dosimetry. For this purpose, pulverized asthma tablets were irradiated with 60Co gamma source at a dose range of 10-1100 Gy and EPR spectra of unirradiated and irradiated samples were recorded by JEOL JesFa-300 EPR spectrometer. While unirradiated drugs has been observed to be EPR silent, the drugs have become paramagnetic due to the radiation effect. The dose-dependent and time-dependent variation of the signals belonging to radiation-induced radical(s) were investigated by dose response and fading studies, respectively, and the values of Minimum Measurable Doses (MMDs) were also determined. In addition, the stability of the radical(s) was also supported by kinetic studies performed at various temperatures. As a result, by considering radiation sensitivity and stability of the radiation-induced radicals in the host structures, it can be suggested that the drugs examined can be used as emergency dosimetry for the selected dose range.

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## P105

**Different variants of cyclosporin studied by NMR:  
Molecular structure and conformational flexibility**

Sergey Efimov\*, Yuriy Zgadzay, Ilya Khodov, Vladimir Klochkov  
*Kazan Federal University*

Cyclosporin A, well known as an immunosuppressive agent, is one of a family of congeners synthesised in certain soil fungi. Three natural variants of cyclosporin (B, C, D) were studied by high resolution NMR spectroscopy in chloroform and dimethyl formamide. CsB has alanine in position 2 instead of  $\alpha$ -aminobutyric acid (Abu2) found in CsA, in CsC this residue is replaced by threonine, and in CsD, by valine.

In general, obtained spectra of the peptides are alike. However, there are some differences seen in  $^1\text{H}$  and 2D  $^1\text{H}$ ,  $^{13}\text{C}$ -HSQC spectra. For instance, the OH proton of residue Thr2 in CsC is visible in  $\text{CDCl}_3$  at 298 K, as well as the OH proton of Bmt1 in CsD. In CsA and CsB, however, hydroxyl protons were not observed directly due to the exchange. CsD was also found to have negligible signals of the minor conformer, while it is clearly seen in the spectra of other three compounds.

Structural data were obtained from ROESY spectra, which allowed building model structures of the investigated molecules.

The case of chemical exchange was considered on the example of CsC in DMF. The pattern of exchange resembles that observed for CsA in polar media such as DMSO. Signals of the NH protons were assigned, and temperature behaviour of their chemical shifts was analysed. It was found that most of the observed conformers have lost their intramolecular H-bonds. Cis-trans isomerisation of the peptide bonds was suggested to be the reason of the conformational exchange.

The work was funded by the Russian Science Foundation (project no. 18-73-10088).

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## P106

**Polarization transfer in [1-<sup>13</sup>C]fumarate using constant-adiabaticity field sweeps**

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Much work in the field of parahydrogen-enhanced NMR involves the transfer of proton polarization to heteronuclei using the molecular J-coupling network. Techniques such as magnetic field cycling [1] and 'SABRE-SHEATH' [2] have emerged for this purpose. We recently presented a new technique: a molecule containing a heteronuclear spin is hydrogenated with parahydrogen at some magnetic field to yield an AA'X spin system, and the field is adiabatically swept through the zero point and up in the opposite direction to transform the proton singlet order into magnetization on the heteronuclear spin[3]. Initial experiments involved: (1) hydrogenating [1-<sup>13</sup>C]acetylene dicarboxylic acid to form [1-<sup>13</sup>C]maleic acid; (2) performing a linear field sweep from -2 to +2  $\mu$ T to polarize the carbonyl <sup>13</sup>C spin; (3) shutting the sample into a Magritek SpinSolve high field benchtop magnet for direct <sup>13</sup>C detection.

We are now implementing this technique on [1-<sup>13</sup>C]fumarate, formed via trans-hydrogenation of [1-<sup>13</sup>C]acetylene dicarboxylic acid with para-enriched hydrogen gas[4]. Fumarate is a metabolite in the citric acid cycle, and has been shown to be a promising candidate for medical imaging experiments[5]. In order to achieve high <sup>13</sup>C polarization levels and minimize the field sweep duration, we have implemented 'constant-adiabaticity' field sweeps optimised for the fumarate J-coupling network.

The preferred method for producing hyperpolarized fumarate for imaging studies is dissolution dynamic nuclear polarization, but this is a notoriously expensive, batch-mode technique. This work opens the possibility for cheap, continuous-flow production of hyperpolarized [1-<sup>13</sup>C]fumarate, by using parahydrogen as the polarization source.

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## P107

## NMR-based study on dietary composition of faecal composition of mice

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Andreas Dötsch<sup>2</sup>, Daniela Graf<sup>2</sup>, Pavleta Tzvetkova<sup>1</sup>

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The metabolic syndrome, which is becoming increasingly important especially in western industrial countries, involves four disorders: fat metabolism, sugar metabolism, fat conductivity and high blood pressure. A healthy lifestyle including a healthy diet should minimize the risks of disease. Several metabolic pathways play an important role in the etiology of the metabolic syndrome, especially food metabolism and its effect on intestinal microbiota in animal models as well as on the human enzymes. Plant components like dietary fibers are indigestible by human enzymes and are thus considered as essential for a healthy diet. Microbiota metabolizes them in the large intestine producing short chain fatty acids (SCFA). New studies show that SCFA (acetate, propionate and butyrate) are well absorbed by the intestinal epithelium and serve as energy carriers. SCFA also play a role as signaling molecules in the regulation of repletion.

Male mice are an established model in food research and allow comparable studies to be performed over long periods of time. In our study we examined fecal mice samples for intestinal changes, especially changes in microbiota and metabolite composition. Mice were fed high fat diets with a diverse range of dietary fiber supplements. We followed by NMR spectroscopy the effects on metabolites composition over several weeks. For this, we first established a robust protocol for extraction with particular emphasis on SCFA [1]. Using this protocol our study presents a cost-effective and robust approach of investigation of different diets over longer periods of time. The monitoring of the changes in the metabolite concentration during the study depending on the dietary fibres concentration in the mice diet was statistically evaluated. The impact of their concentration is demonstrated and shows a direct comparison of the specific metabolites composition between different dietary fibre rich diets.

Reference: [1] A. Hauser et al. (2019), *Metabolites*, 9(3), 55.

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## P108

**Dynamic yet Defined: EPR Spectroscopy on  
Extremely Soft, Self-Assembled Structures in Solution**  
Jana Eisermann, Dariusz Hinderberger

Based on the process of ionic self-assembly (ISA) we characterize the formation of loosely bound ion-based colloid-like globular clusters of several nanometer size, termed globular ionoids.[1-2] Here, our model system contains the ‘Texas-sized molecular box’, developed by Sessler and coworkers[3] as a mechanically interlocked molecule to create elegant non-covalent architectures (e.g. molecular organic frameworks), together with small dianionic salts like methanedisulfonic acid dipotassium salt.

The formation of such size monodisperse supramolecular structures due to long-range electrostatic correlations also requires the right interplay of weaker noncovalent interactions such as solvation, hydrogen bonding or van der Waals interactions. In our solvent mixture of DMSO:glycerol:water 50:43:7 (v/v/v) we can balance out these correlations to gain small self-assembled entities, whereas their size and shape anisotropy can be tuned based on the applied ionic ratio.[4]

To analyze the local structure in our self-assembled system with the ‘Texas-sized molecular box’ at the initial ion-cloud state[2], we apply electron paramagnetic resonance (EPR) spectroscopy utilizing the dianion of Fremy’s salt as observer component. Here, we explore the globally averaged interactions as well as the local environment of our nitroxide radical with continuous wave (CW) EPR spectroscopy at X- and Q-band frequencies. Furthermore, our findings highlight the changes of the solvation shell around Fremy’s salt while adding the ‘large’ multicationic compound through partially deuterated solvent mixtures of DMSO:glycerol:water and utilizing various pulsed EPR spectroscopy methods like 2p-ES-EEM, 3p-ESEEM and HYSCORE.

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## P109

**Design and Performances of Hyperpolarizing Polymers (HYPOP) for dissolution-DNP**

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Hyperpolarization by dissolution dynamic nuclear polarization [1] has emerged in the last decade as a powerful tool for boosting the sensitivity of magnetic resonance by orders of magnitude. This has for instance brought metabolic imaging to reality [2]. Unfortunately, hyperpolarization's lifetimes in all molecules hardly exceed a minute. This means that hyperpolarization needs to be produced 'on-site', which is for many reasons unpractical. We have recently introduced a new concept for producing long-lasting transportable hyperpolarized molecules formulated in the form of micro-powders [3].

We are now generalizing this concept to virtually any soluble or liquid molecule by introducing a new generation of hyperpolarizing matrices made up of morphologically controlled microporous polymers containing stable radicals. These materials can be impregnated with the solution to be hyperpolarized without any need of contaminants such as radicals or glass forming agent, and are stable in a broad range of pH/Temperature. After polarization of the solution inside such materials at ~1.2K and 7T it is possible to delay dissolution for hours by storing the sample at 4K and low magnetic fields.

We will

- 1) present the synthesis of these new porous polarizing matrices based on straightforward and very versatile epoxy-based chemistry.
- 2) show how the morphology of these materials (porosity) can be tuned and how this affects the efficiency and lifetime of hyperpolarization.
- 3) expose DNP results obtained on the matrices, with radical concentration optimization, with ultimately absolute polarization values as high as  $P(1H) > 60\%$ .

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High-resolution NMR structural characterization of Huntingtin’s polyglutamine tract

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Huntington’s disease (HD) is an incurable neurological disorder triggered by the aberrant expansion of the polyglutamine tract present in the huntingtin protein (HTT). The pathological version of HTT (with more than 35 consecutive glutamines) is prone to aggregate and form insoluble fibrils within neurons, affecting their normal functions. However, the precise mechanism by which the expanded tract promotes aggregation, as well as the structural differences between non-pathological and pathological forms of HTT, remain poorly understood. That is mainly due to the low sequence complexity of HTT, which poses a challenge for traditional structural methodologies. To solve this problem, we have developed an approach, combining cell-free expression and nonsense suppression, enabling us to produce site-specific isotopically labeled samples and record simplified NMR spectra. Using this strategy we have studied at atomic resolution two versions of HTT exon 1 with 16- and 46-residue-long polyglutamine tracts. Our study shows that the polyglutamine tract consists in an equilibrium of multiple  $\alpha$ -helices of variable length. All these helices start in N-terminal flanking region of the homo-repeat and propagate towards the polyglutamine. Interestingly, the helical tendency is enhanced in the pathological version of HTT.

In addition, the structural coupling between the polyglutamine and its flanking regions was evaluated by designing several mutants. Their evaluation demonstrates the importance of the 17-residues N-terminal region of exon 1 in the propagation of the  $\alpha$ -helix towards the polyglutamine tract, and the helix-breaking effect of the proline-rich region that succeeds the polyglutamine.

In summary, our study demonstrates that our method, based on site-specific isotopic labeling, can provide high-resolution structural information of low complexity regions such as those found in HTT. This paves the way to study multiple biologically relevant proteins whose structural analysis has been hampered until now.

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Dynamics analysis from nanosecond to second:  
Effect of ligand binding on  $\beta$ -lactamase mobility  
Wouter Elings\*, Marcellus Ubbink  
Leiden University

$\beta$ -lactamases pose a great threat to our healthcare system, yet may simultaneously present potential solutions. We used CEST, CPMG relaxation dispersion and  $T_1$ ,  $T_2$ , NOE Lipari-Szabo analyses to characterise the dynamic behaviour of the  $\beta$ -lactamase of *Mycobacterium tuberculosis*, BlaC, over a wide range of timescales. BlaC is mostly rigid on the fast timescales and shows dynamics around the active site in the low millisecond time range. We also studied the dynamics effects of inhibitor binding and changes that occur in inhibitor-resistant variants. Inhibitor binding affects dynamics at nanosecond as well as micro-millisecond mobility. In point mutants, rigidity on fast timescales is largely conserved, whereas millisecond dynamics were found to vary dramatically. Our results show that NMR-based sampling of dynamics over a wide range of timescales is essential for a better understanding of enzyme activity and can help to detect surprisingly large changes in the protein energy landscape that are not detected by crystal structures.

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P112

An open source, high pressure (50 bar), liquid-N2 cooled parahydrogen generator

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Motivation

Among other factors, the sensitivity of Magnetic Resonance Imaging (MRI) is limited by the low polarization of the sample investigated. Enhancing the polarization offers several orders of magnitude in signal enhancement. In comparison to e.g. Dynamic Nuclear Polarization (DNP), para-hydrogen (pH<sub>2</sub>) based approaches are less cost intensive, scale well and offer high throughput and scalability. A necessary requirement is a device to produce pH<sub>2</sub>. The few commercial solutions available have a wide price range (10<sup>4</sup> – 10<sup>5</sup> €) and use either liquid nitrogen or a closed cycle helium cryostat (Bruker, IDB Budzylek, SpinDynamics e.g.). To facilitate groups entering the field, here, we provide the blueprint for a robust and low cost pH<sub>2</sub> generator based on liquid nitrogen.

Methods

The following criteria were defined to design an open access pH<sub>2</sub> generator:

- 1. Enrichment: min. 50 % enrichment
- 2. Flow: min. 0.2 standard liters per minute
- 3. Pressure: min. 50 bar
- 4. Maximum safety
- 5. Mechanical robustness
- 6. Ease of operation
- 7. Cost: less 1000 €
- 8. Easy to reproduce

Results

Based on these criteria, a prototype (V 0.1) was produced and successfully employed for pH<sub>2</sub> production and hyperpolarization. Criteria 1-6 were met, and criteria 7 and 8 are likely to be addressed in the device presented at the conference (V 1.0). An open access approach was chosen, and all part and construction drawings will be provided.

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## P113

### Contactless $^1\text{H}$ to $^{13}\text{C}$ polarization transfer strategies for dissolution-dynamic nuclear polarization

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Conventional nuclear magnetic resonance (NMR) experiments are limited by low sensitivity. Hyperpolarization techniques such as dynamic nuclear polarization (DNP) enhance NMR signals by several orders of magnitude [Larsen et. al. 2003]. Most dissolution-DNP (*d*DNP) applications involve the use of weakly magnetic isotopes such as  $^{13}\text{C}$ , but excessively long  $T_{\text{DNP}}(^{13}\text{C})$  timescales hinder efficient polarization build up and leads to extended experimental times. Intrinsically sensitive proton nuclei do not suffer from such issues and can polarize quickly and to a greater extent at low temperature.

We recently pioneered the use of cross-polarization (CP) under *d*DNP conditions to boost both  $^{13}\text{C}$  polarizations and build-up rates  $1/T_{\text{DNP}}(^{13}\text{C})$ . The technique requires intense  $B_1$ -matching (typ.  $> 20$  kHz) of simultaneous  $^1\text{H}$  and  $^{13}\text{C}$  spin-locking radiofrequency fields throughout an optimized contact period (typ.  $> 1$  ms) in the superfluid-helium bath. This CP-DNP approach recently turned out to be key in the preparation of transportable hyperpolarization [Ji et. al. 2017].

While our CP approach works efficiently on typical *d*DNP sample volumes (typ.  $< 500$   $\mu\text{L}$ ), we feel today the critical need to scale up to larger samples (typ.  $> 10$  mL) which will ultimately enable parallel hyperpolarization of multiple transportable samples. Unfortunately, scaling-up CP turns out incredibly challenging as it can engender detrimental arcing in superfluid helium.

To hyperpolarize larger samples volumes, efficient sequences with reduced power requirements become mandatory. Lower power alternatives to CP have previously been described in the literature [Vinther et. al. 2019]. We describe the application of these techniques as an alternative to CP in *d*DNP experiments. The observed enhancements compare favourably to CP, considering the less stringent power requirements. With such methods, the hyperpolarization of large dose or multiple samples should become possible due to reduced sample heating and minimized probe arcing.

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## P114

## Hyperpolarizing Water at High Magnetic Fields

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Hyperpolarization techniques are used to enhance NMR signals by several orders of magnitudes. Para-Hydrogen Induced Polarization (PHIP) is a practical and cost-effective method with great potential for medical applications. Moreover, the hyperpolarization of biocompatible solvents has potential for versatile applications in structural biology and medical imaging. Our group recently demonstrated the hyperpolarization of water by means of  $p\text{-H}_2$  via a new mechanism introduced as NEPTUN (Nuclear Exchange Polarization by Transposing Unattached Nuclei). [1, 2] NEPTUN is based on a chemical exchange of a single proton from  $p\text{-H}_2$  with one proton from  $\text{D}_2\text{O}$  at a molecular catalyst, resulting in hyperpolarized HD and HDO. In detail, the hyperpolarization can only be transferred if the symmetry of  $p\text{-H}_2$  is broken and the two spin order  $I_z S_z$  of  $p\text{-H}_2$  evolves into inphase magnetization  $I_z S_z$  prior to the proton exchange.[2] This evolution occurs spontaneously in the strong coupling regime that prevails for most chemical systems at low magnetic fields ( $< 1$  T).

In these systems, the  $T_1$  times of HDO are short in the low magnetic prepolarization fields and only moderate enhancements of bulk HDO were observed. However, the relaxation time of bulk HDO at 14 T is up to 45 s, allowing the storage of hyperpolarization in the bulk phase while generating the HDO hyperpolarization with the molecular catalyst. In this work, we present adapted pulse sequences based on selective excitation of the catalyst-bound hydrides, enabling the hyperpolarization of bulk water at high magnetic fields.

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## P115

**SIFTER a method to study spin labeled biomolecules****Burkhard Endeward<sup>\*1</sup>, Philipp E. Spindler<sup>2</sup>, Thomas F. Prisner<sup>2</sup>**<sup>1</sup>*Goethe University, Frankfurt*, <sup>2</sup>*Institute of Physical and Theoretical Chemistry and Center for Biomolecular Resonance*

SIFTER[1] (single-frequency techniques for refocusing) is a single frequency pulsed EPR method similar to PELDOR[2] for studying dipolar couplings of spin labeled molecules like proteins, RNA or DNA[3]. Due to the single frequency setup, there is no sensitivity lost like in PELDOR with two frequency settings, which limits the excitation of the EPR spectrum and reduces the sensitivity in regards to the selection of the frequency and the resonator profile. However, PELDOR has the possibility to render orientation selective PELDOR traces, enabling to get besides to dipolar distance information also orientation dependent information. Such orientation dependency encoded in the spectral resolution of nitroxide reveal 2D-SIFTER[4] experiments as well.

Studying biomolecules like nuclide acid molecules containing spin labels, which are rigidly linked, by 2D-SIFTER opens possibilities to discover internal orientations, induced changes or flexibilities of that molecule in atomic resolutions. These probabilities of biomolecules are often hidden in x-ray spectroscopy; however, the knowledge is necessary to understand the function of many biomolecules.

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Structure and magnetic properties of ludwigite  $\text{Mn}_{2.25}\text{Co}_{0.75}\text{BO}_5$

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$\text{Mn}_{2.25}\text{Co}_{0.75}\text{BO}_5$  oxyborates belong to the ludwigite family with the general formula  $\text{M1}^{2+}\text{M2}^{3+}\text{BO}_5$ , where M1 and M2 are divalent and trivalent metal ions, respectively. Magnetic properties of oxyborates with the ludwigite structure are usually related with the presence of zigzag walls in their crystal structure formed by metal ions of different valency and also the presence of up to twelve magnetic ions in the unit cell, which occupy four non-equivalent positions. Here we present the detailed investigations of structural and magnetic properties of  $\text{Mn}_{2.25}\text{Co}_{0.75}\text{BO}_5$  by magnetometry and electron magnetic resonance methods. Magnetic measurements in  $\text{Mn}_{2.25}\text{Co}_{0.75}\text{BO}_5$  showed that this compounds are ferrimagnetic with Curie temperature 61 K. The temperature dependence of magnetic susceptibility can be well fitted by the Curie-Weiss above 60 K, that is fitted by the linear temperature dependence of the inverse magnetic susceptibility. The fitting parameters Curie-Weiss temperature is equal -145 K. The Curie-Weiss temperature is negative, that suggests the presence of antiferromagnetic correlations in the investigated samples. We shown one ESR line in powder sample, and two lines in monocrystal between 100 and 300 K.

The study was supported by the Russian Foundation for Basic Research (RFBR), grant No 17-02-00953.

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Investigation of ytterbium doped  $Y_2SiO_5$  by EPR method

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The ability to convert quantum states from microwave photons to optical photons is important for the hybrid system approaches to quantum information processing. The inverse transformation allows one to implement quantum memory of photon states based on electron and nuclear spins with a very long coherence storage time. The problem of quantum state conversion of microwave photons into optical photons and vice versa can be resolved by using impurity rare-earth ions in dielectric crystals.  $Y_2SiO_5$  (YSO) single crystal doped by rare earth ions shows the possibility of using for quantum computing, in particular for storing information. The YSO crystal structure is characterized by the space group  $I 2/a$  with lattice parameters (in Angstrom)  $a = 10.3880$ ;  $b = 6.71$ ;  $c = 12.4686$ ;  $\alpha=90$ ;  $\beta= 102.678$ ;  $\gamma = 90$ . There are two structurally non-equivalent Y position in the YSO crystal cell. Y1 site is coordinated by six oxygen atoms, Y2 site is coordinated by seven oxygen atoms. Here we present the results of investigations of the YSO single crystals doped with 0.005% of isotopically pure  $^{171/173}Yb^{3+}$  ions obtained by CW and pulse EPR technique. The CW EPR spectrum consists of four groups of two equidistant lines, which represent the hyper fine structure (HFS) components due to the odd neodymium isotope  $^{171/173}Yb$  (the nuclear spin  $I=1/2$ ) for two magnetically and structural nonequivalent positions Yb(1) and Yb(2) substituting  $Y^{3+}$  ions in the two distinct crystallographic sites. The relaxation time  $T_2$  was measured by the primary echo decay for two sites of  $Yb^{3+}$  ions. It was found that the spin-lattice relaxation times for ytterbium ions in sites I and differ by an order of magnitude. The samples were grown by Zavartsev and Kutovoi in Prokhorov General Physics Institute RAS, Moscow.

This work was supported by the Russian Science Foundation (project no. 16-12-00041).

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Fragment Screening of a Kinase Target by 19F NMR:  
Hit Rate Comparison of 19F and 1H NMR Screens.  
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A fragment screen using ligand detected 19F NMR versus a kinase target will be described. In addition, practicalities of sample preparation and mixture design will be discussed. Furthermore, a TopSpin/Perl script package for automatic analysis of 19F NMR based screening data will be presented.

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## P119

## NMR and challenging biological systems

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Thibault Viennet<sup>2</sup>, Aldino Viegas<sup>2</sup>, Manuel Etzkorn<sup>\*,3</sup>

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In general, structure and function of biomolecules can be strongly influenced by their environment. This is in particular true for membrane (associated) proteins and soluble proteins inside a densely-packed cell. Modern structural biology therefore requires advanced biochemical tools to generate adequate complex environments as well as techniques that can be used in these environments and still report on structural details with high resolution.

In the last years our research has contributed to both aspects and recent advancements will be addressed, in particular:

1. The use of adequate environments including the potential of the lipid bilayer nanodiscs system for NMR-based structural studies of membrane proteins [1-4] and protein-membrane interactions as modulators in signaling or protein aggregation [5, 6].
2. The usage of most suitable NMR techniques including the effective use of the available magnetization in challenging systems [7] and the possibility to selectively hyperpolarize a target protein in a cellular context [8].

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Dynamics Studies of UV Pretreated Polyethylene Terephthalate  
Using Solid-State NMR Spectroscopy

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Polyethylene terephthalate (PET) is one of the most widely used plastics worldwide and is applied, *i.a.*, as packaging material in the food industry.[1] Like many polymers, PET is susceptible to photochemical degradation and absorbs in the ultraviolet (UV) range.[2]

As already known, UV irradiation of PET leads to shorter molecular chains and thus to a higher number of carboxyl end groups,[3,4] which was confirmed here with attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). To clarify whether this pretreatment method also affects the chain dynamics of PET, we used centerband-only detection of exchange (CODEX) and rotating-frame spin-lattice relaxation times for protons ( $T_{1\rho}(\text{H})$ ) to investigate PET chain dynamics on various timescales at ambient temperature (30 °C) as well as close to its glass transition temperature (70 °C) before and after UV illumination. CODEX is a one-dimensional exchange experiment which allows for characterization of slow segmental reorientations on the millisecond to second timescale,[5] whereas  $T_{1\rho}(\text{H})$  times provide information about molecular dynamics on the microsecond to millisecond timescale.[6]

Both MAS NMR experiments revealed an increased mobility of the PET chains at 70 °C compared to 30 °C. Furthermore,  $T_{1\rho}(\text{H})$  experiments showed slower motions after UV treatment at both temperatures and CODEX experiments indicated lower mobility for the UV illuminated sample at 70 °C.

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YAP:TEAD Binding is Facilitated by the  
Structural Preformation of a Surprisingly Compact State

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Matthias Schmid<sup>1</sup>, Fedir Bokhovchuk<sup>2</sup>, Patrick Chène<sup>2</sup>, Robert Konrat<sup>1</sup>  
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Yes-associated protein (YAP) contains intrinsically disordered protein (IDP) regions that play a major role in the Hippo pathway that regulates organ size, cell proliferation, apoptosis, and is associated with a wide range of cancers. Therefore, the binding between YAP and transcriptional enhanced associate domain (TEAD) proteins is an interesting target for cancer therapy. For further characterization of the apo state of the YAP 50-171 fragment that contains the TEAD-binding domain, we applied paramagnetic relaxation enhancement (PRE), selective labeling with late metabolic precursors, utilization of <sup>15</sup>N relaxation, and site-directed mutagenesis of key residues for the interaction. Our findings reveal a surprisingly compact state in YAP that is even more compact than the bound form of YAP. The formation of this state is dependent on preformation of two canonical ( $\alpha$ -helix and  $\beta$ -strand) and one non-canonical ( $\Omega$ -loop) secondary structure elements. We identified the structural prearrangement of the latter by site-specific <sup>13</sup>C labeling of sidechain atoms of Phe, Leu, and Met with late metabolic precursors. These three structured segments are interdependent and form a locally compact state that was mapped by PRE. In addition, we have shown that the formation of the compact state can be disrupted by mutating crucial residues at the YAP:TEAD interaction interface. These effects of spatial expansion are supported by PRE and DOSY data. In summary, our data suggest that the three YAP:TEAD interaction interfaces are co-stabilizing each other in the apo state and, therefore, they preformation of this surprisingly compact state may facilitate the formation of the YAP:TEAD complex.

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Simultaneous snapshots of intrinsically disordered proteins in action

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Rainer Kuemmerle<sup>2</sup>, Wolfgang Berme<sup>3</sup>, Roberta Pierattelli<sup>1</sup>, Isabella Felli<sup>\*,1</sup>

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<sup>2</sup>*Bruker BioSpin AG,* <sup>3</sup>*Bruker BioSpin GmbH*

Intrinsically disordered proteins (IDPs) as well as intrinsically disordered regions (IDRs) of complex protein machineries have recently been recognized as key players in many cellular functions. NMR represents a unique tool to access atomic resolution structural and dynamic information on highly flexible IDPs/IDRs. Improvements in instrumental sensitivity made heteronuclear direct detection possible for biomolecular NMR applications. The CON experiment has become one of the most useful NMR experiments to get a snapshot of an IDP/IDR in conditions approaching physiological ones. The availability of NMR spectrometers equipped with multiple receivers now enables the acquisition of several experiments simultaneously instead of one after the other. Here we propose several variants of the CON experiment in which, during the recovery delay, a second 2D experiment is acquired, either based on 1H detection (CON//HN) or on 15N detection (CON//btNH, CON//(H)CAN). The possibility to collect simultaneous snapshots of an IDP/IDR through different 2D spectra provides a novel tool to follow chemical reactions, such as the occurrence of post-translational modifications, as well as to study samples of limited lifetime such as cell lysates or whole cells.

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### Enhanced Performances in Blend Fuel Cells Studied by Electrochemical Nuclear Magnetic Resonance Spectroscopy

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Currently, proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) have been combined with electrochemistry (EC) to observe reaction pathways and identify products generated due to its high selectivity, capacity in chemical structure determination [1], and quantitation [2]. We have utilized NMR in our electro-catalysis researches, with a representative example that electro-catalytic performances of polymer-modified electrodes have been comparatively studied with  $^1\text{H-NMR}$  spectroscopy as the evaluator [3]. Recently, fuel blends have shown enhanced electrochemical responses, yet mechanisms concerned are still awaiting investigation [4]. Given the advantages of  $^1\text{H-NMR}$  mentioned above, herein, a study on enhancements in fuel blends has been carried out through NMR monitoring.

Fuel blends consisting of ethanol, formic acid, as well as isopropanol or 1, 2-propanediol with different molar ratios were obtained at first. Following that, half cells with those blends were fabricated, with EC-NMR technique applied to compare their electrochemical performances and study possible mechanisms.

Cyclic voltammograms depicts increased electrochemical current responses in half cells with ternary fuel blends, and obviously risen current densities have also been observed in those three-fuel systems during electro-oxidation at constant potentials afterwards. Product distributions varied with electro-oxidation time periods have been figured out according to  $^1\text{H-NMR}$  signal intensities, and results show that as electro-oxidation time goes on, concentrations of all products grow gradually. The relationship between those improved electrochemical performances mentioned above and product yields is now under investigation.

This work is supported by NNSF of China (U1632274, 21505109, 11761141010).

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New potential biomarkers for Alzheimer’s disease from MR spectroscopy in vivo

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Although the incidence of Alzheimer’s Disease (AD) increases[1], the underlying mechanisms of the neurodegenerative process are still not understood. In vivo magnetic resonance spectroscopy (MR) is ideally suited for investigating of neurochemical disease processes, due to its non-invasive combination of chemical sensitivity and imaging. So far, however, significant magnetic resonance spectroscopy (MRS) contributions are still missing in this field. Changes in concentrations of N-acetyl-aspartate (NAA), and myo-inositol (Ins) in AD have been reported previously[2,3]. However, group differences between AD patients and healthy controls (HC) remain small. Utilizing higher field strengths, i.e. 7 T, allows the quantification of additional relevant metabolites– such as the neurotransmitters glutamate (Glu) and γ-amino-butyric acid (GABA)[4]. Here, we propose the combination of several AD-associated metabolites into concentration product-ratios as a promising new biomarker for AD and report results from the EMPIR NeuroMET project.

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Mistic – Interaction studies in native *E. coli* membranes using 400 and 800 MHz DNP  
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Mistic is an  $\alpha$ -helical self-inserting integral membrane protein of *B. subtilis* triggering biofilm formation together with YugO, a K<sup>+</sup>-channel located downstream in the *mstX-yugO* two-gene operon[1]. Its self-inserting properties attracted attention as a fusion tag to support production and incorporation of recombinant membrane proteins into lipid bilayers[2]. NMR studies revealed that Mistic interacts tightly with detergent[3]. However, it shows an unusual polar surface when solubilised in detergent, therefore Mistic seems to combine features of soluble and integral membrane proteins[3]. Additionally, it was shown that polar interactions with headgroups of anionic and zwitterionic detergents overcome the hydrophobic effect in stabilizing Mistic[4,5]. Nevertheless, the aliphatic chain length of detergents still is of importance, as shown with non-ionic detergents[4,5].

We applied 400 MHz and 800 MHz DNP solid-state NMR experiments on isolated *E. coli* membranes containing selectively <sup>13</sup>C-,<sup>15</sup>N-labeled HEKS, TILDA, and STGV Mistic. These labeling patterns cover the loops and secondary structure elements and will be used to determine the secondary structure boundaries and the membrane topology.

We show the beneficial effect of the increased resolution at 800 MHz yielding well-resolved <sup>13</sup>C-<sup>15</sup>N correlations, allowing sequential assignment together with 3D and 4D data recorded at 400 MHz (in progress). We analyzed the incorporation of labels as well as scrambling into undesired amino acids and demonstrate that Mistic is present in a soluble and a membrane bound state. With our studies we aim to improve our understanding of how membrane proteins autonomously fold and we demonstrate that with DNP it is possible to study membrane proteins in their native environment.

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Structure of a transmembrane protein by solid-state NMR  
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Transmembrane (TM) proteins form an important class of proteins, due to their involvement in many cellular pathways and human diseases. They are targeted by more than 50% of commercially available drugs. Due to their hydrophobic nature, structural studies of TM proteins have been severely limited in the past. Plenty of studies relied on detergent-solubilized proteins. Evidence tells us that these exhibit non-native conformational states, thus studies in near-to-native conditions are sought for. Lipid bilayers constitute the native environment for TM proteins *in vivo*. Recent advances in solid-state NMR spectroscopy now enable us to study TM proteins reconstituted into liposomes. Magic angle spinning (MAS) at frequencies of 55 kHz and higher makes proton detection in solid-state NMR possible, benefiting from the intrinsically higher sensitivity of protons as compared to the commonly used <sup>13</sup>C and <sup>15</sup>N nuclei [1]. Here, we show that the reconstitution of a 29 kDa, eight-stranded beta-barrel TM protein, which interacts with cell-adhesion molecules *in vivo*, into DMPC-liposomes enables the recording of proton-detected solid-state NMR spectra for resonance assignment of the protein backbone. We worked on the assignments of the protein, and these reveal that visible peaks are mostly emerging from residues within the more rigid beta-barrel region, as opposed to the large extracellular loop regions. These exhibit large amplitude motions in the micellar structure and show no stable conformational states. Future studies will focus on reconstitution of the TM protein into lipids closer to native membranes. We expect a change in loop flexibility, thus making further progress in what can be measured with proton detected MAS solid-state NMR.

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**Model of the Dynamics of a Bound Protein in a  
Complex Constrained by Dipole-Dipole Recoupling and Relaxation**

W. Trent Franks\*, Angelo Gallo, Jacqueline Tognetti, Carl Oster,  
Becky Stevens, Simone Kosol, Jonah Trenouth, Jozef Lewandowski  
*University of Warwick*

The function of a protein depends on both its structure and the internal dynamics of the peptide chain. Solid-state NMR is one of the few techniques to determine ps to ms dynamics of large biomolecule complexes with atomic-resolution at physiologically relevant conditions. The development of fast MAS (>60 kHz spinning) has contributed to the ability to constrain the protein dynamics in at least two ways: it provides for high resolution and high sensitivity  $^1\text{H}$  detection, and coherent effects that compromise quantitative measurements are reduced at higher spinning frequencies.

Dynamics can be characterized at different time-scales using  $R_1$  and  $R_{1\rho}$  relaxation and dipolar coupling measurements. The quantification of the dynamics relies on simultaneous application of these complementary techniques, which present their own challenges. Dipole-dipole recoupling experiments by and large are difficult to implement at high-spinning frequencies.[1] We present experiments with optimized symmetry-based recoupling scaling factors at fast spinning. Additionally, we consider  $R_{1\rho}$  relaxation measurements both in the Bloch-McConnell and near rotary resonance (NERRD).[2] To improve the rate of data acquisition in such experiments, we will present multiple acquisition experiments.

$R_1$ ,  $R_{1\rho}$ , NERRD, and symmetry-based dipole recoupling data are combined to create a model of the dynamics of a small protein (GB1) in a > 300 kDa complex with its binding partner (IgG).[3] The data indicates that additional motions acquired by GB1 upon binding involve restricted overall motion rather than an increase of the breathing mode across the  $\beta$ -sheet.

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Data Processing to Overcome Insufficient Signal Strength in ESR, NMR, and MRI  
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Cornell University

Overcoming noise in experimental signals has been an age-old problem in magnetic resonance. Apart from improving instrument sensitivity, data processing approaches such as model fitting and signal filtering have been applied with limited success. Current data processing methods focus on capturing the signal from the noisy data, which often requires a priori information and may be limited to a particular type of experiment. This limits their ability to recover signals given initially low Signal-to-Noise Ratio (SNRs). We present an alternative approach that captures and removes noise, leaving signal as the residue, without the need of a priori information. This can be accomplished by using wavelet transforms that can effectively distinguish the randomness of the noise from the coherence of the signal. Randomness is represented by many small coefficients in the wavelet domain, enabling its separation from strong signal coefficients. Although wavelet denoising has been around for 25 years and has previously been used to remove noise from signal, it has failed to achieve its true potential in the past. We overcame its limitations by developing an objective method to accurately calculate noise thresholds for separating the noise coefficients from those of the signal, avoiding both under-denoising or signal distortion. This has led to signal recovery with excellent fidelity at SNRs ~ 1. Our denoising methods achieve more than two orders-of-magnitude improvement in SNR, and/or being able to reduce signal acquisition time by similar orders of magnitude. We will demonstrate the denoising procedure and its application to 1D and 2D ESR and NMR signals, and to MRI. The talk will include representation and analysis in the wavelet domain, and identification and elimination of noise coefficients from signal coefficients in the wavelet domain. Since our method is designed to remove random noise, it can be applied to all types of signals.

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Structural behavior of full-length murine prion protein aggregates in the absence and in the presence of DNA aptamers probed by solution and solid-state NMR

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*UFRJ*

The prion protein is a macromolecule related to several pathologies, such as Kuru, Creutzfeldt-Jakob disease, Scrapie and Mad Cow disease. According to the “Protein only hypothesis”, the prion protein by itself can adopt a non-native infectious conformation that can be transmitted from one animal to another. However, it has been hypothesized the existence of an X-factor that could speed up the conversion from the native into the infectious species. There exist several X-Factor candidates, with nucleic acids being one of them. Our group is interested in understanding the interaction between DNA aptamers and the prion protein. Our results indicate that in the presence of aptamers the prion solution increases turbidity but not because of protein aggregation itself but due to something else. In order to understand this behavior, we were able to prepare less polymorphic fibrils of full-length murine prion protein in the absence of denaturants but using successive cycles of seeding. The 8th generation of seeding resulted in well-ordered fibrils as observed by C-C correlation NMR spectra with 20 ms DARR mixing, in which the sharp peaks suggested a less polymorphic sample. In order to produce a qualitative comparison between the amino acids belonging to the core of the 8th generation of fibrils and the aggregates formed in the presence of aptamers, we lyophilized the sample right away after the addition of the DNA aptamers as centrifugation induces the loss of turbidity. The results indicate that a small amount of prion-aptamers are forming aggregates that could be observed by solid-state NMR, but the fibrillar core seems to be different from the fibrils formed by seeding. The next step of our work will be to see how prion-aptamer-conjugates behave under pressure to see if the interaction only induces a liquid-liquid phase but neither small aggregates nor fibrils.

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Co-Aggregation of Ganglioside Membranes and Alpha-Synuclein  
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Lund University

Parkinson’s disease and several other neurodegenerative diseases are accompanied by the misfolding and aggregation of the natively monomeric protein  $\alpha$ -synuclein. Many disease-related mutants of  $\alpha$ -synuclein have modified N-terminals which will impact their interaction with membranes, while oligomers have been shown to cause membrane permeabilization which can potentially disrupt cell homeostasis. In vivo  $\alpha$ -synuclein aggregates such as Lewy bodies also include membrane lipids, while little is known about the process of lipid/ $\alpha$ -synuclein co-aggregation and its implications for the formation of mature amyloid fibrils and any toxic intermediate forms of  $\alpha$ -synuclein such as oligomers. Ganglioside lipids are abundant in neuronal membranes and have been found to accelerate fibril formation and to accumulate in Parkinson’s patients.

We probe  $\alpha$ -synuclein/lipid interactions by incubating GM3-POPC model membranes with monomeric  $\alpha$ -synuclein and evaluating the effects of co-aggregation on the mobility (regarding the order parameter and the correlation time of C-H bond reorientation) of incorporated lipids using polarization-transfer  $^{13}\text{C}$  solid-state NMR.  $^{31}\text{P}$  NMR was used to monitor the lipid phase and the spinning sidebands at 1250 Hz were used to fit the chemical shift anisotropy, while cryo-TEM was used to image the co-aggregates.

It was found that both lipids are co-assembled with  $\alpha$ -synuclein in large amorphous aggregates where lipids display reduced headgroup and acyl chain mobility, and that GM3-rich membranes increase the uptake of membrane lipids into co-aggregates.  $^{31}\text{P}$  shows that lipids are arranged isotropically in co-aggregates with a reduced  $T_2$  relaxation time. These findings suggest that GM3 can influence the speciation of  $\alpha$ -synuclein into toxic intermediates and fibrils by modifying their physiochemical properties during co-aggregation, with effects that could propagate to the permeabilization/disruption of membranes suggested in synucleinopathies.

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**Combined EPR/DFT/CV Study of  
Electron-Transfer Properties of Germanium-based Photoinitiators**  
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Acylgermanes are efficient germanium-based photoinitiators (PIs) that are suitable for medical applications such as dental fillings.[1–4] Remarkably, this class of compounds displays electron-transfer properties being comparable with phosphorus-based photoinitiators.[5]

Here we present a combined EPR/DFT/CV approach to characterize the redox properties of acylgermanes. Preliminary CV investigations reveal that mono- and bisacylgermanes show reduction peaks in the range of -1.8 to -2.3 V (vs  $\text{Fc}^+/\text{Fc}$ ) depending on the electronic properties of the substituents at the aromatic rings. A reduction of the acylgermanes by elemental potassium leads to one-electron reduced species which could be characterized by EPR. Results from both EPR measurements and DFT calculations indicate that the electron density of these radical-anionic species is only confined to one of the aromatic rings and not delocalized over the whole  $\pi$ -system.

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## Triplet Dynamic Nuclear Polarization of Metal-Organic Frameworks

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Nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) are versatile methods in modern chemistry and biology fields. Nevertheless, they suffer from intrinsically limited sensitivity due to the low nuclear spin polarization at ambient temperature. One of the promising methods to overcome this limitation is dynamic nuclear polarization (DNP). In particular, DNP based on photoexcited triplet electrons (triplet-DNP) has the potential to hyperpolarize nuclear spins of target substrates in the low magnetic field at room temperature. Room-temperature triplet-DNP has provided high enhancements in organic crystals such as p-terphenyl. However, it remains difficult for such organic crystals to accommodate target molecules to be monitored. While amorphous solids such as o-terphenyl were employed as host matrices to accommodate target substrates, the flexible structure requires the cooling of the sample for triplet-DNP (~120 K). Therefore, despite these efforts, it remains a grand challenge to develop a room-temperature triplet-DNP system with accessibility for polarizing targets. In this work, we report the first example of triplet-DNP of nanoporous metal-organic frameworks (J. Am. Chem. Soc. 2018, 140, 15606-15610). We modified the typical polarizing agent pentacene with metal-coordinating carboxylate moieties (4,4'-(pentacene-6,13-diyl)dibenzoic acid (PDBA)) for its introduction into MOFs. A relatively long <sup>1</sup>H T<sub>1</sub> has been reported for a prototypical diamagnetic Zn<sup>2+</sup>-based MOF, [Zn(MeIM)<sub>2</sub>]<sub>n</sub> (ZIF-8; MeIM = 2-methylimidazolate). A partial deuteration of MeIM ligands allows the elongation of T<sub>1</sub> of partially deuterated D-ZIF-8. Polarization transfer from PDBA triplet electrons to <sup>1</sup>H nuclei in D-ZIF-8 resulted in a clear enhancement (ε = 58) of <sup>1</sup>H NMR signals of D-ZIF-8 at a low magnetic field of 0.67 T and room temperature.

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Photo-thermo refractive Glasses: NMR and EPR Mechanistic Studies

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Photo-thermo refractive (PTR) glasses are photosensitive glasses of composition  $70\text{SiO}_2 \cdot 15\text{Na}_2\text{O} \cdot 5\text{NaF} \cdot 5\text{ZnO} \cdot 4\text{Al}_2\text{O}_3 \cdot 1\text{KBr}$  that are doped with small quantities of  $\text{CeO}_2$ ,  $\text{Ag}_2\text{O}$ ,  $\text{Sb}_2\text{O}_3$ , and  $\text{SnO}_2$  (0.01 mol %). Their refractive index can be modified with high spatial resolution using focused UV irradiation and subsequent annealing close to the glass transition temperature. This enables manufacturing of various important optical components and devices (e.g. Bragg gratings, holograms and components to control the length of laser pulses).[1] However, the structure of the glass, the origin of the refractive index change, and the role of  $\text{Sb}_2\text{O}_3$ ,  $\text{SnO}_2$  and KBr are not yet fully understood.

Here, the short- and medium-range order of the glass system is studied for the first time by multinuclear single- and double-resonance solid-state nuclear magnetic resonance (NMR) spectroscopy.[2]  $^{23}\text{Na}\{^{19}\text{F}\}$  REDOR experiments are useful for measuring the fraction of F-bonded  $\text{Na}^+$  ions, giving insights into their spatial distribution.

By means of CW and pulsed EPR spectroscopy, the paramagnetic species, formed by UV irradiation, are identified.[3] The vicinity of photosensitive and photoactivated centers ( $\text{Ce}^{3+}$  and “ $\text{Sb}^{4+}$ ”) is further studied by Electron Spin Echo Envelope Modulation (ESEEM) and HYperfine Sub-level Correlation (HYSCORE) Spectroscopies. Based on the electron’s hyperfine interactions with nearby nuclear spins, we obtain new insights on the role of the dopants in the initial steps of photoexcitation. Finally, combining  $^{19}\text{F}$  MAS NMR and CW EPR spectroscopy the formation of silver clusters (nucleation upon thermal annealing) can be monitored.

The authors are grateful for the support by FAPESP grant 2013/07793-6 and thank Professor Leonid Glebov (University of Central Florida) for providing the samples. LMF thanks the Stiftung der Deutschen Wirtschaft for a doctoral stipend.

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### Structural basis for the interaction between a peptidyl carrier protein and condensation domain in the enacyloxin hybrid PKS-NRPS

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Modular polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are giant bacterial multi-enzymes (1-3 MDa), which biosynthesize structurally complex bioactive natural products. Modular nature of PKSs makes them suitable for bioengineering provided important factors responsible for control of the biosynthesis, such as protein-protein interactions, are considered. Here we report on protein-protein interactions implicated in the chain termination in the biosynthesis of enacyloxin; an antibiotic that is active against multidrug-resistant *A. baumannii*: the number one organism on the list of the WHO priority pathogens.

Combining solution NMR, solid-state NMR, mass spectrometry based carbene footprinting, X-ray crystallography and molecular dynamics (MD) we are able to elucidate the interactions between a peptidyl carrier protein (Bamb\_5917 PCP, ~11 kDa) and a condensation domain (Bamb\_5915 C, ~56 kDa).

Solution NMR and carbene footprinting studies show that interaction between the two proteins involve the C-terminal intrinsically disordered docking domain of Bamb\_5917 PCP and beta-hairpin docking domain of Bamb\_5915 C and as well as globular segments of the proteins. <sup>15</sup>N CEST on Bamb\_5917 PCP provides clues about changes in conformation of the C-terminal intrinsically disordered region upon binding. All these data suggest large conformational changes of both Bamb\_5917 PCP and Bamb\_5915 C upon binding, which are consistent with correlated motions observed in MD simulations. Fast and ultra-fast MAS solid-state NMR of sedimented the protein complex is used to obtain an atomic resolution view of Bamb\_5917 PCP within the ~70 kDa complex. Docking calculations using HADDOCK provide insights into the interactions between the two proteins and biochemical assays demonstrate that the condensation reaction critically depends on the presence of the docking domains.

Our results suggest an intriguing general allosteric regulation mechanism responsible for directionality of a condensation reaction and provide a basis for a synthetic biology approach to create hybrid PKS/NRPSs systems to produce new antibiotics.

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**P137**

**Solid State NMR study of Curcumin partition inside  
solid lipid nanoparticle@mesostructured silica matrix**

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Pierrick Durand, Sabine Bonnet, Andreea Pasc, Axel Gansmüller\*  
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Curcumin is a hydrophobic Active Pharmaceutical Ingredient with anti-oxidant and anti-inflammatory properties. To overcome challenges in its oral delivery, a novel drug delivery system has been developed [1]. It consists of solid lipid nanoparticles (SLN) embedded in a porous silica matrix (SBA-15). The material is structured in a network of meso-pores, filled with surfactant micelles, linking macropores containing the lipid cores. When curcumin is included, its release kinetics varies depending on the lipid chemical nature (cetyl palmitate or stearic acid). In this study, we show that the partitioning of curcumin, a factor controlling the release rates, is tied to the inner structure of the SLN.

We first show how combining DSC with temperature dependent NMR measurements allows the detection and identification of two co-existing lipid polymorphs. In particular, the comparison of, direct, CP-MAS and INEPT <sup>13</sup>C polarization schemes, permits to focus on the different elements of the heterogeneous materials. Additionally, by performing <sup>1</sup>H-<sup>1</sup>H dipolar correlation experiments with <sup>13</sup>C detection, we establish a two layered structural model for the SLNs into a crystalline bulk-like lipid core, surrounded by a polymorphic corona. These experiments also allow a comparison between the structure of the cetyl palmitate and the stearic acid based materials, as the polymorphic phase is more pronounced for the latter.

The second part is dedicated to the study of curcumin loaded materials. <sup>13</sup>C isotopic labeling, along with our <sup>1</sup>H-DQF <sup>1</sup>H/<sup>13</sup>C hetero experiment, allows us to determine curcumin partition in the materials, despite its very low content (~1.7%). While we find that most of the compound is in an amorphous state, dispersed in the different compartments of the hybrid material, we also show that curcumin has a preferential partition within the SLN corona and the surfactant micelles.

**Reference:** [1] S. Kim et al., J Mater Chem B. 2, 7910–7917 (2014)

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Revealing the evolution of the solid-electrolyte interphase in sodium metal batteries by ex situ/in situ NMR  
Lina Gao, Juner Chen, Xueqian Kong

Due to the increasing demands for large scale grid energy storage, sodium metal batteries (SMBs), are competitive for economic and environmental reasons. Stabilizing the reactive metal anode is a critical challenge for the development of SMBs. Our work demonstrates that sodium-difluoro(oxalato)borate (NaDFOB)-based carbonate-ester electrolyte possesses favourable electrochemical stability and effectively passivates the Na metal anode by forming a compact, robust and conductive solid-electrolyte interphase (SEI) layer, enabling high rate performance and long cycle life. We performed  $^{19}\text{F}$ ,  $^{23}\text{Na}$  and  $^{11}\text{B}$  NMR experiments to investigate the chemical composition of the NaDFOB-derived SEI film, which includes a mixed phase of primarily sodium diborate, tetrafluoroborate and carbonate. The identification for the sodium and boron species was accomplished through the combination of 1D spectra and 2D triple-quantum magic-angle spinning (2D-3QMAS) experiments. In addition, we monitored the SEI evolution and electrolyte depletion during battery cycling by ex-situ and in-situ NMR. Quantitative  $^1\text{H}$  and  $^{19}\text{F}$  NMR were employed to quantify the electrolyte consumption. This study explores the formation mechanisms of SEI and the relations between SEI properties and battery performance, providing an insightful viewpoint for the design of SEI.

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## P139

Design of Ultra-Broadband  $^{19}\text{F}$ -CPMG and  $^{19}\text{F}$ -PROJECT Pulse Sequences

Stella Slad\*, Burkhard Luy  
*Karlsruhe Institute of Technology*

Recently the access to  $^{19}\text{F}$ -containing screening libraries has started a great pharmaceutical interest for  $^{19}\text{F}$ -based screening methods. Especially the  $^{19}\text{F}$ -CPMG experiment can be used for detection of protein-ligand interactions. However, when screening large libraries of fluorinated organic compounds, pulses that cover the  $^{19}\text{F}$ -bandwidths of 120 kHz on a 600 MHz spectrometer are needed. This bandwidth is hardly covered by standard broadband excitation and refocussing pulses. In addition, a second experiment of interest is PROJECT [1], which prevents phase distortions due to J-modulation in polyfluorinated molecules, but which requires the application of very broadband universal rotation 90 degree pulses.

The GRadient Ascent Pulse Engineering (GRAPE) algorithm has been shown to be a very effective tool for pulse optimization [2]. The method has already been used extensively for the design of robust broadband excitation and inversion pulses (BIBOP) [3,4] as well as universal rotation pulses (BURBOP) [5]. In general, pulses optimized for a large bandwidth will have long pulse lengths in order to achieve comparable performance. In addition, with increasing bandwidth it becomes harder to find an optimal solution. Good high resolution NMR pulses for bandwidths larger than 60 kHz are therefore difficult to obtain. In our work, different ultra-broadband pulses have been optimized and compared with each other. In addition, resulting CPMG and PROJECT sequences have been designed and tested theoretically using density matrix simulations.

**References:** [1] Aguilar et al., *Chem. Commun.* 48, 811–813 (2012). [2] Khaneja et al., *J. Magn. Reson.* 172, 296–305 (2005). [3] Kobzar et al., *J. Magn. Reson.* 170, 236–243 (2004). [4] Kobzar et al., *J. Magn. Reson.* 194, 58–66 (2008). [5] Kobzar et al., *J. Magn. Reson.* 225, 142 (2012).

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P140

NMR structural studies on lipoprotein signal  
peptidase II in micelles and reconstituted into a cubic phase

Coilin Boland<sup>1</sup>, Hamed Kooshapur<sup>2</sup>, Walter Teague<sup>3</sup>,  
Jonathan Bailey<sup>4</sup>, Martin Caffrey<sup>4</sup>, Klaus Gawrisch<sup>4,3</sup>

<sup>1</sup>Trinity College Dublin, <sup>2</sup>NHLBI/NIH, <sup>3</sup>NIAAA/NIH, <sup>4</sup>Trinity College, Dublin

The lipoprotein signal peptidase II (LspA), a membrane protein, is a key enzyme in *Pseudomonas aeruginosa*, a human pathogen. Its substrate are lipoproteins of the bacterium. The antibiotic globomycin inhibits LspA. The goal of the project is to gather structural information related to LspA inhibition and lipoprotein processing. From recent structural studies by crystallography and solution state NMR, it is known that globomycin-bound LspA has four transmembrane helices and a beta-sheet on the periplasmic site of the bacterial membrane. The LspA was expressed in a cell-free expression system using isotopically labeled amino acids, chromatographically purified and solubilized in Fos-Choline-12 detergent. Protein structure was studied both by solution-state NMR in detergent micelles and by solid-state NMR reconstituted into a monooleine cubic phase. Spectra of both the apo form of LspA as well as the globomycin-bound form were obtained. Structural differences between both states will be discussed.

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P141

**High-resolution structure of a farnesylated protein: selective isotope labeling strategies for proteins with unlabeled moieties or ligands**

Gerd Gemmecker<sup>\*,1</sup>, Konstantinos Tripsianes<sup>2</sup>, Leonidas Emmanouilidis<sup>3</sup>, Ulrike Schütz<sup>3</sup>, Michael Sattler<sup>4</sup>

<sup>1</sup>BNMRZ, TU München, <sup>2</sup>CEITEC, <sup>3</sup>BNMRZ,

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The structural investigation of the C-terminally farnesylated peroxisomal protein PEX19 was complicated by the fact that isotopically labeled *apo* PEX19 had to be enzymatically farnesylated *in vitro* with farnesyl pyrophosphate. Since this reagent is only available in natural abundance, the reaction will inevitably lead to an isotopically unlabeled farnesyl moiety, while the protein moiety can be expressed with various combinations of isotope labels.

Therefore, for the structural studies of farnesylated PEX19, a combination of different isotope labeling tricks was used, including deuteration, residue and sidechain specific <sup>13</sup>C labeling and selective protonation. With appropriately filtered NMR experiments it was possible to achieve the complete assignment of the unlabeled farnesyl moiety, as well as unambiguously detect NOEs between the farnesyl part and neighbouring protein sidechains.

In the case of PEX19 this resulted in the first high-resolution NMR structure of a farnesylated protein. However, the approach described in this poster will also be useful when dealing with other post-translational modifications or protein-ligand complexes where the ligand is not available in isotope-labeled form.

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P142

Protonation Dynamics in PSII Subunit PsbO

Lisa Gerland<sup>\*,1</sup>, Daniel Friedrich<sup>1</sup>, Anne Diehl<sup>1</sup>, Natalja Erdmann<sup>1</sup>, Linus Hopf<sup>1</sup>, Holger Dau<sup>2</sup>, Peter Schmieder<sup>1</sup>, Hartmut Oschkinat<sup>1</sup>  
<sup>1</sup>FMP Berlin, <sup>2</sup>FU Berlin

PsbO is an extrinsic subunit of the Photosystem II (PSII) complex and is known to act as ‘manganese-stabilizing protein’. It is the only extrinsic protein of PSII that is present in all photosynthetic organisms. PsbO plays a central role photosynthesis and has therefore been studied over many decades. Its molecular mechanisms remain to be fully elucidated. PsbO has been suggested to functionally control the oxygen evolution center (OEC) of PSII, regulating the chloride and calcium concentration and thereby stabilizing the manganese-complex.

The crystal structure of PsbO revealed the presence of numerous surface-clusters of glutamate and aspartate residues, suggesting an additional function of PsbO as proton antenna of PSII. These clusters could serve as proton storage, enable a directed proton transport at the protein surface or interact with luminal bulk water contributing to a proton transfer.

To characterize these surface-clusters and the role of protonation dynamics in PsbO function, we determined the pKa values of carboxyl-groups in glutamate and aspartate residues using solution NMR pH-titration experiments. We determined the majority of pKa values ranging from 3 – 5 for these residues in the 19 kDa beta-barrel PsbO protein. Exceptions include residues forming a salt bridge and in a proposed structural switch region. Further structure-related analysis of the obtained pKa values did not yield a gradient along the beta-barrel for a proposed directed proton transport. Interestingly lower pKa values cluster on one side of the protein, whereas the other side harbors residues with higher pKa values.

Firstly, our results indicate a buffer capability of PsbO, stabilizing the luminal pH via proton storage. Secondly, the pKa distribution could induce a macro dipole along the beta-barrel of PsbO, potentially leading to a higher dielectric constant of the luminal solution. This could affect the protonation events during photolysis of H2O in the OEC.

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## P143

**Photo-Induced Reactivity and NMR/EPR: Examples and Ways for Optimization**Georg Gescheidt, Eduard Stadler, Max Schmallegger, Christian Holly

Photo-induced reactions have (again) become rather popular within the recent years. NMR, CIDNP, and EPR providing information at the molecular level have been utilized as ideal methods for following such reactions. [1-2]

We have investigated electron-transfer processes in phospholipid membranes using EPR establishing the corresponding rate constants. In terms of NMR, we have performed experiments and strategies to enhance the efficiency for following photo-induced reactions. This includes appropriate sample preparations, the quantification of their optical and photo-physical properties, adjusting NMR and photo-active volumes, and the modification of NMR sequences including light pulses. [3-5]

In this presentation examples of our recent developments will be presented.

**References:** [1] Feldmeier, C.; Bartling, H.; Magerl, K.; Gschwind, R. M., *Angew. Chem. Int. Ed.* 2015, 54, 1347-1351. [2] Kind, J.; Kaltschnee, L.; Leyendecker, M.; Thiele, C. M., *Chem. Commun.* 2016, 52, 12506-12509. [3] Stadler, E.; Eibel, A.; Fast, D.; Freissmuth, H.; Holly, C.; Wiech, M.; Moszner, N.; Gescheidt, G., *Photochem. Photobiol. Sci.* 2018, 17, 660-669. [4] Stadler, E.; Dommaschk, M.; Fruhwirt, P.; Herges, R.; Gescheidt, G., *Chemphyschem* 2018, 19, 571-574. [5] Stadler, E.; Eibel, A.; Neshchadin, D.; Gescheidt, G., *Z. Phys. Chem.* 2017, 231 (3), 625-636.

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P144

Facile quantitation by NMR, using neat liquids as external standards  
Ion Ghiviriga\*  
*University of Florida*

We propose an easy and precise method of quantitation by NMR, using the neat protonated solvent as external concentration reference. Spectra of the analyte in a deuterated solvent, e.g. chloroform-d, and the neat solvent, in this case protonated chloroform, are taken in the same conditions, i.e. the same relaxation delay, pulse width, acquisition time and receiver gain. The linearity of the receiver and the effective resolution of the ADC achieved by over-sampling allows for quantitative comparison of samples with a concentration difference larger than 3 orders of magnitude. Good tuning and matching of the probe improves the precision of the result, but does not affect the accuracy. Concentrations of analyte down to 10 mM can be measured with a precision of 1%. Using a proper receiver gain, the limit of quantitation can be decreased to 0.5 mM, without the need for any correction or solvent suppression.

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EPR probes two ‘closed’ ATP/ADP conformations and small nucleotide-driven structural changes in yeast Hsp90 chaperone

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Weizmann Institute of Science

Hsp90 is a central chaperone performing its activity hydrolyzing ATP with Mg(II) as cofactor. Hsp90 is homo-dimeric and each monomer consists of 3 domains (CTD, MD, NTD). The ATPase site is in the NTDs, while the CTDs are dimerized. In absence of nucleotide Hsp90 is in ‘open’ conformation shifting to an ATP-bound ‘closed’ conformation by dimerization of the NTDs.[1] Yet, there are still open questions on whether the ATP hydrolysis induces global conformational changes and whether the ADP state is ‘open’ or ‘closed’.

We address this question by investigating the local structural changes at the ATPase site and the concomitant conformational changes at various nucleotide-bound states in yeast Hsp90 using EPR techniques. We substituted Mg(II) with Mn(II) and performed hyperfine and pulse dipolar EPR. Specifically, we tracked ATP hydrolysis using <sup>31</sup>P ENDOR and investigated Mn(II)-protein interactions by <sup>14/15</sup>N EDNMR. We, also, measured the distance between the Mn(II) cofactors using DEER/PELDOR spectroscopy. Using site-directed spin labelling (SDSL) with nitroxide and Gd(III) labels and DEER we monitored inter-monomer rearrangements at different states of the ATPase cycle.

We observed large disorder of Hsp90 as well as spin label flexibility and found that the presence of nucleotide merely dictates the conformational ensemble. This is in contrast to a well-defined Mn(II)-Mn(II) distance in the ATP state, which shortened and broadened in the ADP state, providing experimental evidence to the existence of two different ‘closed’ conformations. The exploitation of the intrinsic metal binding site allowed us probe local and global interactions from a single sample and obtain new structural insights previously challenging to observe with SDSL-DEER/FRET. DEER on mutants revealed small nucleotide-driven structural preferences implying the role of co-chaperones and/or substrates to be the trigger for large conformational changes.

Reference: [1] K.A. Verba et al. Science, 2016, 352, 1542; M.M.U. Ali et al. Nature, 2006, 440, 1013.

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**EPR study of ferroelectric phase transition in  
Mn<sup>2+</sup>-doped [NH<sub>4</sub>][Zn(HCOO)<sub>3</sub>] formate framework**

Laisvydas Giriūnas<sup>\*,1</sup>, Marius Navickas<sup>1</sup>, Mirosław Maćzka<sup>2</sup>,  
Jūras Banys<sup>1</sup>, Andreas Pöppl<sup>3</sup>, Mantas Šimėnas<sup>1</sup>

<sup>1</sup>Vilnius University, <sup>2</sup>Polish Academy of Sciences, <sup>3</sup>Leipzig University

Dense metal-formate frameworks of general formula [A][M(HCOO)<sub>3</sub>]<sub>n</sub> attracted significant attention of the scientific community due to the indications of the multiferroic behavior [1]. These compounds consist of transition metal ions (M<sup>2+</sup>) linked by formate groups into porous frameworks, where each pore confines a single molecular cation (A<sup>n+</sup>). The majority of these frameworks exhibit structural phase transitions, which involve long-range molecular cation ordering and metal-formate framework deformation. A useful method to study local structural changes and dynamic effects in formate frameworks is EPR spectroscopy [2].

In this study, we employ X-band and Q-band CW EPR to study a ferroelectric phase transition in ammonium zinc formate [NH<sub>4</sub>][Zn(HCOO)<sub>3</sub>] (AmZn) framework doped with a small amount of Mn<sup>2+</sup> ions. The obtained temperature dependent EPR spectra of AmZn:Mn<sup>2+</sup> powder shows typical patterns of Mn<sup>2+</sup> ions in the 3d<sup>5</sup> electronic configuration. Upon cooling, CW EPR spectra exhibit a continuous change at the phase transition temperature of about 190 K, demonstrating that Mn<sup>2+</sup> centers are susceptible to the phase transition. Temperature dependent EPR linewidth continuously increases upon cooling, revealing an anomalous peak at 190 K, which indicates a second-order character of the phase transition.

The spin Hamiltonian with terms describing the electron Zeeman interaction, fine and hyperfine structures was used to simulate the experimental CW EPR spectra of AmZn:Mn<sup>2+</sup>. The determined value of isotropic hyperfine coupling constant indicates Mn-O coordination [2], suggesting that Mn<sup>2+</sup> ions successfully replaced Zn<sup>2+</sup> centers and formed MnO<sub>6</sub> octahedra. The temperature dependence of the axial zero-field splitting parameter *D*, measuring the axial distortion of the MnO<sub>6</sub> octahedra, exhibits a continuous increase at about 190 K, which confirms a second-order character of the phase transition in AmZn.

**References:** [1] Jain *et al.*, *J. Am. Chem. Soc.*, 131(38), 13625 (2009). [2] Šimėnas *et al.*, *J. Phys. Chem. C*, 120(35), 19751 (2016).

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**P147**

**Difference approach enhanced by DNP to eliminating radical  
induced relaxivity in relaxation dispersion studies**

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*Ilmenau University of Technology*

Fast field cycling (FFC) NMR relaxometry enhanced by DNP [1] has recently been reported to obtain  $T_1$  relaxation time dispersion with increased sensitivity and selectivity to study molecular dynamics in various systems. First studies were devoted to the measurement of the DNP spectrum and enhancement as well as DNP-enhanced dispersion curves of polymer systems in the presence of radicals [2], effects of fluorination on interaction in simple organic liquid and features of interaction between asphaltenes and low weight molecular part of oil [3]. The perceptible levels of DNP enhancement up to several hundred were reached in the studied system, showing the high potential of FFC DNP. Furthermore, additional relaxivity, i.e., a significant decrease of the relaxation time due to the addition of radicals in the system of interest, inducing drastic changes in the spin-lattice relaxation dispersion, usually renders the analysis of molecular dynamics properties difficult or impossible. Using a new difference approach, the dynamics in the system without radicals can be recovered, and the radical relaxation effects can be isolated. The main area of interest for the implementation of the difference approach is the study of molecular dynamics in systems with exhibit low thermal polarization.

In this contribution the proof of principle is discussed based on the molecular dynamics study of a block-copolymer (PS-PB-PS), while the first results of actual X nuclei systems with  $^2\text{H}$ ,  $^7\text{Li}$  and  $^{13}\text{C}$  target nuclei are presented.

**References:** [1] O. Neudert, M. Reh, H. W. Spiess, K. Münnemann, *Macromol. Rapid Commun.*, 36 (2015), 885–889. [2] B. Gizatullin, O. Neudert, C. Mattea, S. Stapf, *Chem. Phys. Chem.*, 2017, 18, 2347–2356. [3] S. Stapf, A. Ordikhani-Seyedlar, N. Ryan, C. Mattea, R. Kausik, D. E. Freed, Y. -Q. Song, M. D. Hürlimann, *Energy Fuel*, 2014, 28, 2395–2401.

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Size Dependent Ultrafast Water Flow in Carbon Nanotubes A Combined 2D <sup>1</sup>H NMR Diffusion-Relaxation (D-T<sub>2</sub>) and Molecular Dynamics Simulations Study

Lydia Gkoura<sup>\*,1</sup>, Jamal Hassan<sup>2</sup>, Margarita Beazi-Katsioti<sup>3</sup>,  
Michael Fardis<sup>1</sup>, George Papavassiliou<sup>1</sup>

<sup>1</sup>Institute of Nanoscience and Nanotechnology, NCSR Demokritos, <sup>2</sup>Department of Physics, Khalifa University, <sup>3</sup>School of Chemical Engineering, National Technical University of Athens

Several theoretical studies using molecular dynamics MD simulations showed an enhancement of water dynamics inside hydrophobic channels and a peak of the liquid self-diffusion coefficient is reported at certain carbon nanotube sizes. The studies have shown that this enhancement depends on the size of the hydrophobic nanochannels. However, experimental evidence of this dependence is lacking so far. Combining two-dimensional Nuclear Magnetic Resonance (NMR) diffusion–relaxation (D-T<sub>2</sub>) spectroscopy, and Molecular Dynamics (MD) simulations we analyzed the size dependence of water dynamics inside carbon nanotubes (CNTs) of different diameters (1.1 nm to 6.0 nm), in the temperature range of 265K to 305K. These NMR method provide a unique way to distinguish water in the interior of CNTs from bulk water and water adsorbed on the external surface of the CNTs. Most notable, a favourable CNTs diameter range (3.0-4.5nm) is experimentally verified for the first time, in which water molecule dynamics at the centre of the CNTs exhibit distinctly non-Arrhenius behaviour, characterized by ultrafast diffusion and extraordinary fragility, a result of significant importance in the efforts to understand the water behaviour in hydrophobic nanochannels.

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DNP-MAS NMR on cryo-trapped photointermediates reveals  
photocycle-dependent cross-protomer interactions in microbial rhodopsins  
Jakob Maciejko<sup>1</sup>, Jagdeep Kaur<sup>1</sup>, Johanna Becker-Baldus<sup>2</sup>, Clemens Glaubitz<sup>\*,1</sup>

<sup>1</sup>Goethe University Frankfurt, <sup>2</sup>Goethe University

Proteorhodopsin (PR) is found in marine bacteria in various ecosystems and is one of the most abundant photoreceptors. It converts light into a transmembrane, electrochemical gradient and forms functionally unresolved higher oligomers. Although the PR monomer is able to undergo a full photocycle, the question arises whether the pentameric complex formed in the membrane via specific cross-protomer interactions plays a role for its functional mechanism. Here, we use DNP-MAS-NMR in combination with light-induced cryo-trapping of photo-intermediates to address this topic. The highly conserved residue H75 is located at the protomer interface. We show that it switches from the (tau)- to the (pi)-tautomer and changes its ring orientation in the M-state. It couples to W34 across the oligomerisation interface based on specific His/Trp ring orientations, while stabilizing the pKa of the primary proton acceptor D97 within the same protomer. We further show that specific W34 mutations have a drastic effect on D97 and proton transfer mediated through H75. The residue H75 defines a novel cross-protomer Asp-His-Trp triad, which potentially serves as a pH-dependent regulator for proton transfer. Our data represent the first light dependent, functionally relevant cross-talk between protomers of a rhodopsin homo-oligomer and our experimental approach is a showcase for the application of DNP to resolve catalytic mechanisms in membrane proteins.

**References:** [1] Maciejko et al. (2019) Photocycle-dependent conformational changes in the proteorhodopsin cross-protomer Asp-His-Trp triad revealed by DNP-enhanced MAS-NMR. Proc Natl Acad Sci USA: 201817665. [2] Mehler et al. (2017) Chromophore Distortions in Photointermediates of Proteorhodopsin Visualized by Dynamic Nuclear Polarization-Enhanced Solid-State NMR. J Am Chem Soc 139: 16143-16153. [3] Maciejko et al. (2015) Visualizing Specific Cross-Protomer Interactions in the Homo-Oligomeric Membrane Protein Proteorhodopsin by Dynamic-Nuclear-Polarization-Enhanced Solid-State NMR. J Am Chem Soc 137: 9032-43.

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## P150

## Nuclear Spin Singlet States – From Molecular Switches to Biological Probes

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*Max Planck Institute for Biophysical Chemistry*

Nuclear spin singlet states consist of two spin- $\frac{1}{2}$  nuclei and form a spin 0 which can only be probed indirectly[1]. The equilibration time ( $T_s$ ) between the triplet and singlet state often exceeds the longitudinal relaxation time  $T_1$ [1].

We are going to present our recent investigations on using the singlet phenomenon as contrast mechanism. In initial experiments we have designed a variety of molecular spin switches in which changes in  $T_s$  can be measured once a stimulus acts on the molecules[2,3]. The stimuli involve light or temperature and the processes are reversible, hence resemble a switch.

So far, the main drawback of the singlet state phenomenon is the presence of paramagnetic ions which significantly accelerates  $T_s$ . Recently, we have introduced the concept of nuclear spin singlet multimers (NUSIMERS)[4] consisting of several singlet states per molecule that can be populated at the same time. We have succeeded in completely shielding singlet states against paramagnetic ions in such constructs[5]. Moreover, we still detect long-lived singlets once a fluorophore is attached to the NUSIMER. This discovery led us to develop a new kind of bimodal bio-probe combining fluorescence microscopy and singlet state NMR and we show its applicability in a variety of cell lines.

Lastly, we demonstrate first successful singlet experiments in brain matter[6]. We have developed a singlet experiment which allowed us to probe specific metabolites in the brain. Due to different proton-proton couplings, certain metabolites can be filtered and undesired signals from e.g. water suppressed.

**References:** [1] M. H. Levitt, Annu. Rev. Phys. Chem. 63, 89-105 (2012). [2] S. Mamone, S. Glöggler, Phys. Chem. Chem. Phys. 20, 22463 (2018). [3] S. Yang et al. Angew. Chem. Int. Ed. 58, 2879 (2019). [4] P. Saul et al. Chem. Sci. 10, 4131 (2019). [5] P. Saul et al. (submitted). [6] S. Mamone et al. (submitted)

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Elucidating structure and dynamics of  
extracellular matrix collagen using solid state NMR

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<sup>1</sup>University of Cambridge, <sup>2</sup>FMP Berlin

Collagens are the most abundant components of the extracellular matrix (ECM). Due to their diverse structures and compositions, collagens serve many functions, providing structural and mechanical support for surrounding cells, and playing important roles in cell-to-cell communication. Nonetheless, despite being at first glance a simple protein formed by three homologous polypeptide chains of repeating three-amino-acid triads trimerized into a triple helix, it is a highly versatile and complex system. With over 3000 amino acids per triple helix, it is insoluble and does not crystallize. Due to its complexity and size, and in spite of technological advances, there is still poor understanding of collagen structure, flexibility, and dynamics at the atomic level.

The work presented here focuses on <sup>15</sup>N ssNMR spectral assignments in synthetic collagen model peptides, U-<sup>13</sup>C, <sup>15</sup>N labelled collagen and mouse bone. As nitrogen is a component of peptide bonds, <sup>15</sup>N relaxation is a sensitive probe of collagen protein backbone dynamics. However, in such heterogeneous systems, the relaxation studies can be challenging due to the overlapping resonances resulting in one relaxation constant. Therefore, we use a nuclear spin relaxation-based separation method to extract information from complex unresolved spectra. Inverse Laplace transform of these relaxation decays show a separation of different relaxing components with different relaxation constants for the species with similar chemical shifts. We show that this methodology can be employed successfully to separate T<sub>1</sub> relaxation of synthetic model peptides, where this methodology allows characterization of sequence and neighbor effects on <sup>15</sup>N relaxation. Furthermore, this methodology can be employed further on the isotopically enriched in-vitro grown collagen and mouse bone.

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How does the mood stabilizer Lithium bind ATP, the “energy currency” of the cell

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*Tel Aviv University, School of Chemistry*

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Lithium is a leading drug for the treatment of bipolar disorder and affects many biochemical pathways via inhibition of phosphatases and kinases. Lithium can replace magnesium cations in enzymes and small molecules, among them ATP. Yet, despite its fundamental pharmaceutical importance, the mode of binding of lithium to ATP has never been directly observed. Here we present the binding environment of lithium in Lithium-ATP, and determine the identity of its phosphate ligands. Using a multi-nuclear solid-state magic-angle spinning NMR approach, including distance measurements, correlation experiments, and MQMAS, we determine that lithium coordinates with the first ( $P_{\alpha}$ ) and second ( $P_{\beta}$ ) phosphates of one ATP molecule, and with the second ( $P_{\beta'}$ ) phosphate of a second ATP molecule in the unit cell. The Li-P distance is 3 Å. The fourth coordination is probably to water. Such binding is similar to the coordination and distances of lithium in the environment of carboxyl groups, both in a small inorganic lithium-glycine-water complex and in the putative target for lithium therapy – the enzyme inositol monophosphates. In Lithium-ATP we also show that the phosphate chains are non-linear and that  $P_{\gamma}$  of one ATP molecule resides in between  $P_{\alpha}$  and  $P_{\gamma'}$  of a second ATP molecule. Despite the use of excess lithium in the preparations, sodium ions still remain bound to the sample, at distances of 4.3-5 Å from Li, and coordinate  $P_{\gamma}$ ,  $P_{\gamma'}$ , and  $P_{\alpha}$ .

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Applications of differential  $^{19}\text{F}$  labelling of proteins for complex mixture analysisAlexander Golovanov<sup>\*</sup>, John Edwards, Jack Bramham*The University of Manchester*

Proteins in biological environments exist as part of crowded mixtures. Biopharmaceutical proteins for injections also often have to be formulated at very high concentrations (100-400 mg/mL) and as mixtures. Characterizing protein self-interactions vs cross-interactions in such complex systems in situ is challenging, but is required for adequate system description.

Recently our group and collaborators pioneered the use of differential labelling of several proteins at once with covalently-attached extrinsic  $^{19}\text{F}$  tags, which allows observing a characteristic non-overlapping  $^{19}\text{F}$  NMR signal from each protein component independently and simultaneously in a mixture, without any background signals (Edwards et al. 2018 Mol. Pharmaceutics, 15(7):2785). Proteins even as large as 145 kDa monoclonal antibodies can be individually monitored and characterized using such measurables as translational diffusion coefficients and transverse relaxation rates, which all are sensitive to changes in protein size or cluster formation. Moreover, recent application of Dark State Exchange Saturation Transfer (DEST) NMR to such  $^{19}\text{F}$ -labelled proteins in mixtures enabled protein-specific quantification of very large NMR-invisible protein clusters in high-concentration co-formulations of monoclonal antibodies (Edwards et al. 2019 Anal. Chem., 91(7):4702). In another application, monitoring three proteins at once, human albumin, transferrin and IgG in serum revealed that their temperature-dependent association behavior is significantly different from that if these three are just formulated in a simple buffer (Edwards et al. 2019 ChemPlusChem, 84(5):443-446). The basis of this new approach and its applications will be presented.

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## P154

## Two distinct mechanisms of transcriptional regulation by the redox sensor YodB

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For bacteria, cysteine thiol groups in proteins are commonly used as thiol-based switches for redox sensing to activate specific detoxification pathways and restore the redox balance. Among the known thiol-based regulatory systems, the MarR/DUF24 family regulators have been reported to sense and respond to reactive electrophilic species, including diamide, quinones, and aldehydes, with high specificity. Here, we report that the prototypical regulator YodB of the MarR/DUF24 family from *Bacillus subtilis* uses two distinct pathways to regulate transcription in response to two reactive electrophilic species (diamide or methyl-p-benzoquinone), as revealed by X-ray crystallography, NMR spectroscopy, and biochemical experiments. Diamide induces structural changes in the YodB dimer by promoting the formation of disulfide bonds, whereas methyl-p-benzoquinone allows the YodB dimer to be dissociated from DNA, with little effect on the YodB dimer. The results indicate that *B. subtilis* may discriminate toxic quinones, such as methyl-p-benzoquinone, from diamide to efficiently manage multiple oxidative signals. These results also provide evidence that different thiol-reactive compounds induce dissimilar conformational changes in the regulator to trigger the separate regulation of target DNA. This specific control of YodB is dependent upon the type of thiol-reactive compound present, is linked to its direct transcriptional activity, and is important for the survival of *B. subtilis*. This study of *B. subtilis* YodB also provides a structural basis for the relationship that exists between the ligand-induced conformational changes adopted by the protein and its functional switch.

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## P155

### DNP-Enhanced NMR of Metamorphosing Young Corals Shows that Skeleton Construction Entails Modulation of Organic Material

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The ability of corals to maintain homeostasis and mineralize has been compromised by ocean acidification and temperature rise causing reefs to recede and even vanish. Therefore, corals have been serving lately as important proxies of environmental impact on marine life. As newborn, corals undergo transformation from a swimming organism (planula) to an benthic immobile one (polyp), that lives in colonies and forms exoskeleton for protection. During this metamorphosis process, they are vulnerable leaving them exposed to detrimental external changes. It is therefore very important to carefully characterize the two developmental states – planula and polyp- and to be able to assist corals withstand ongoing hazardous variations in their surroundings.

Recently, we have shown using <sup>13</sup>C MAS NMR on whole <sup>13</sup>C-labeled young Stylophora pistillata corals that mineralization starts before the coral settles. We also found using 2D <sup>13</sup>C DARR that Glu-rich proteins bind soft amorphous mineral in the planula and Asp-rich proteins bind aragonite crystals in the polyp. Regulation of the two mineral states by the disparate proteins suited well the needs of the coral changing from motile to sessile.

Here, we expand investigations of the metamorphosis using DNP-enhanced MAS NMR measurements, obtaining favorable signal enhancements of about  $\times 16 - \times 48$ . Using 2D <sup>13</sup>C DQ-SQ and PDSD measurements on the intact corals, labeled either via <sup>13</sup>C carbonate/glycine or via <sup>13</sup>C6 glucose/glycine, we follow the changes in organic level production related to the transformation and the onset of aragonite precipitation. We monitor contribution of symbiotic dinoflagellates via carbonate metabolism by using the latter as a food source.

The carbon fingerprint changes observed relate to increased carbohydrate production and certain proteins which were not detected without enhancement. These changes are indicative of the transitions entailing onset of colonization and expedited mineralization effort initiated after settling on the bottom of the ocean.

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A molecular ruler for ligand efficacy: Using NMR to probe ligand induced changes in  $\alpha 1A$ -adrenoceptor conformational equilibria

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$\alpha 1$ -adrenoceptors ( $\alpha 1$ -ARs) comprise three G protein-coupled receptor (GPCRs) subtypes that stimulate smooth muscle contraction in response to binding adrenaline and noradrenaline.  $\alpha 1A$ -AR and  $\alpha 1B$ -AR are clinically targeted for treating hypertension and benign prostatic hyperplasia but are putative drug targets for neurodegenerative diseases. New subtype-selective tool compounds are required to probe the role of these receptors in the brain and to validate them as drug targets for neurodegenerative diseases. GPCRs are allosteric machines that sample multiple conformations existing in equilibrium. Agonist binding shifts the equilibrium to active states to promote G protein signalling. Recent crystal structures give us snapshots of inactive and active states, but not the dynamics that underlie GPCR activation. Here, we isotopically labelled six methionines in  $\alpha 1A$ -AR to probe how different ligands modulate the conformational equilibrium of this GPCR using NMR. Met292 sits in the orthosteric ligand binding pocket and its chemical shift was unique upon binding different ligands. Met203 on-the-other-hand, is located on the intracellular side of the receptor, near the NPxxY microswitch, and where G proteins interact. We found the resonance of Met203 shifts upfield in the presence of inverse agonists, shifts downfield and broadens upon agonist binding and that the chemical shift changes correlated well with ligand efficacy. The linear dependence of the chemical shifts is consistent with a conformational selection mechanism, while the resonance broadening in the presence of agonist suggests increased microsecond motion. We subsequently used this molecular efficacy ruler to validate the pharmacology of two novel hits from a trial fragment screen and the peptide toxin,  $\tau$ -Tia. In conclusion, this study validates the current conformational equilibrium-based hypothesis of GPCR function and establishes NMR for screening and characterizing novel GPCR ligands.

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## P157

**Benchtop NMR reaction monitoring with enhanced sensitivity and spectral resolution**Dariusz Gołowicz<sup>\*,1</sup>, Krzysztof Kazimierczuk<sup>2</sup>, Mateusz Urbańczyk<sup>3</sup>, Tomasz Ratajczyk<sup>4</sup><sup>1</sup>*Faculty of Chemistry, University of Warsaw; Centre of New Technologies,**University of Warsaw, <sup>2</sup>University of Warsaw, <sup>3</sup>NMR Research Unit, University of Oulu,**<sup>4</sup>Institute of Physical Chemistry, Polish Academy of Sciences*

Benchtop NMR spectrometers are a promising tool for chemical laboratories worldwide because of their moderate price and low handling cost. The easy setup of flow measurements makes them convenient to use for reaction/process monitoring. However, their applications are mainly limited to simple tasks. This is due to the insufficient spectral resolution and sensitivity, which are a consequence of a relatively low magnetic field strength.

Here, we present that the sensitivity, as well as the spectral and temporal resolution in benchtop NMR reaction monitoring, can be improved by coupling several methods<sup>1</sup>, i.e.: parahydrogen induced polarization (PHIP)<sup>2</sup>, non-uniform sampling (NUS) and non-standard data processing. As an example, we performed 2D time-resolved NUS DQF-COSY NMR monitoring of parahydrogenation reactions (ALTADENA) in a continuous flow mode. Moreover, we implemented the interleaved acquisition of 1D NMR with 2D NUS NMR to get a better insight into the occurring reaction. The NUS data in this approach is divided into overlapping subsets and each of them is reconstructed separately. It significantly improves temporal resolution and is often referred to as “moving frame” method<sup>3</sup>. We also show that interleaving of 1D NMR spectra may be beneficial for post-processing reduction of  $t_1$ -noise artifacts present in 2D NUS NMR spectra. Undoubtedly, the combination of these methods would be even more beneficial if it were used with  $^1\text{H}$ - $^{13}\text{C}$  correlation experiments.

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31P MAS Multiple-Quantum NMR Analysis of  
Phosphate Containing ADMET Polyethylene Samples

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Double Quantum NMR is a well-established method to study spatial proximities and local molecular dynamics in solid samples. In many cases, more or less restricted local dynamics of polymer chains has been analyzed. Here we studied the local packing of phosphate groups during the crystallization of precise polyethylene obtained from acrylic diene metathesis (ADMET) polycondensation with isolated phosphate ethyl ester groups in the main chain regularly spaced by 20 methylene groups. Simple 31P MAS and double-quantum filtered 31P MAS NMR spectra indicate, that 31P sites in the non-crystalline phase have only very weak homo-nuclear dipolar couplings to neighboring 31P sites and do not contribute to the substantial double-quantum signal, which is obtained from the crystalline fraction only.

Using the BABA-xy16 pulse sequence [1] with the common DQ intensity referencing method [2], the 31P DQ build-up curve of the samples could be determined. The DQ build-up behavior was analyzed in terms of 31P dipolar second moment. Comparing the extracted values to ideal 31P dipolar second moments computed based on the lattice constants determined from TEM studies of the samples, we find that PE crystallites own a highly ordered crystal surface with a lamellar arrangement of phosphate sites and that these crystallites stack, in such a way that phosphate sites of one crystallite are on top of the phosphate site of the neighboring with a spacing of > 0.5 nm.

**References:** [1] K. Saalwächter et al., J. Magn. Reson. 212 (2011) 204. [2] Prog. Nuc. Magn. Reson. Spectroscopy 51 (2007) 1–35.

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Comparison of backbone dynamics of the p50 dimerization domain of  
NF-κB in the homodimeric transcription factor NF-κB1 and in its  
heterodimeric complex with RelA (p65)

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Mörtel, Gerard Kroon, Jane H. Dyson, Raphael Stoll

The nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB) protein family comprises transcription factors that play an important role during human immune response. The family encompasses homodimers and heterodimers of five component proteins that mediate various transcriptional responses. These proteins are structurally very similar, suggesting these dimeric complexes may differ in their dynamic behaviors rather than structural differences. This study presents the first near-complete <sup>15</sup>N, <sup>13</sup>Cα/β, and HN backbone resonance assignments of the homodimer from the dimerization domain of the NF-κB1 (p50) protein that comprises residues 244-353. We have also analysed the pico-second-nanosecond timescale dynamics of this homodimer and compared it to that of the corresponding heterodimer formed by the p50 and p65 dimerization domains. In conclusion, chemical shifts were identified that led to identification of both the dimerization interface and the DNA binding site. Furthermore, we report and discuss the relaxation properties of both complexes. This work now paves the way for further structural studies of NF-κB proteins in order to address important biological questions, such as residue-based DNA binding, mutations of binding DNA motif, point mutations, and binding of potential drugs. Thus, this study will form the basis for future studies on the interaction of the dimerization of NF-κB with different physiologically-relevant ligands.

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### Understanding Adsorption Mechanisms in Multicomponent Metal organic frameworks by Solid State NMR

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Incorporating structurally distinct ligands with metal oxide clusters can produce multicomponent porous metal organic frameworks which have heterogeneous adsorption properties. For example, different adsorbates methanol, N,N-dimethylaniline or acridine orange may be attracted to different sites. We used solid-state  $^2\text{H}$  and multidimensional  $^1\text{H}$ - $^{13}\text{C}$  NMR techniques to probe the dynamics of ligands and analyze how different fragments are affected by the guest molecules. By analysis of the  $^2\text{H}$  line shape and spin-lattice relaxation as well as  $^1\text{H}$ - $^{13}\text{C}$  dipolar coupling, we can quantitatively characterize the dynamics of different components, and compare the differences before and after adsorption. Combining SSNMR with molecular dynamics simulations, our analyses can provide a whole picture of adsorption mechanisms in such complex systems.

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Comparing Molecular Structure of the Transmembrane  $\alpha$ -Helices of SPPL2a/b Substrate TNF $\alpha$  and Its Mutant TNF $\alpha$ S34P by Solution State NMR Spectroscopy  
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Intramembrane proteases catalyse the hydrolysis of their substrates within the hydrophobic environment of the lipid bilayer and are involved in several diseases and the functional activation of substrates [1]. SPPL proteases belong to the class of GxGD aspartyl proteases. It is still unknown how members of this family distinguish between substrates and non-substrates, or which factors determine the rate of proteolysis.

To understand the molecular properties that select substrates, we determine the transmembrane domain (TMD) structures of TNF $\alpha$ , a substrate of SPPL2a/b, and a mutant substrate that is processed by a different mechanism, TNF $\alpha$ S34P, by NMR spectroscopy. TNF $\alpha$ <sub>28-60</sub> in 80% TFE shows a TMD divided into two  $\alpha$ -helical regions connected via a flexible area. Protease cleavage sites [2] map to breaks in the helical structure, at the respective ends of each helix. Spectra of the mutant TNF $\alpha$ S34P<sub>28-60</sub> reveal that major structural changes at the cleavage sites do not occur. Structure determination will show whether the mutation induces changes in the mutual orientation of the two helical regions that could be responsible for the altered processing.

We further study TNF $\alpha$ <sub>28-60</sub> and TNF $\alpha$ S34P<sub>28-60</sub> in DHPC:DMPC bicelles to investigate the influence of the natural lipid environment on the structures. Besides providing valuable insights into the substrate selection process of protease SPPL2a/b our results may be extended to related intramembrane proteases.

**References:** [1] D. Langosch, C. Scharnagl, H. Steiner and M. K. Lemberg (2015) Trends Biochem. Sci. 4, 318-327. [2] R. Fluhrer, G. Grammer, L. Israel, M. M. Condrón, C. Haffner, E. Friedmann, C. Bohland, A. Imhof, B. Martoglio, D. B. Teplow and C. Haass (2006) Nat. Cell Biol. 8, 894-896.

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**Solid-state Dynamic Nuclear Polarization for  
resolving complex Structures of Hybrid Nanoparticles**  
Torsten Gutmann<sup>\*,1</sup>, Timmy Schäfer<sup>2</sup>, Vytautas Klimavicius<sup>2</sup>,  
Steffen Vowinkel<sup>2</sup>, Markus Gallei<sup>3</sup>

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In the past few years, the interest in research of inorganic/organic hybrid materials has increased tremendously. Especially, novel nanoparticles based on inorganic/organic hybrid-materials have been designed for applications ranging from heterogeneous catalysts for environmentally friendly chemical transformations, to materials with optical or sensoric properties.

The crucial step for tuning nanoparticles for these applications is the detailed knowledge on their structure. The investigation of the complex structure of nanoparticles at a molecular level is still a challenging task and requires advanced analysis techniques that deliver the necessary resolution and sensitivity. The combination of dynamic nuclear polarization (DNP) with solid-state NMR covers these requirements. [1,2] Especially, the selective signal enhancement obtained by DNP enables the resolution of single components in multi-component materials. [3]

The presentation will demonstrate how selectively enhanced DNP enables the structure determination of core-shell nanoparticles containing either inorganic/organic particle architectures or only organic particle architectures. Combining selectively enhanced DNP with 1D and 2D multinuclear solid-state NMR, the structures of the shell and the core of the particle are individually addressed. [4,5] Based on these studies the potential of DNP enhanced solid-state NMR for the characterization of a variety of nanoparticle materials including catalysts is highlighted.

**References:** [1] A. Lesage et al., J. Amer. Chem. Soc. 2010, 132, 15459-15461. [2] R. G. Griffin, Nature 2010, 468, 381-382. [3] T. Gutmann et al., J. Phys. Chem. C 2017, 121, 3896-3903. [4] S. Vowinkel et al. Nanomaterials 2017, 7, 390. [5] T. Schäfer et al., J. Phys. Chem. C 2019, 123, 644-652.

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## P163

## Simplified NMR assignment of small to medium-sized molecules by edited HSQC-CLIP-COSY experiments

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CLIP-COSY[1] experiment, providing high quality homonuclear correlation spectrum with clean in-phase multiplets, accelerates the assignment of  $J$ -coupled proton spin network and so, the structure elucidation of small and medium-sized molecules. Further resolution enhancement and, hence, simplification of spectra can be obtained by implementing the recently introduced PSYCHE broadband homonuclear decoupling approach into the CLIP-COSY and relayed experiments.

In the homonuclear CLIP-COSY spectra, resolution is limited by the small chemical shift dispersion of proton resonances. Therefore utilizing the resolving power of a heteronucleus, we have devised HSQC-variants of the CLIP-COSY experiment.[2] These novel pulse sequences also allow editing of signal phases in the correlation spectra according to even or odd multiplicity of X-nuclei and/or editing of direct HSQC correlations vs. CLIP-COSY peaks. The different phases imposed on the direct and relay peaks facilitate to track the connectivity network of protonated carbons, making the assignment of both  $^1\text{H}$  and  $^{13}\text{C}$  resonances possible from a single spectrum. In order to avoid signal cancellation of accidentally overlapping peaks with opposite phases ultra-high resolution spectra obtained with non-uniform sampling (NUS) can be utilized.

Moreover, the HSQC-CLIP-COSY sequence can be combined with any HMBC-type experiment into NOAH[3] (NMR by Ordered Acquisition using  $^1\text{H}$ -detection) supersequences to get maximal spectral information in the shortest possible data collection time.

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**Dependence of the linker length and stiffness on the molecular recognition of UIM-SH3 with Ub and Ub2-K63**  
Maggy HOLOGNE\*<sup>1</sup>, Minh-Ha NGUYEN<sup>1</sup>, Marie MARTIN<sup>1</sup>,  
Henry KIM<sup>2</sup>, Frank GABEL<sup>3</sup>, Olivier WALKER<sup>1</sup>  
<sup>1</sup>ISA - University of Lyon, <sup>2</sup>ISA - University of Lyon, <sup>3</sup>IBS

A wide ensemble of protein-protein interaction networks warrants communication and information exchange between cells. These interactions are mainly mediated by protein domains, which could be considered as protein modular building blocks assembled in different fashions. They decode specific signals emerging from post-translational modifications involved in receptor signaling, endocytosis or DNA damage for instance. These identical or different domains are linked together by disordered segments characterized by variable lengths and sequences, also termed linkers. Due to their inherent variable flexibility, linkers can confer large conformational rearrangement to proteins to induce intra- or inter-domain interactions. Little has been done to investigate the role played by flexible linkers upon binding to other multidomain partners and some questions are pending: what is the role played by the linker flexibility and dynamics to control allostery, cooperativity or avidity? To shed light on this dangling question we have chosen to explore the interaction of STAM2\_UIM-SH3, a dual domain protein, with ubiquitin and K63-diubiquitin linked chains. Indeed, STAM2 is part of the ESCRT-0 complex, which is the most upstream component of the ESCRT system involved in lysosomal degradation. Moreover, the proteins that are directed for degradation are first tagged by Lys63-polyubiquitin linked chains. STAM2 harbors three ubiquitin binding domains (UBDs) that recognize and interact with ubiquitin (Ub) moieties, namely the VHS, UIM and SH3 domains. In addition to the UIM-SH3 wild type (US-wt), we have engineered four other constructs where the tether that bridge UIM and SH3 has been shortened (US-D1 and US-D2), and where UIM (US-D3) or half of UIM (US-D4) has been deleted. A combination of NMR (titration, 15N relaxation) and SAXS experiments have been recorded to get insights into the role of the linker flexibility and dynamics in molecular recognition during lysosomal degradation process.

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High-resolution structure of a modified G-quadruplex:  
implications for conformational preferences of  
2'-F-riboguanosine and V-loop flanking residues

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Universität Greifswald

Among the various alternative DNA structures, G-quadruplexes exhibit a remarkable variety of topologies. In this study, two adjacent guanosine residues adopting *syn* glycosidic bond conformations in a G-quadruplex of (3+1)-hybrid type were substituted by *anti*-favoring 2'-F-riboguanosine nucleosides (FrG). Residue-specific <sup>15</sup>N labeling along with 2D NOE, <sup>1</sup>H-<sup>13</sup>C HSQC and HMBC spectra allowed for the complete resonance assignment of the FrG-modified sequence. Strikingly, it adopts an unusual quadruplex fold with G1 as part of the central tetrad and a zero-nucleotide V-shaped loop connecting the two FrG residues. While some identical or similar topologies with V-loops have been reported in the past years, it is remarkable that this particular modification provokes a fold with one of the incorporated FrG residues forced into the unfavoured *syn* conformation.

In an attempt to better understand the driving force for this rearrangement, conformations of the two FrG residues flanking the V-loop were analyzed in detail based on 2D NOE and DQF-COSY spectra as well as on F2'-H3' and F2'-H1' heteronuclear scalar couplings extracted from selectively <sup>1</sup>H-decoupled <sup>19</sup>F spectra. In contrast to S-puckered unmodified residues, both FrGs adopt a sugar pucker in the favoured *north* domain. The C3'-*endo* pucker of the FrG residue at the 3'-end of the V-loop seems essential for the following sharp turn of the phosphate backbone, as seen in the high-resolution structure calculated from NOE-derived distance restraints. The NMR-structure also demonstrates, that even the *syn* glycosidic torsion angle of the FrG residue at the V-loop 5'-end is compatible with an N-type sugar pucker. Apparently, the preference of FrG analogs for a C3'-*endo* conformation outweighs its propensity for an *anti* glycosidic torsion angle, allowing for the unexpected refolding to the V-loop topology. Finally, stabilization of this fold by *north*-favouring FrG residues points to a general importance of the C3'-*endo* conformation for V-loop formation.

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Intercepting second messenger signaling by a c-di-GMP sequestering peptide

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Bacterial second messengers regulate a wide range of cellular functions from biofilm formation to growth and survival. Targeting second messenger networks is challenging because the system involves a multitude of components with often overlapping functions. Here, we present a novel strategy to intercept cyclic diguanylate (c-di-GMP) signaling pathways by directly targeting the second messenger. C-di-GMP is a central regulator of biofilm formation in bacteria including many pathogens that are associated with severe clinical complications. To target the second messenger directly, we developed a c-di-GMP sequestering peptide (CSP), that was derived from a CheY-like c-di-GMP effector protein. CSP binds c-di-GMP with nanomolar (sub-micromolar?) affinity. Elucidating the structure of the CSP- $\Sigma$ -c-di-GMP complex led to the identification of a novel c-di-GMP binding motif consisting of a single core peptide. An intercalated c-di-GMP dimer is tightly bound to the peptide through a network of H-bonds and pi-stacking involving arginine and aromatic residues. Structure based mutagenesis yielded a variant with considerably higher affinity and a shortened variant (19 residues) with almost uncompromised affinity.

Using *P. aeruginosa*, a model for serious biofilm-associated medical implications, we demonstrated that endogenously expressed CSP intercepts c-di-GMP signaling and by that effectively inhibits biofilm formation. Notably, the expression of CSP also inhibits biofilm formation triggered by sub-inhibitory concentrations of antibiotics. To our knowledge, this is the first example of direct inhibition of a signaling molecule by using a small peptide. This strategy could be applied to other signaling molecules that are often difficult to target with conventional approaches and could provide the basis for specific interference with bacterial persistence.

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**Hyperstore: Long-term storage of nuclear spin polarisation**Andrew Hall\*, Francesco Giustiniano, Aliko Moysiadi, Giuseppe Pileio  
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Hyperpolarisation provides the means to dramatically increase the signal-to-noise ratio in an NMR spectrum, however, the useful lifetime of the hyperpolarized state is limited by relaxation processes that lead to the re-establishment of thermal equilibrium over time. In order to make hyperpolarisation useful for experiments requiring lengthy delays for magnetisation transfer, chemical exchange or diffusion processes it is necessary to find a means of storing the hyperpolarisation.

It has been shown that nuclear singlet states offer a means of storing magnetisation for a time much longer than  $T_1$ , and that by careful molecular design, singlet lifetimes can be extended to over an hour.[1] Achieving even longer lifetimes requires careful control of sample environment to minimise relaxation mechanisms. In particular, the solvent viscosity and magnetic field strength that the sample is exposed to are important factors in determining which relaxation mechanisms dominate.

In this work we investigate the singlet lifetime of a doubly carbon-13 labelled naphthalene complex in ultra-low viscosity carbon dioxide solution, using custom built apparatus that allows relaxation to be measured as a function of the magnetic field strength. We aim to study how the  $T_1$  relaxation time and singlet relaxation time ( $T_s$ ) vary as a function of magnetic field strength and solvent viscosity, with the goal of identifying conditions where relaxation mechanisms are minimised to prolong the lifetime of the hyperpolarisation.

**Reference:** [1] Stevanato, Gabriele; Hill-Cousins, Joseph T; Hakansson, Paer; Roy, Soumya Singha; Brown, Lynda J; Brown, Richard C D; Pileio, Giuseppe; Levitt, Malcolm H, *A Nuclear Singlet Lifetime of More than One Hour in Room-Temperature Solution*, Angew. Chem. Int. Ed., 2015, 54 (12), pp. 3740-3743

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**Real-time pure shift  $^1\text{H}$ ,  $^{15}\text{N}$  BEST-HSQC and  
 $^1\text{H}$ ,  $^{15}\text{N}$  BEST-TROSY for  $^{13}\text{C}$  and  $^{15}\text{N}$  labeled samples.**

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A key feature of NMR is that information is accessible at atomic resolution. However, ambiguities arise if signals overlap and, therefore, great effort has been made to increase either signal dispersion (e.g. high magnetic fields, n-dimensional NMR, detection of heteronuclei) or to reduce signal width (e.g. pure shift NMR, TROSY). Despite the fact that individual homonuclear couplings might not be resolved for biomolecules, in sum they can still contribute to linebroadening [1-3]. Recently, it was shown [3] that signal width and sensitivity can further improve when also heteronuclear long-range couplings are considered. We here present a BIRD-based pure shift acquisition scheme for the detection of amide protons in  $^{13}\text{C}$  and  $^{15}\text{N}$  labeled proteins which is incorporated into a  $^1\text{H}$ ,  $^{15}\text{N}$  BEST-HSQC [4] and  $^1\text{H}$ ,  $^{15}\text{N}$  BEST-TROSY [5], maintaining the favorable relaxation properties of the TROSY component. In contrast to hitherto existing sequences the water signal is not flipped in between chunks and artifacts due to radiation damping are drastically reduced so that homodecoupling can be applied without saturating water.

**References:** [1] J. Ying J. Roche, A. Bax (2014). J. Magn. Reson. 241, 97. [2] P. Király, R. W. Adams, L. Paudel, M. Foroozandeh, J. A. Aguilar, I. Timári, M. J. Cliff, M. Nilsson, P. Sándor, G. Batta, J. P. Waltho, K. E. Kövér, G. A. Morris (2015). J. Biomol. NMR 62, 43. [3] J. D. Haller, A. Bodor, B. Luy (2019). J. Magn. Reson. 302, 64. [4] P. Schanda, H. Van Melckebeke, B. Brutscher (2006). J. Am. Chem. Soc. 128, 9042. [5] J. Farjon, J. Boisbouvier, P. Schanda, A. Pardi, J.-P. Simorre, B. Brutscher (2009). J. Am. Chem. Soc. 131, 8571.

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Solvent Influence on the Structure and Dynamics of  
PFSA Membranes Investigated by Low-Field <sup>19</sup>F Double-Quantum NMR  
Regine Hammer, Michael Ryan Hansen, Monika Schönhoff

Perfluorinated sulfonic acid (PFSA) membranes are polymers known for their high proton conductivity and mechanical stability. The benchmark material is Nafion, which is often described as a long side-chain (LSC) polymer with two fluoroether groups. However, these fluoroether groups suffer from several drawbacks in fuel cell application. Therefore, a new type of PFSA membrane was introduced, which includes solely a single fluoroether group (short side-chain, SSC). For the given application, the understanding of structure and dynamics of the PFSA membranes especially in the presence of solvents plays a crucial role for improvement of performance and electrochemical properties.<sup>1</sup>

In this work, we have characterized Nafion 117 (LSC), Nafion 212 (LSC) and Aquivion (SSC) swollen in solvents of different polarity regarding their structural and dynamical properties. Temperature-dependent time-domain <sup>1</sup>H multi-quantum NMR spectroscopy is well-established in characterization of protonated polymers;<sup>2,3</sup> however, so far, this method has not been applied to fluorinated polymers. From analysis of <sup>19</sup>F double-quantum build-up curves, the residual <sup>19</sup>F-<sup>19</sup>F dipolar couplings (Dres) are obtained, which depend on the polymer density and therefore contain about the structural information. In the same solvent, all PFSA membranes show identical Dres values, indicating that their swelling behavior is independent of the side-chain length. However, the swelling with solvents of different polarities leads to solvent-dependent Dres. The dry-state membranes show the highest Dres, while swelling with polar-protic solvents leads to decreased Dres. In PFSA membranes swollen with solvents of lower polarity a higher Dres is observed. Thus, it is concluded that the observed structures are mainly dominated by the crystalline PTFE-like domains, which are identical for all membranes. The swelling with polar-protic solvents, especially ethanol, results in less densely packed domains, while the presence of solvents with lower polarities leads to dense packed domains.

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**Manatee: A toolbox for statistical analysis of 2D NMR data**

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Manatee is a program written in Matlab that facilitates various types of statistical analyses of 2D NMR data by transforming a set of 2D spectra (or a selected region thereof) into a data matrix suitable for various analyses such as multivariate analyses (PCA, OPLS etc.) or a built-in peak correlation analysis. These types of analyses have several different applications when studying complex mixtures in order to either identify individual components or classifying samples in multivariate models. For example, the extra information content available in 2D spectra, such as HSQC-type experiments are crucial for identification of individual components in biofluids and plant cell walls.

The imported set of processed 2D spectra (Bruker format) will, after optional modifications, be transformed to a 2D data matrix where each row consists of the data from each spectrum. A number of pre-processing as well as visualization options are available. For example, the user can export a sub-region of the spectra that contain the peaks of interest and remove regions containing artifacts or solvent peaks. The final data matrix can be further compressed by removing data-points below a user-defined S/N ratio. The final data matrix can be saved in various formats for analyses in external software or be utilized in the built-in peak correlation feature that with a simple click in the spectrum calculates the Pearson correlation coefficients from the defined data-point to all other points in the spectra. This feature facilitates identification of individual components of mixtures as peaks from the same molecule should, in theory, have almost perfect correlations. Manatee also supports plotting loadings from multivariate models performed in SIMCA (Sartorius-Stedim Biotech). All plots can be exported as images or 2rr files for visualization in Topspin (Bruker Biospin).

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**Determination of Redox Potentials of  
Plant-type Ferredoxin Isoforms via EPR Spectroscopy**  
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Ferredoxins (Fdxs) are soluble iron-sulfur proteins that function as electron acceptors and donors in diverse metabolic pathways. They are characterized by their acidity and low redox potentials (230 to 420 mV). The unicellular green alga *Chlamydomonas reinhardtii* contains 12 different plant-type [2Fe2S]-Fdx isoforms.[1] The most abundant isoform Fdx1 has been characterized extensively due to its essential role as electron acceptor of the photosynthetic transport chain. Under anaerobiosis and nutrient deprivation the [FeFe]-hydrogenase HydA1, which catalyzes the reversible reduction of protons to molecular hydrogen (H<sub>2</sub>), becomes another redox partner of Fdx1.[2] Since electron supply is one of the main limiting factors for commercial H<sub>2</sub> production applications, midpoint potentials of Fdx1 and other Fdx isoforms, which might function as electron donors of HydA, are of great interest.

The redox potentials of Fdx1, Fdx2 and Fdx5 have been previously detected via EPR and (spectro-) electrochemical techniques.[3] However, the reported midpoint potentials are not identical. Here, we employed small volume EPR-based potentiometric titrations to identify midpoint potentials of selected Fdx isoforms. In order to make a precise and certain detection, we made a detailed investigation of parameters that might affect the identified midpoint potentials. The reduction potential we have determined for Fdx2 in a temperature independent buffer at pH 7.5 agrees very well with the only reported value that is also based on EPR-based potentiometric titrations.[4] The work presented here establishes a protocol for the accurate determination of redox potentials of FeS proteins via EPR, and also reports the midpoint potentials of several Fdx isoforms for the first time.

**References:** [1] A. Sawyer, M. Winkler, Photosynth. Res., 2017, 134, 307–316. [2] S. Rumpel et al., ChemBioChem, 2015, 16(11), 1663–1669. [3] A. Terauchi et al., J. Biol. Chem., 2009, 284, 25867–25878. [4] M. Boehm, et al., Photosynth. Res., 2016, 128(1), 45–57.

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## Site-specific DNP in a Gd(III)-labeled protein

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Recently, site-specific dynamic nuclear polarization (DNP) has shifted into the scientific focus, with the aim to restrict hyperpolarizing to a specific region of interest, or even extract distance information by directly analyzing DNP parameters, such as enhancement factor and polarization build-up rate.[1]

In this work we present recent results of site-directed spin labeling on a protein to show that site-selective DNP can be observed with the transition metal Gd(III) as a polarizing agent (PA) in a biological system. Therefore, ubiquitin is well suited as a biological model system. The absence of cysteines allows for specific mutations for site-directed spin labeling with a chelate tag.[2]

In a first optimization step we investigate the effect of different level of deuteration of the protein on spin-lattice relaxation and proton-driven spin diffusion of enhanced polarization. Both of these mechanisms, which effectively compete with site-specific DNP, are effectively attenuated by perdeuteration. Based on a detailed experimental study we provide evidence that <sup>1</sup>H-<sup>13</sup>C cross relaxation occurring within mobile methyl groups is prohibiting the accumulation of enhanced <sup>13</sup>C polarization if aliphatic protons are present.

In a second step, we compare three different single-site mutants with labeling sites in the alpha-helical, beta-sheet, and C-terminal region, respectively. By focusing especially on <sup>15</sup>N nuclei, which contains a small gyromagnetic ratio and is rather diluted over the uniformly isotope enriched protein, we observe extraordinarily large enhancement factors and distinct side chain resonances of the amino acids lysine, arginine, and histidine. This allows to distinguish specific localized effects between the different mutants. Analysis of polarization build-up dynamics reveal quantitative information about the distance dependence of the initial electron-nuclear DNP transfer process and subsequent propagation by spin-diffusion.

**References:** [1] Lilly Thankamony et al., Progr. Nucl. Magn. Reson. Spectrosc., 102-103, 120 (2017). [2] M. Kaushik et al. Phys. Chem. Chem. Phys., 18, 27205 (2016).

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Disorder and Misfolding of Proteins Seen by  
(DNP-enhanced) Solid-State NMR-Spectroscopy

Henrike Heise\*

Heinrich Heine Universität Düsseldorf

We apply solid-state MAS NMR-spectroscopy at different temperatures to investigate protein folding and misfolding on a molecular level [1].

On the one hand, we exploit DNP-enhanced MAS NMR spectroscopy at low temperatures (~100K) to investigate conformational ensembles of intrinsically disordered proteins (IDPs). Such proteins are not represented by a single well-defined structure but rather by a full conformational ensemble of structures which can interconvert rapidly. As IDPs are not stabilized by secondary structures, they are often prone to aggregation and amyloid formation. Traditional biophysical methods like solution NMR spectroscopy and FRET measurements usually determine ensemble averages and do not give direct insight into the conformational distributions. In frozen solution the full conformational ensemble is trapped and can be examined simultaneously, for example by (DNP-enhanced) solid-state NMR-spectroscopy [1,2]. First, we studied the distribution of backbone conformations in the intrinsically disordered protein  $\alpha$ -synuclein in frozen solution in different surroundings by evaluating the inhomogeneously broadened line-shapes of the  $C\alpha/C\beta$  cross peak [2,3]. Furthermore, we investigated the rotameric states sampled by amino acid side chains in different globular as well as unfolded proteins which drastically depends on the local and global structure of the protein.

We also investigated mature amyloid fibrils from  $A\beta(1-42)$  fibrils by room-temperature MAS-NMR-spectroscopy. Recently, a 3D structure with a resolution of 4 Å was obtained on amyloid fibrils grown at low pH by cryo-EM [4]. We now investigated the fibril stability with respect to different pH values salt concentrations on a residue-specific level.

**References:** [1] A. König et al. (2019) Solid State Nucl. Magn. Reson. 98, 1-11. [2] B. Uluca et al. (2018) Biophys. J. 114, 1614-1623. [3] T. Viennet et al. (2018) Communications Biology 1, 44. [4] L. Gremer et al (2017) Science 2017, 358, 116-119.

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**A single NaK channel conformation is not enough for non-selective ion conduction**  
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NaK and other non-selective channels are able to conduct both sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) with equally high efficiency. In contrast to previous crystallographic results, we show that the selectivity filter (SF) of NaK in native-like lipid membranes adopts two distinct conformations that are stabilized by either Na<sup>+</sup> or K<sup>+</sup> ions. The atomic differences of these conformations are resolved by solid-state NMR (ssNMR) spectroscopy and molecular dynamics (MD) simulations. Besides the canonical K<sup>+</sup> permeation pathway, we identify a side entry ion-conduction pathway for Na<sup>+</sup> permeation unique to NaK. Moreover, under otherwise identical conditions ssNMR spectra of the K<sup>+</sup> selective NaK mutant (NaK2K) reveal only a single conformational state. Therefore, we propose that structural plasticity within the SF and the selection of these conformations by different ions are key molecular determinants for highly efficient conduction of different ions in non-selective cation channels.

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NMR and CRISPR-Cas9 reveal the role of a  
Dynein transient helix in cellular cargo transportation.

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Transportation of cellular cargo towards the minus end of microtubules is mediated by specific adaptors that engage with dynein to recruit and activate the motor. Malfunction of this machinery is known to cause diseases such as tumors and spinal muscular atrophy, but the molecular mechanisms remain incompletely understood.

Here, we use structural and dynamics nuclear magnetic resonance (NMR) analysis to characterize for the first time the C-terminal region of human dynein light intermediate chain 1 (LIC1). Our data shows that this segment is intrinsically disordered and contains two short conserved segments with helical propensity. NMR titration, CEST and CPMG experiments reveal that the first helical segment (helix 1) constitutes the main interaction site for the adaptors Spindly (SPDL1), bicaudal D homolog 2 (BICD2), and Hook homolog 3 (HOOK3). In vitro binding assays show that helix 1, but not helix 2, is essential in both LIC1 and LIC2 for binding to the tested adaptors although an additional disordered segment preceding helix 1 is required for efficient binding. Point mutations in the C-terminal helix 1 of *Caenorhabditis elegans* LIC, introduced by genome editing, severely affect development, locomotion, and life span of the animal and disrupt the distribution and transport kinetics of membrane cargo in axons of mechanosensory neurons, identical to what is observed when the entire LIC C-terminal region is deleted. We conclude that helix 1 in the intrinsically disordered region of LIC provides a conserved link between dynein and structurally diverse cargo adaptor families that is critical for dynein function in vivo.

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Tissue Metabolomics

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In addition to the analysis of liquid extracts, methods based on the principle of nuclear magnetic resonance can be used for the analysis of intact tissue. Since HR-MAS NMR allows metabolite quantification from intact specimens, the need for an extraction step is abolished, which decreases the analysis time and avoids potential source of poor reproducibility. The nondestructive nature of the method allows the analysis of metabolite levels in tissue to be combined with other analytical techniques (i.e. transcriptomics) based on the same tissue specimen, which may be important when tissue availability is limited. In addition, new  $\mu$ -strip techniques enables in-vitro studies of biomimetic 3D-cell-culture models (e.g.organoids, 3D-printed) mimicking complex tissues allowing for the measurement of transport and distribution of metabolomics markers or metabolized pharmacological compounds.

Tissue samples consisting of a variety of cell types as for instance tumor cells, infiltrated immune cell populations, endothelial cells and cancer-associated fibroblasts offer a special analytical challenge. In addition, lipids have to be considered as an important, high abundant component performing essential functions like signaling. They can be challenging from an analytical perspective, since lipid signals with a linewidth up to 35 Hz frequently overlap with metabolite regions in HR-MAS NMR spectra of intact tumor tissue; a problem, which is further amplified in tumor tissue with marked adipocyte admixture. Different strategies to overcome this severe limitation will be discussed.

We will demonstrate the application of NMR-based tissue metabolomics for different tumors (i.e. breast cancer, thymomia), for non-alcoholic fatty liver disease (NAFLD) the combination with transcriptomic, and we will discuss the potential of biomimetic tissue models in combination with NMR.

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### Performance of a Maleimide-Functionalized Trityl Spin Label in Pulsed Dipolar EPR Spectroscopy

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Pulsed dipolar electron paramagnetic resonance spectroscopy (PDS) in combination with site-directed spin labeling (SDSL) is a powerful tool in structural biology. It can be applied to biomolecules in vivo, does not require crystallization and can thus provide information on structure and dynamics in the native environment.[1,2]

To date, the most widely employed type of spin labels are nitroxides such as the well-established MTSL tag. However, alternative spin labels based on trityl radicals are getting more important and address the intrinsic drawbacks of nitroxides.[3,4] Depending on their functionalization, trityls can show an increased stability under reducing conditions as found in cells. Owing to their narrow EPR spectrum, they are ideal candidates for single-frequency PDS experiments like Double Quantum Coherence (DQC)[5] and the Single Frequency Technique for Refocusing Dipolar Couplings (SIFTER)[6] providing a high EPR sensitivity.

Here, we present a new maleimide-functionalized trityl spin label and compare its chemical stability to that of nitroxides and trityls with different linker groups. Its highly cysteine-specific labeling properties are shown via PDS measurements upon bioconjugation to the *Yersinia* outer protein O (YopO, ~72 kDa). Finally, the label performance in PDS is assessed relative to the conventional nitroxide MTSL label.

**References:** [1] O. Schiemann *et al.* *Quart. Rev. Biophys.* **2007**, 40, 1. [2] O. Krumkacheva *et al.* *J. Magn. Reson.* **2017**, 280, 117. [3] A. Giannoulis *et al.* *Phys. Chem. Chem. Phys.* **2019**, 21, 10217. [4] Z. Yang *et al.* *J. Am. Chem. Soc.* **2012**, 134, 9950. [5] P. P. Borbat *et al.* *Chem. Phys. Lett.* **1999**, 313, 145. [6] G. Jeschke *et al.* *Chem. Phys. Lett.* **2000**, 331, 243.

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## P178

## A Low-field NMR detector for probing in situ SABRE hyperpolarisation

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A signature trait of NMR spectroscopy is its limited sensitivity due to low, field-dependent polarisation across spin states. Several hyperpolarisation methods have been developed to overcome this barrier, however, such techniques typically require expensive and complex instrumentation. The introduction of Parahydrogen (p-H<sub>2</sub>) based methodologies, however, provide pathways for hyperpolarisation at low cost. Notably, Signal Amplification By Reversible Exchange (SABRE), provides a promising method to generate renewable hyperpolarisation on time-scales of tens of seconds. SABRE catalytically transfers spin order from p-H<sub>2</sub>, the nuclear singlet isomer of H<sub>2</sub>, to the target analyte via reversible exchange reactions mediated by a transition metal catalyst in solution. Polarisation transfer occurs in the very-low-field regime (0–10mT) prior to signal detection in the NMR spectrometer. SABRE has demonstrated polarisation levels of >10% for <sup>1</sup>H and <sup>15</sup>N in a range of N-heterocyclic compounds. The signature challenge of SABRE-based techniques is the optimisation of polarisation transfer to new classes of molecules and nuclei.

This work describes an instrument that allows SABRE polarisation transfer to be probed in-situ. This instrumentation allows for a comprehensive investigation into the polarisation transfer process, including the interplay between spin dynamics driving polarisation transfer, chemical exchange kinetics between substrate and p-H<sub>2</sub>, and NMR relaxation. Crucially, this scheme facilitates investigation of magnetic states created and relaxation behaviour without complication of sample transport through ill-defined magnetic field gradients typically experienced when the sample is transferred between the polarisation-transfer-field and the NMR detector. The major components of this system consist of a low cost, liquid nitrogen p-H<sub>2</sub> generator to provide variable (~52-60%) level of p-H<sub>2</sub> enrichment, an automated sample polarizer and a variable field NMR detector, which operates over the range ~40μT-10mT. This work will focus on the combination of these elements to produce an integrated, flexible system that may probe all aspects of the polarisation transfer process.

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The dynamic mechanism of 4E-BP1 recognition by mTORC1

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Translation of proteins is a crucial cellular process that requires tight regulation. In eukaryotes, its activation requires phosphorylation of the cellular pool of the intrinsically disordered protein 4E-BP1. This is achieved by the 1 MDa kinase complex mTORC1 (mammalian Target of Rapamycin Complex 1), a central cellular signaling hub that integrates multiple signaling pathways. mTORC1 thus needs to combine substrate specificity with efficient processing speed. Phosphorylation of 4E-BP1 leads to release of eIF4E, initiating translation.

Here, we monitor 4E-BP1 by solution NMR spectroscopy while it is interacting with mTORC1 or its subunit Raptor to resolve mechanistic details of the substrate recognition and its regulation. 4E-BP1 binds to Raptor via two interaction sites at its N- and C-terminus, tethering the substrate specifically to mTORC1 but leaving flexibility in its central region to allow the kinase reaching multiple phosphorylation sites. Furthermore, we demonstrate that Raptor and mTORC1 not only bind apo 4E-BP1, but also the fully assembled 4E-BP1:eIF4E complex, as well as phosphorylated 4E-BP1. This multi-species recognition is enabled by the spatial separation of recognition and phosphorylation sites. Importantly, the recognition of the 4E-BP1:eIF4E complex ensures that the cellular pool of 4E-BP1 can be efficiently phosphorylated, not limited by the slow release kinetics of 4E-BP1 from eIF4E, enabling an overall rapid signalling response.

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## P180

## Low-Field Enantiodifferentiation via Chiral Lanthanide Shift Reagents

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Especially in pharmaceuticals, chirality has a high impact as enantiomers can have tremendously different effects on biological systems. To differentiate enantiomers in NMR, methods make use of chiral environments like chiral solvents, chiral solvating agents or chiral alignment media. A very well-known class of solvating agents are lanthanide shift reagents (LSRs) that have been developed until the mid-1980s for permanent magnet systems. Although chemistry has evolved very potent chiral inorganic lanthanide complexes for enantio-selective synthesis, since then the development of LSRs has essentially been stalled [1]. In the context of benchtop NMR, however, LSRs become of interest again.

Next to the excellent chemical shift differentiation due to (pseudo) contact shifts, the paramagnetic ions of LSR complexes induce nuclear relaxation (PRE) which leads to line broadening proportional to the square of the magnetic field ( $B_0$ )<sup>2</sup>, limiting LSRs to low-field application [2]. Furthermore, LSRs are hard Lewis acids whereas best enantiomeric differentiation is achieved for analytes with hard Lewis base characteristics like amine or hydroxyl groups [3]. Well-known Europium(III) tris[3-(heptafluoropropyl)hydroxymethylene]-d-camphorate] (Eu(hfc)<sub>3</sub>) as well as newly synthesized Europium(III) tris[(R)-1,1'-bi-2-naphtholate]Li<sub>3</sub> (Eu(BINOL)<sub>3</sub>) were added to small molecule analytes in CDCl<sub>3</sub>. 1D and 2D spectra were measured at Magritek NMR Spinsolve 60 benchtop spectrometer.

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**P181****Longitudinal Detection of EPR Signals for Dissolution DNP**

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Dissolution DNP aims at enhancing the inherently low NMR sensitivity for nuclear spins using the high spin polarization of unpaired electrons. Especially preparation of hyperpolarized samples for in vivo MRI requires highest possible polarization of the target molecule in the shortest time possible. Transformation of dissolution DNP into a standard technique requires further progress in hardware design due to constant method development and special sample treatments.

We have designed a polarizer operating at 7 T field, enabling higher enhancements compared to commercial 3.5 T systems, with the tradeoff for longer buildup times. In addition to a NMR coil we use a separated coil for longitudinal detection (LOD) of EPR signals. LOD relies on the periodic modulation of the electron  $M_z$  magnetization, with frequencies around 1 kHz. This technique brings insight into the electron spin properties for better understanding of the limiting factors of nuclear enhancement, without the need for dedicated resonant structures used in conventional EPR. Most importantly the detection is done at the exact same conditions (temperature, magnetic field, microwave excitation) as the DNP experiments.

We will show improvements to the performance of the LOD system used in our polarizers. On the one hand, implementing a new more powerful solid-state microwave with a range of 10 GHz at a center frequency of 197 GHz allows exciting electron spins over a much wider range of radicals. On the other, modifying the waveguide, increasing the available sample capacity and upgrading the detection circuit with a lock-in amplifier increased the signal to noise ratio by orders of magnitude.

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**NMR structural studies of the parasitic TGF- $\beta$  mimic TGM**  
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TGF- $\beta$  stimulates the proliferation and differentiation of regulatory T-cells (T<sub>regs</sub>) to promote peripheral immune tolerance. The mouse parasite *Heligmosomoides polygyrus* manipulates the host immune system through the induction of Foxp3+ T<sub>regs</sub> using the five-domain complement control protein (CCP) protein, TGM. Though lacking any homology to the TGF- $\beta$ s, TGM binds directly to the TGF- $\beta$  receptors, T $\beta$ RI and T $\beta$ RII, to induce immunosuppressive signaling. To determine how the five domains of TGM, TGM-D1-D5, bind and assemble T $\beta$ RI and T $\beta$ RII into a signaling complex, TGM-D1, -D2, and -D3 were produced in *E. coli* and refolded. Through NMR, ITC, and SPR, TGM-D2 and TGM-D3 were shown to be responsible for binding T $\beta$ RI and T $\beta$ RII, respectively, though the binding of T $\beta$ RI by TGM-D2 was further potentiated by TGM-D1. Through NMR, we further showed that T $\beta$ RII uses the same exposed  $\beta$ -strand to bind TGM-D3 as it does to bind TGF- $\beta$ s, consistent with TGM-D3 and TGF- $\beta$  competing for T $\beta$ RII. Through SPR, we showed that TGM-D1D2 and TGF- $\beta$ :T $\beta$ RII compete for binding to T $\beta$ RI, suggesting that T $\beta$ RI uses its pre-helix extension to bind TGF- $\beta$ :T $\beta$ RII and TGM-D1D2. TGM-D3 was shown by NMR structural analysis to adopt a canonical CCP fold, but differed in that it forms a continuous half  $\beta$ -barrel rather than two unconnected antiparallel  $\beta$ -strands. Through assignment of TGM-D3 bound to T $\beta$ RII, we showed that the majority of resonances perturbed by T $\beta$ RII reside within a cleft on the outer surface of the barrel. This cleft is formed by two loop regions which are extended in TGM compared to canonical CCP domains, suggesting that these loop insertions represent important adaptations for binding the TGF- $\beta$  receptors. The information derived from these studies is important not only for guiding efforts to determine the structure of TGM with T $\beta$ RI and T $\beta$ RII, but will also aid in the development of TGM as an immunosuppressive therapeutic.

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**P183**

**Second-Order Effects in  $^{11}\text{B}$  NMR Exploited to Study Fast and Slow Dynamics of the Inorganic Molecular Glass Former Trimethoxyboroxine**  
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NMR is a powerful tool to unravel the molecular dynamics in viscous liquids, polymers, and melts. Most of the applications in this field focus on organic materials and typically they employ spin-1/2 nuclei. Conversely, for many inorganic glass formers one has to use compounds for which quadrupolar interactions prevail. Here we explore the static and dynamic properties of liquid and glassy trimethoxyboroxine (TMB). In contrast to most inorganic boron-containing covalently bonded networks, TMB represents a molecular glass former, held together by van der Waals attractions. Consequently, TMB shows a low glass transition temperature which, using broadband dielectric spectroscopy, is determined to take place near 205 K. Low-temperature  $^{11}\text{B}$  MAS experiments are carried out in order to obtain the quadrupolar anisotropy and asymmetry parameters. At higher temperatures, however, the MAS line shapes are analyzed for the determination of motional time scales and geometries. The same goal is pursued by carrying out stimulated-echo measurements and two-dimensional exchange spectroscopy on nonrotating samples. To extend the range in which the molecular dynamics of TMB can be studied up to much higher temperatures, we exploit dynamic second-order shifts, non-monotonic linewidth effects, and spin relaxation times. Several of the experimental results are compared with random-walk simulations to further deepen our understanding of the dynamics for TMB, a member of this barely studied class of inorganic glass formers.

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## Structural study of KIX domain by NMR

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The KIX domain of transcriptional co-activator CREB binding protein (CBP) mediates transcription by recruiting transcription factors such as oncogenes c-Myb and mixed lineage leukemia (MLL) [1-2]. These binding partners are largely disordered and attain secondary structure after binding to the KIX domain. The KIX domain can bind transcription factors simultaneously to its two opposite binding sites which communicate allosterically through hydrophobic core [3]. Many transactivation domains for transcription factors interacting with KIX domain belong to established Nine amino acid Transactivation Domain (9aaTAD) family [4].

Standard triple resonance experiments (HNCACB, HN(CO)CACB, HNCO, HNCA, HN(CO)CA) were measured on double labelled <sup>13</sup>C, <sup>15</sup>N KIX domain in order to assign protein backbone. Subsequently, <sup>15</sup>N KIX domain was titrated with c-Myb and MLL WT and mutated ligands to observe structural changes and dynamics using 2D HN-HSQC experiment.

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## Fast NMR experiments in research education

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The modern era chemistry laboratory curriculum combines pedagogically rich experiments with modern analysis techniques, such as MS and NMR. While 1-dimensional NMR has long been used, 2D techniques are not yet standard as they are more complex and usually time-consuming. But these modern experiments allow for unambiguous assignments of resonances and resolution of overlapped regions in more complex molecules, and hence can lead to a deeper understanding of reactions and the associated concepts, such as regio-specificity and formation of product mixtures. The biggest obstacle for routine inclusion of 2D experiments is their cost in spectrometer time since they take 10-25min per sample to acquire. Multiplied by the number of students in a class (>200/class), the total time needed becomes a limiting factor for most NMR facilities.

We therefore investigated the suitability of very fast Acceleration by Shared Adjacent Polarization (ASAP)-HSQC[1], ASAP-COSY[2] and ASAP-HMBC[3] for use in the undergraduate curriculum. ASAP experiments speed up acquisition by greatly reducing the length of the relaxation delay. Further improvements can be obtained by combining ASAP with an optimized Ernst angle. Although ASAP experiments can be dangerous to NMR hardware, we show how they can be tested and run safely. Run times per ASAP-setup of 0.5 to 2 min have been compared with the normal experiments, and large numbers of ASAP-spectra have been collected. For moderately concentrated samples, the experiments show very good agreement with traditional 2D spectra.

In conclusion, ASAP technique makes it possible for important 2D experiments to become a routine component of the undergraduate laboratory curriculum, even when large class sizes are involved.

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### Detection of Potential Pancreatogenic Diabetes Patients Based on Their Metabolomic Profiles

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The relationship of pancreatic cancer (PC) and diabetes mellitus (DM) is obvious, as more than 2/3<sup>rd</sup> of the cancer cases are diagnosed with DM. This so-called pancreatogenic diabetes (T3cDM) appears prior to PC within a period of three years and is accompanied by characteristic features such as absence of obesity or frequent infections[1]. PC belongs to the most lethal types of cancer, since its 5-year survival rate is only 3-5%<sup>2</sup>. The diagnosis of PC is usually too late as the early symptoms (weakness, abdominal pain or nausea) are rather generic and the specific local symptoms (pain, jaundice, cachexia or cholangitis) manifest in later stages of the cancer[2]. Therefore, the search for early specific symptoms and characteristic biomarkers remains a subject of intense research.

In our recent study, <sup>1</sup>H NMR metabolomics was applied to plasma samples of patients with PC, individuals with long term DM type 2 (lasting more than 5 years) and subjects of the risk group - patients with recently diagnosed DM (less than 3 years after diagnosis of DM). Plasma sample of healthy subjects was also analysed as a control study group. First two groups of patients served to establish a solid statistical model with good discrimination parameters based on multivariate statistical methods (mainly PCA and OPLS-DA). Such a model was then used to classify the patients of the risk group to either of the previous groups. This proposed model could possibly serve as a pre-screening method to reveal patients with a high risk of developing PC. Consequently, specific biomarker panel for pancreatic cancer was also investigated.

#### Acknowledgement

This study was financially supported by the Ministry of Health of the Czech Republic (16-31028A) and specific university research (MSMT No 21-SVV/2019).

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**The phosphorylation kinetics of tyrosine hydroxylase and its interaction with 14-3-3zeta protein studied by NMR.**

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Human tyrosine hydroxylase 1 (hTH1) is activated by phosphorylation of its N-tail regulatory domain (RD-hTH1, 169 residues) and by the interaction with regulatory 14-3-3 protein. In order to understand the nature of changes introduced by phosphorylation of residues Ser19 and Ser40, we assigned the RD-hTH1 protein. This task was complicated due to the fact, that about 70 residues at the N-tail are intrinsically disordered, while the rest is well folded and structured. This obstacle was

overcome for the disordered part by usage of two 5D experiments, measured with non-uniform sampling approach followed by sparse multidimensional Fourier transform. These techniques allowed to acquire all the information necessary for backbone and side-chain assignment in less than 3 days of spectrometer time. For the assignment of structured part we had to rely only on standard set of 3D spectra.

We measured kinetic rates of the phosphorylation of Ser19 by PRAK kinase. These were derived from intensity changes of signals of several residues close to the phosphorylation site. Also, we compared the HSQC spectra of non-phosphorylated, Ser40-phosphorylated and doubly phosphorylated sample to find the most affected residues. To reveal the interaction mode with 14-3-3zeta protein we titrated highly concentrated 14-3-3zeta protein to doubly phosphorylated RD-hTH1. The changes in peak intensities indicate the regions of RD-hTH1 most involved in the interaction.

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One-dimensional Nanoscale Localization of  
Nitrogen-Vacancy Centers in Diamond by Phase Encoding

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University of Science and Technology of China

We achieve one-dimensional nanoscale localization of the nitrogen-vacancy (NV) defect centers in diamond by means of phasing encoding approach under gradient magnetic field. Coplanar and gradient micro-wires deposited on the diamond surface are connected in series with a power supply and voltage controlled current source. Magnetic gradient fields for NV centers phase encoding are produced by the current sent through the gradient micro-wires. We perform one dimensional localization of single NV center with nanoscale resolution of 1.5nm. The experiment results demonstrate that this technique can be extended to wide-field imaging technology, thus enabling applications in diverse areas ranging from quantum sensing to biological and life sciences.

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### Investigation of amyloid beta 1-42 binding interactions with potential aggregation modulators by solution NMR

Marie-Theres Hutchison<sup>\*1</sup>, Elke Stirnal<sup>2</sup>, Sridhar Sreeramulu<sup>2</sup>, Christian Richter<sup>2</sup>, Tobias Lieblein<sup>3</sup>, Rene Zangl<sup>3</sup>, Janosch Martin<sup>3</sup>, Frank Kaiser<sup>3</sup>, Tina Stark<sup>3</sup>, Verena Linhard<sup>2</sup>, Michael Göbel<sup>3</sup>, Nina Morgner<sup>3</sup>, Thomas Schrader<sup>4</sup>, Harald Schwalbe<sup>2</sup>

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The aberrant folding of certain proteins results in toxic oligomers and aggregates involved in a plethora of diseases. Alzheimer's disease, the most prevalent debilitating and fatal neurodegenerative disorder is linked to misfolding of the protein amyloid beta. Pathological indicators which confirm AD include brain shrinkage, plaques and neurofibrillary tangles, whereby the plaques are mainly comprised of the A $\beta$  isoforms A $\beta$ 40 and A $\beta$ 42. The aggregation rate of the more amyloidogenic isoform, A $\beta$ 42, is significantly faster than that of the less-toxic A $\beta$ 40. [1] This, coupled with A $\beta$ 42's lower aqueous solubility often leads to the A $\beta$ 40 peptide being chosen as a model system for studying primary and secondary nucleation, aggregation kinetics and inhibitor studies. The lysine-specific molecular tweezer, CLR01, has previously been studied with respect to A $\beta$  binding and its capability of inhibiting and reversing aggregation. [2] These NMR studies were performed using A $\beta$ 40. Here, we report the effects of CLR01 with respect to A $\beta$ 42 binding and aggregation determined by solution state NMR. Furthermore, a comparative analysis of the binding modes of the known A $\beta$  KLVFF sequence derived peptide inhibitors, OR1 and OR2 [3] is presented.

**References:** [1] Iryna Benilova, Eric Karran<sup>1</sup> & Bart De Strooper, The toxic A $\beta$  oligomer and Alzheimer's disease: an emperor in need of clothes, *Nature Neuroscience*, 2012, 15, 349–357. [2] S. Sinha, D. H. J. Lopes, Z. Du, E. S. Pang, A. Shanmugam, A. Lomakin, P. Talbiersky, A. Tennstaedt, K. McDaniel, R. Bakshi, P.-Y. Kuo, M. Ehrmann, G. B. Benedek, J. A. Loo, F.-G. Klärner, T. Schrader, C. Wang, G. Bitan, Lysine-Specific Molecular Tweezers are Broad-Spectrum Inhibitors of Assembly and Toxicity of Amyloid Proteins., *J. Am. Chem. Soc.* 2011, 133, 16958–16969. [3] Austen BM, Paleologou KE, Sumaya AE, Qureshi MM, Allsop D, El-Agnaf OMA. Designing peptide inhibitors for oligomerisation and toxicity of Alzheimer's  $\beta$ -amyloid peptide. *Biochemistry* 2008;47:1984–92.

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Characterization of H/D exchange in type 1 pili by  
proton-detected solid-state NMR and molecular dynamics simulations  
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Uropathogenic *Escherichia coli* invades and colonizes hosts by attaching to cells using adhesive pili on the bacterial surface. Although many biophysical techniques have been used to study the structure and mechanical properties of pili, many important details are still unknown. Here we use proton-detected solid-state NMR experiments to investigate solvent accessibility and structural dynamics. Deuterium back-exchange at labile sites of the perdeuterated, fully proton back-exchanged pili was conducted to investigate hydrogen/deuterium (H/D) exchange patterns of backbone amide protons in pre-assembled pili. We found distinct H/D exchange patterns in lateral and axial intermolecular interfaces in pili. Amide protons protected from H/D exchange in pili are mainly located in the core region of the monomeric subunit and in the lateral intermolecular interface, whereas the axial intermolecular interface and the exterior region of pili are highly exposed to H/D exchange. Additionally, we performed molecular dynamics simulations of the type 1 pilus rod and estimated the probability of H/D exchange based on hydrogen bond dynamics. The comparison of the experimental observables and simulation data provides insights into stability and mechanical properties of pili.

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Novel approach of screening the effect of small molecules on RNA via <sup>1</sup>H-NMR

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Riboswitches belong to the group of sensory RNA motifs that are able to form two different conformations in response to physiological signals like differing metabolite concentrations. While the RNA adopting one conformation will encourage expression of the associated genes, adopting the other one leads to termination of either transcription or translation. Until today, various metabolites acting as ligands for riboswitches have been discovered in different bacteria.[1] Riboswitch RNAs can be structurally subdivided into an aptamer domain and the expression platform. The aptamer domain binds a specific metabolite that initiates an allosteric restructuring of the downstream expression platform and thus a Rho dependent or independent terminator-hairpin will be formed that destabilizes the transcription-elongation-complex.[2] Recently, riboswitches got into the focus of drug target research due to their ability to bind specific metabolites and to undergo structural refolding mechanisms that lead to the downregulation of their associated genes. RR Breaker *et. al* showed, that gene expression can be modulated and bacterial growth interrupted by targeting riboswitches with metabolite analogues.[3,4]

Herein we show an approach of screening the 2'-deoxyguanosine-sensing riboswitch RNA (transcriptional off-switch), by small molecules via <sup>1</sup>H-NMR. The NMR experiments are based on CSP, which makes this method a very sensitive and robust tool to observe several interactions between RNA and small molecules. We screened mixtures of up to 12 components plus RNA at the same time to precisely point out single hits.

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### Strategy pursuit for NMR spectroscopic identification of polar metabolites from mixture spectra

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An approach that represents a large field of research is the analysis of the composition and alteration of metabolic products. With the aid of this metabolic researches, it is possible to identify signals from chemical markers, which are of interest for biological studies.[1] A large number of metabolites have not yet been recorded. In doing so an identification by plant materials is of particular interest as plant materials have a large variety of secondary metabolites.

The challenge in the analysis of metabolites is the identification from mixed spectra. On the one hand mixed spectra show a superimposition of the specific metabolite signals, on the other hand the metabolites may be present in different concentrations, so that the structural elucidation of individual metabolites is made more difficult.

In order to facilitate identification, the polar extract of the hazelnut is time-fractionated by high-performance liquid chromatography and the concentration is increased by repetition. The fractions obtained subsequently exhibited a lower number of metabolites, which simplifies mass spectrometric and NMR spectroscopic identification. It was shown that identification can be simplified by evaluating <sup>1</sup>H-NMR spectra of the single fractions in combination with selective NMR spectra (*selective* TOCSY - and *selective* NOESY spectra). Using selective NMR methods, spin couplings and spatial couplings of a metabolite can be identified, so that the metabolite can be composed retrospectively. This approach can be used to identify metabolites that were not previously stored in databases in addition to those already known.

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**$^{47,49}\text{Ti}$  Solid State NMR and DFT Study of Ziegler-Natta Catalyst**

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In Ti-based Ziegler-Natta catalysts (ZNCs), disagreements exist concerning which lateral surface of the  $\text{MgCl}_2$  support adsorbs Ti species so as to be an active site for the catalysis of polymerization. In the present paper, we investigated the local structure of  $\text{TiCl}_4$  adsorbed onto the surface of  $\text{MgCl}_2$  by  $^{47,49}\text{Ti}$  solid-state nuclear magnetic resonance (NMR) spectra at 21.8 T along with density functional theory (DFT) calculations. The magic-angle-spinning NMR spectrum of the  $\text{TiCl}_4/\text{MgCl}_2$  adduct sample prepared by 20 h of milling, which exhibited broadened and shifted peaks compared to that of the sample without milling, was simulated by a Czek model considering the distribution of quadrupole interaction parameters. The electric field gradient and chemical shielding tensors of  $^{49}\text{Ti}$  were obtained via DFT calculations for model molecules of  $\text{TiCl}_4$ ,  $2\text{TiCl}_3$ , and  $\text{Ti}_2\text{Cl}_8$  adsorbed onto the (110), (104), and (104)-step defect surfaces of  $\text{MgCl}_2$ . By comparing the obtained NMR parameters, the  $^{47,49}\text{Ti}$  NMR spectrum of the milled sample was assigned to  $\text{TiCl}_4$  adsorbed onto the (104) surface of  $\text{MgCl}_2$ , which may not be a principal component of adsorption.

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## Dynamics of carbon dioxides in a metal-organic framework under high pressure

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We investigate the dynamics of CO<sub>2</sub> adsorbed in a metal-organic framework (MOF) in high pressure range (~1 MPa) utilizing the combination of MAS NMR and CSA analyses. MAS NMR spectra show adsorbed CO<sub>2</sub> adsorbed and free mobile gas-phase CO<sub>2</sub>. Analyses for the dynamics by T<sub>1</sub> measurement suggest that the adsorbed CO<sub>2</sub> in pores has very slow mobility corresponding to the values reported for some solid materials. CSA analyses reflect local rotation of CO<sub>2</sub> with various angles. The finding and analyses would help to enhance the function of separation in MOFs via the control of dynamics and kinetics for CO<sub>2</sub>.

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Solid-state NMR Studies of Cross-seeding of Aβ40 with Aβ42 fibril

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Misfolded β-amyloid (Aβ) is associated with Alzheimer’s disease (AD) and the most abundant species (isoforms) in brains are 40-residue Aβ40 and 42-residue Aβ42. As those Aβs have different properties, despite similarities in their amino acid sequences, aggregation kinetics and structures of both Aβ40 and Aβ42 fibrils have been studied separately to investigate the mechanism of AD. However, both Aβ40 and Aβ42 coexist in AD. Thus, it is important to understand interactions between the different isoforms of Aβs. Misfolding kinetics of Aβ42 or Aβ40 monomers seeded with fibril of the same Aβ isoform suggests nucleation-dependent polymerization from monomers. On the other hand, recent studies indicate that the kinetics of Aβ40 monomers cross-seeded with Aβ42 fibril was unaffected despite the presence of the seed Aβ42 fibril. To examine cross-seeding mechanism at atomic level, in this study, we analyzed 13C SSNMR spectra for Aβ40 cross-seeded with Aβ42 fibril as fingerprints. The solid-state NMR data with ThT fluorescence data suggested that Aβ40 monomer is likely to interact with Aβ42 fibril, but “cap” the terminus of Aβ42 fibril by forming a disrupted structure that was not fully templated by Aβ42 fibril to prevent the resultant further elongation.

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# Development of Meanderline Coils for Millimeter-Wave ESR/NMR Double Magnetic Resonance Measurements of Thin Samples

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We have been developing a millimeter (mm)-wave ESR/NMR double magnetic resonance system in ultra-low temperature and high magnetic field regions aiming for direct detection of diluted nuclear spins.

Increase of nuclear magnetization by using dynamic nuclear polarization (DNP) may enable direct observation of NMR.

One of our targets is a silicon crystal doped lightly with phosphorus (Si:P).

Si:P has been attracting much interest especially after the proposal of a quantum computer model using Si:P (B.Kane, Nature 1998) in which the <sup>31</sup>P nuclear spin ( $I=1/2$ ) stores the quantum state. Behavior of <sup>31</sup>P spins is important for evaluating the realization of the quantum computer, though there is no result of direct observation of NMR signals from diluted <sup>31</sup>P nuclear spins.

In order to perform <sup>31</sup>P-DNP-NMR, we have developed a resonator that can simultaneously measure ESR and NMR, where the Fabry-Pérot-type mm-wave resonator composed of spherical-plane mirrors is combined with a serpentine rectangular conductor line (we call "Meanderline").

Owing to the flat shape of the meanderline, the meanderline can be put close to a thin plate sample, which is advantageous to better NMR sensitivity. Further, we made a gold layer ( $t=160\text{nm}$ ) on a kapton film as a flat mirror. With an optimal thickness of the gold layer, millimeter waves around 128GHz can be reflected and radio waves around 140MHz can be transmitted due to frequency dependence of the skin depth.

We can put the meanderline just outside the flat mirror without disturbing the standing wave for Fabry-Pérot resonator.

The developed resonator incorporated into a <sup>3</sup>He/<sup>4</sup>He dilution refrigerator, and we performed electron-nuclear double resonance (ENDOR) and <sup>31</sup>P-DNP-NMR measurements of Si:P.

The ENDOR observed the <sup>31</sup>P-NMR frequency. In addition, single-shot pulse NMR measurements have been attempted in a polarization state of about 90% and we have observed the <sup>31</sup>P-DNP-NMR signals successfully.

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**$^1\text{H}$ - $^{35}\text{Cl}$  Heteronuclear Correlation Experiments to  
Probe Structure of Pharmaceutical Salts**

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Chloride salts have wide applications in pharmaceuticals, whether they are used for isolating and purifying the active pharmaceutical ingredients, or for providing more stability and/or solubility to the drugs.  $^{35}\text{Cl}$  nucleus has a 75% natural abundance and it is quadrupolar, with a nuclear spin  $I=3/2$ . In many organic compounds where it is covalently bound, the  $^{35}\text{Cl}$  nucleus feels a very large quadrupolar interaction not amenable to MAS experiments. However, in many pharmaceutical salts, as well as in biological materials, Chlorine atoms are weakly ionic bonded and mobile, therefore the quadrupolar interaction is reduced and the  $^{35}\text{Cl}$  MAS NMR signal can be measured at high magnetic fields and under very fast MAS. Encouraged by the success of the  $^1\text{H}$ - $^{14}\text{N}$  Dipolar HMQC MAS experiments to probe hydrogen bonding in pharmaceutical co-crystals and amorphous dispersions we explore the applicability of  $^1\text{H}$ - $^{35}\text{Cl}$  D-HMQC experiments to measure proximities between Hydrogen and Chlorine atoms in model pharmaceuticals, like Cimetidine and Amitriptyline as well as on Glycine-HCl. Numerical simulations of the  $^1\text{H}$ - $^{35}\text{Cl}$  MAS D-HMQC experiment can be performed on each quadrupolar orientation independently and this gives the possibility to group the orientations of the quadrupolar tensors in a statistical way and evaluate the efficiency of the recoupling sequence ( $\text{SR}4_1^2$  or  $\text{R}^3$ ) at different mixing times. Numerical simulations can also be used to quantify and understand how WURST saturation of  $^{35}\text{Cl}$  satellite transition enhances the coupling between  $^1\text{H}$  and  $^{35}\text{Cl}$  central transition and lessens the signal coming from the coupling between  $^1\text{H}$  and  $^{35}\text{Cl}$  satellite transitions. Theoretical results will be compared with experimental results obtained on Tyrosine-HCl.

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## P198

**Singlet-state NMR with pairs of nearly equivalent nuclear spins**

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Singlet-state NMR is an important emerging methodology for overcoming limitations imposed by finite nuclear spin relaxation times. Specifically, one can exploit advantages of long-lived spin states (LLSs) and long-lived coherences (LLCs). LLSs exist when the symmetry of the static and fluctuating Hamiltonian coincide; consequently, such states become immune to particular kinds of relaxation. An LLC is a spin coherence with a lifetime much longer than  $T_2$ , giving rise to very narrow lines.

An important class of systems for singlet-state NMR is given by molecules containing pairs of “nearly-equivalent” spins, being strongly coupled even at high field (i.e., the J-coupling strength is much greater than the difference in their NMR frequencies). Efficient use of such molecules, however, requires rational design of special NMR methods for efficient generation of singlet spin order and robust excitation of singlet-triplet coherences.

In this work, we report progress in designing such NMR methods:

- We present an optimization of magnetization-to-singlet (M2S) conversion and backward S2M conversion. The optimization utilizes constant-adiabaticity RF-pulses, which significantly increase the conversion efficiency, up to 95%.
- By using repetitive M2S/S2M conversion with optimized pulses, we achieve a further increase of the spin order (above the value of 2/3 given by unitary bounds of spin evolution) and enhance spin magnetization by approximately 25%.
- We present NMR pulse sequences for optimal excitation of singlet- $T_z$  transitions, which reveals itself in the increased intensity of “forbidden” NMR transitions.
- Such pulse sequences can be used as building blocks for more complex sequences that can be exploited to excite the long-lived singlet- $T_0$  coherence; here we report coherence lifetimes reaching 200 s.
- We also propose singlet presaturation methods for fast repetition of experiments with LLSs.

The proposed methods have been tested by running experiments with a specially designed naphthalene derivative having two nearly equivalent  $^{13}\text{C}$ -nuclei.

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**Real-time online reaction monitoring by single-scan 2D NMR under flow conditions**Corentin Jacquemmoz<sup>\*,1</sup>, François Giraud<sup>2</sup>, Jean-Nicolas Dumez<sup>1</sup><sup>1</sup>CEISAM, CNRS UMR6230, Université de Nantes, <sup>2</sup>ICSN, CNRS UPR2301

NMR spectroscopy is a powerful tool for the monitoring of organic chemical reactions. Multidimensional experiments have the potential to bring more information on reacting mixtures, but the duration required for classic experiments (several minutes) limits the accessible timescales. Ultrafast 2D NMR, originally proposed by Frydman in 2002 [1], makes it possible to collect 2D spectra in less than one second, by spatial encoding of NMR interactions. This potentially extends the scope of real-time reaction monitoring.

Here we show recent developments on the use of UF2DNMR for online monitoring of reaction mixtures under flow conditions. We use a commercial "Flow Unit" [2]: which consists in a pump that circulates the reaction mixture from a reaction vessel to the NMR system in a "flowtube" through a temperature regulated transfer line. The use of such equipment allows reaction monitoring in a wider variety of conditions (heat transfer, stirring...) than running the reaction in an NMR tube or sampling the reaction mixture at given times. Spatial encoding on a flowing sample however raises several challenges, such as pulsations induced by the piston pump or intensity losses linked to the spatial encoding scheme, which are addressed with pulse sequence developments assisted by numerical simulation. In addition, the choice of spatial encoding axis and its influence on repeatability are investigated. The use of UF2DNMR for a flowing sample will be first shown on a model stable mixture, and the limitations encountered will be discussed. Then, as a proof of concept, the real-time 2D NMR monitoring of a model reaction using UFNMR will be shown.

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## P200

**Hyperpolarization of 15N-pyridinium and 15N-anilinium derivatives by using parahydrogen: Novel opportunities to store hyperpolarization in aqueous media**Anil P. Jagtap\*, Lukas Kaltschnee, Stefan Glöggler*Max-Planck-Institute for Biophysical Chemistry and Center for  
Biostructural Imaging of Neurodegeneration*

Hyperpolarization techniques have the potential to improve the sensitivity of magnetic resonance imaging (MRI) by more than four orders of magnitude.[1] Various hyperpolarization techniques have been used to generate hyperpolarized contrast agents to study and diagnose diseases in vivo.[2] Amongst the challenges of designing hyperpolarized contrast agents, two are persistent: the need to create large polarizations and the need to store it sufficiently long. In para-hydrogen induced polarization (PHIP)[3] method, 1H polarization is generated using para-hydrogen. However, 1H-T1 has a relative short lifetime of a few seconds. Therefore, it is essential to store such 1H-hyperpolarization on nuclei having longer relaxation times (T1), e.g. 13C and 15N. Reports on 15N-T1 times of tens of minutes,[4] attracted our attention to develop 15N-based contrast probe for biological applications.

Herein, we present syntheses and hyperpolarization studies of two families of 15N-derived compounds namely: tert-amine aniline derivatives and a quaternary pyridinium compound. For PHIP studies, we chose two types of Rh-based catalyst systems; a homogeneous catalyst in MeOH and Rh-nanoparticles (NAC@Rh) in water. Remarkably, a quaternary pyridinium compound showed 15N-T1 of 8 min and yielded over 8% polarization on 15N-nuclei utilizing our recently developed polarization transfer sequence termed ESOTHERIC (Efficient Spin Order Transfer via Relayed Inept Chains).[5] Further, it gave 3% polarization on 15N nuclei using NAC@Rh under biocompatible aqueous conditions. Pyridinium is a great motive, which is present in a variety of drug molecules and also used in various drug delivery approaches.[6]

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## P201

### Strategic approach to identify polar metabolites from a complex mixture of tuber melanosporum by means of one- and two-dimensional NMR spectroscopy

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Metabolomics studies have grown progressively over the past few decades. This research field has not only become valuable for medicine and pharmaceuticals, but also, for example, for food analysis. A strong interest lays in plant material, since it possesses a large amount of secondary metabolites where a significant part have not yet been identified and therefore their potential biological activities are still unknown. The biological system of truffles is far from being completely identified. Nowadays, not only the unique aroma and nutritional characteristics of truffles are at the center of research, but also their biological potential, such as antiviral and antimicrobial activities.

The challenge in identifying metabolites from a complex matrix by means of NMR spectroscopy is that it leads to unwanted signal superimposition, which makes it difficult to interpret the obtained spectra. In addition, the difference of the individual metabolite concentrations varies strongly, making it nearly impossible to detect low concentrated metabolites in the extract. In order to avoid these challenges, the polar extract of a truffle sample was fractionated by HPLC before being measured via NMR spectroscopy. Due to this separation, the individual fractions were simplified enormously. Subsequently, all obtained fractions were measured by <sup>1</sup>H-NMR experiment with water suppression in combination of one-dimensional experiments, such as *selective* TOCSY and *selective* NOESY, as well as two-dimensional experiments. With this strategic approach, several metabolites from a complex mixture of *tuber melanosporum* have been identified.

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## P202

**Block copolymer domain morphology studied by  
NMR spin diffusion combined with Atomic Force Microscopy**

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Block copolymers (BCP) due to their unique self-assembling properties represent important group among synthetic materials and they are believed to play significant role in future nanolithography. It appears that below certain critical temperature TODT (order-disorder transition temperature) two dissimilar subchains (blocks) become immiscible and tend to separate one from another gathering with alike chemical species accordingly forming domain like structure. These microphase separated systems can be effectively monitored using various experimental methods namely TEM, SAXS, SANS, AFM or SEM. However all mentioned methods can provide only a tentative picture regarding interfacial space thickness, which might be a parameter of greatest importance for nanolithographic design purposes. Therefore, comprehensive BCP structural study should be supported by NMR spin diffusion experiment which enables estimation of both BCP domain size and interfacial region dimensions. This particular technique relies on molecular dynamics contrast observed between dissimilar domains. In the spin diffusion experiment the polymer chain mobility itself plays a role of a contrast agent because it differentiates the dipolar coupling strength across the domains. This feature enables application of dipolar filter sequence which selectively depolarizes nuclear magnetic moments within rigid phase only. Accordingly, it is possible to introduce nonuniform spatial distribution of magnetization within domain system. This thermodynamically non-equilibrium state evolves towards magnetization equilibration via nuclear magnetic moments 'flip-flop' mechanism which is driven by dipolar interactions. The spin diffusion process lasts until the magnetization is gradually leveled out throughout the domains. This process can be directly monitored via analysis of the NMR spectra evolution.

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Truncated field concept in NMR

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In order to thoroughly comprehend and adequately interpret NMR data, it is necessary to perceive the complex structure of spin Hamiltonian. Although NMR principles have been extensively discussed in a number of distinguished introductory publications, it still remains difficult to find illustrative graphical models revealing the tensorial nature of spin interaction. Exposure of the structure standing behind mathematical formulas can clarify intangible concepts and provide a coherent image of basic phenomena. This approach is essential when it comes to hard to manage, time-dependent processes such as Magic Angle Spinning (MAS), where the anisotropic character of the spin system interactions couple with experimentally introduced time evolution processes. The concept of truncated field which represents the effective local magnetic field generated by dipolar nucleus ( $I=1/2$ ) under the influence of Zeeman field, a) illustrates the tensorial character of dipolar coupling, b) clarifies the symmetry of internuclear interactions, c) clearly explains the influence of MAS on spin system. Poster reveals several important examples which prove reliability of the concept.

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NMR study of Transcription factor protein DLX3 and its binding site wt-DNA and mutant-DNA  
Ho-Seong Jin\*, Joon-Hwa Lee, Seo-Ree Choi

Transcription is the first step of gene expression, and RNA polymerase specifically copies a particular part of DNA into mRNA. Transcription Factor (TF) is a protein that controls the rate of transcription by binding to a specific DNA sequence. Of the transcription factor protein, we studied *Drosophila* distal-less homeobox (DLX) gene. Genes in the DLX family encode homeodomain transcription factors related to the *Drosophila* distal-less (Dll) gene. The family has been related to a number of developmental features such as jaws and limbs. *Drosophila* distal-less homeobox 3 (DLX3) is expressed throughout development in a series of structures derived from epithelial mesenchymal interaction such as the teeth, hair follicles, and limb buds. To understand the molecular mechanisms of specific DNA recognition of DLX3, we have performed  $^1\text{H}/^{15}\text{N}$  heteronuclear single quantum correlation (HSQC) experiment and imino proton NMR experiments between DLX3 and consensus DLX3 Binding Site (DBS) in DNA. The exchange rate constants of the imino protons for the wild type and mutant were measured by using water magnetization experiment. We compared the binding affinity of DLX3 for both wild type and mutant and characterized its target DNA recognition.

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Functional details of the *Mycobacterium tuberculosis*  
VapBC26 toxin-antitoxin system based on a structural study  
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*Seoul National University*

Toxin-antitoxin (TA) systems are essential for bacterial persistence under stressful conditions. In particular, *M. tuberculosis* express VapBC TA genes that encode the stable VapC toxin and the labile VapB antitoxin. Under normal conditions, these proteins interact to form a non-toxic TA complex, but the toxin is activated by release from the antitoxin in response to unfavorable conditions. Here, we present the crystal structure of the *M. tuberculosis* VapBC26 complex and show that the VapC26 toxin contains a pilus retraction protein (PilT) N-terminal (PIN) domain that is essential for ribonuclease activity and that, the VapB26 antitoxin folds into a ribbon-helix-helix DNA-binding motif at the N-terminus. The active site of VapC26 is sterically blocked by the flexible C-terminal region of VapB26. The C-terminal region of free VapB26 adopts an unfolded conformation but forms a helix upon binding to VapC26. The results of RNase activity assays show that Mg<sup>2+</sup> and Mn<sup>2+</sup> are essential for the ribonuclease activity of VapC26. As shown in the nuclear magnetic resonance (NMR) spectra, several residues of VapB26 participate in the specific binding to the promoter region of the VapBC26 operon. In addition, toxin-mimicking peptides were designed that inhibit TA complex formation and thereby increase toxin activity, providing a novel approach to the development of new antibiotics.

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## P206

### Investigation of HCV Nonstructural Protein 4B in Proteoliposomes by Proton-Detected Solid-State NMR at 100 kHz MAS

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The hepatitis C virus (HCV) causes acute and chronic hepatitis with approximately 70 million infected people worldwide. HCV life cycle in infected cells is functionally linked to membrane structures and lipid metabolism. Transmembrane protein NS4B is one of major contributors to formation of membranous replication organelles and thus playing a crucial role in viral genome replication and virion assembly. The exact mode of action of NS4B is still unknown and further structure analysis is a pre-requisite to understand its molecular mechanism.

The NS4B was expressed in a wheat germ cell-free system to overcome its high cytotoxicity to *E. coli*. The translation was performed in a presence of detergent to obtain soluble protein followed by one-step purification on the Strep-Tactin column. The deuterated, 100% protonated on exchangeable protons, and uniformly or selectively labelled proteins were then reconstituted into proteoliposomes by detergent removal with methyl- $\beta$ -cyclodextrin.

Various lipid compositions, types of detergent, lipid-to-protein ratios (LPR) as well as types and amounts of cyclodextrin were tested by two-dimensional <sup>1</sup>H-<sup>15</sup>N correlation spectroscopy to yield spectra with optimal line widths and signal-to-noise ratio. Well-resolved two- and three-dimensional spectra were recorded. Out of expected 280 resonances, 190 and 155 resonances were picked by the automatic peak picking with the CCPN software in the hCANH and hCONH spectra, respectively. The signal overlap was severe even in 3D spectra due to the predominantly  $\alpha$ -helical structure of NS4B protein. Still about 60 isolated peaks could be connected between the hCANH and hCONH spectra, demonstrating spectra quality and the potential for further backbone assignment in the context of spectra with decreased signal density. To decrease spectra complexity, selective labeling in cell-free context was applied to introduce anchoring points for assignment and to reduce signal overlap, allowing future de-novo backbone resonance assignment.

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## P207

**A detailed NMR characterisation of a duplex  
DNA containing a newly discovered metallo base pair**  
Olivia P. Schmidt, Simon Jurt, Silke Johannsen\*, Ashkan Karimi,  
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The large diversity of nucleic acid structures can be expanded even further by the introduction of metal-mediated base pairs in which base pairs are generated by coordination to metal ions instead or in addition to hydrogen bonds.[1,2] These metal-mediated base pairs consist of either natural or artificial nucleobases and are frequently used for the programmable and site-specific insertion of metal-based functionality into nucleic acids and therefore play a key role in the development of advanced DNA-based materials and devices.

Here we report about the NMR characterization of duplex DNA containing a newly discovered metallo base pair [3] that revealed four new coupling constants and resulted in three-dimensional structures of metal-bound and metal-free (apo) DNA species. Our comprehensive study disclosed also a very unique dynamic behavior of the metal-bound DNA species, which was further analyzed by determination of rate constants. Given the high quality of both the structural and dynamics information, this system is also a highly attractive model system for future computational studies.

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## P208

**Sterically overcrowded, isopropyl-substituted lanthanide chelating tags for protein PCS nuclear magnetic resonance spectroscopy: Synthesis of their macrocyclic scaffold and benchmarking on ubiquitin and human carbonic anhydrase II**

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*University of Basel*

Pseudocontact shifts (PCS) and residual dipolar couplings (RDC) generated by lanthanide chelating tags (LCT) yield valuable structural restraints for the analysis of structure, dynamics and ligand-binding of proteins in solution. A sterically overcrowded, isopropyl-substituted lanthanide chelating tag (P4M4) was synthesized in order to investigate the influence on the obtained pseudocontact shifts and the anisotropic part of magnetic susceptibility tensor when compared to the predecessor DOTA-M8-(4R4S)-SSPy. For the first time, a concise synthetic route is presented for isopropyl-substituted cyclen, the macrocyclic scaffold of the lanthanide chelating tag, delivering the macrocycle in overall yield of 6% over 11 steps. The geometry of the lutetium complex was assigned by ROESY experiments to adopt exclusively a  $\Lambda(\delta\delta\delta\delta)$  conformation and DFT calculations confirmed a stabilization of 32.6 kJ/mol compared to the  $\Delta(\delta\delta\delta\delta)$  conformer.

The steric overcrowding of the isopropyl substituents was then combined with a newly developed reduction-stable thiazolo linker (P4T) that enables the application of the tags in reductive environments, as requested for example in in-cell NMR studies. Besides the application in reductive environments, the improved linker further enhances the observed anisotropy parameters on the protein, since rotational averaging is strongly suppressed when compared to the more flexible disulfide linker. P4T was benchmarked on ubiquitin as well as human carbonic anhydrase II (hCA II) and its structure and conformational coordination bias was investigated by NMR and DFT.

Both highly rigidified lanthanide chelating tag induce strong pseudocontact shifts on ubiquitin and human carbonic anhydrase II, show significantly improved tensor properties when compared to DOTA-M8-(4R4S)-SSPy and constitute a highly promising starting point for further developments of lanthanide chelating tags.

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## P209

### Development of an *in operando* NMR setup for the investigation of the electrolytic carbon dioxide reduction at silver electrodes

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The electrolytic reduction of carbon dioxide (CO<sub>2</sub>) is a promising option for the future of sustainable energy as chemical energy storage. Specifically, the electrolysis of CO<sub>2</sub> in aqueous media using silver electrodes yields carbon monoxide (CO), which can be used to produce CO<sub>2</sub>-neutral fuels via Fischer-Tropsch synthesis. The technical implementation of this technology is currently limited by long-time stability and efficiency. This is due to a lack of understanding of the molecular reactions and dynamics taking place at the electrode.

As electrochemical processes can only be studied during operation, the investigation of the electrolytic CO<sub>2</sub> reduction requires *in operando* techniques. NMR spectroscopy is chosen as method for the *in operando* studies due to its versatile toolkit, which allows to investigate a broad range of aspects of the electrolysis.

The combination of NMR and electrochemistry introduces several new challenges. Conductive materials, which are required for electrolysis, distort the magnetic field homogeneity and introduce high external noise to the NMR measurement by acting as antennas. Additionally, the electrolysis cell needs to be adapted to the limitations of the probe head.

In this contribution an *in operando* cell for the CO<sub>2</sub> electrolysis with minimized metal content was developed. It fits standard liquid state probe heads and can be easily adapted to other electrochemical systems. Furthermore, an *in operando* setup was constructed to minimize noise and distortions in the NMR measurement caused by external radio waves and the electrochemical measurement.

The system dynamics of CO<sub>2</sub> solved in the electrolyte (1M KHCO<sub>3</sub>(aq)) were characterized using relaxometry, diffusometry and exchange measurements. It is shown that the electrolysis cell and the *in operando* setup did not influence the dynamics of the solved carbon dioxide. Finally, first *in operando* NMR experiments, performed on the electrolytic reduction of carbon dioxide are presented.

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P210

NMR Structural Studies and Mechanisms of Antimicrobial Peptides with higher activity  
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The availability of antibiotics has allowed for the successful treatment of many bacterial infections as well as the ability to perform invasive medical procedures including surgery and chemotherapy. However, their wide use has led to pathogens' increased drug resistance and the need to find novel classes of antimicrobial peptides as alternatives to antibiotics. Lactophorin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, corresponds to the carboxy terminal 113–135 region of component-3 of proteose-peptone and is a good candidate as a peptide antibiotic because it has an antibacterial activity but no hemolytic activity. Several analogs of LPcin, that has mutant amino acids and shorter mutant amino acids are developed by using peptide engineering techniques in our laboratory and show better antibiotic activities than wild-type LPcin and no toxicity at all.

Recently, we designed and selected more antimicrobial peptides based on LPcin-YK3. Designed all analogues were expressed and purified using several biophysical techniques and characterized using antimicrobial activity tests and various spectroscopic methods like MALDI-TOF MS and CD spectrometry, as well as 1D/2D solution NMR and solid-state NMR techniques in membrane environments.

In here, we will present the optimizing processes with high-yield expression and purification of new LPcin analogs and solid- state NMR structural studies to figure out antibacterial killing mechanisms.

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## P211

## Structural and biochemical analysis of 3' splice site recognition

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Recognition and removal of intron from pre-mRNA is an essential step in splicing system to generate a functional protein. The 3' splice site of pre-mRNA introns consists of a branch point site (BPS), a Py-tract and the yAG splice site, which are recognized by the SF1, U2AF65 and U2AF35 RNA binding proteins, respectively. Dynamic conformational shifts of multi-domain arrangements, cooperative and transient interactions are characteristic features of the assembly of this regulatory ribonucleoprotein complex. Thus NMR is an ideal tool to understand the molecular mechanisms at atomic level resolution. SF1 exhibits protein-protein and protein RNA interactions. The N-terminal ULM peptide motif of SF1 interacts via C-terminal UHM domain of U2AF65, while its KH-Qua2 module recognizes the "CURAY" BPS RNA site. The helix-hairpin (HH) domain preceding the KH-Qua2 region has a linker with a conserved SPSP motif, which undergoes phosphorylation at Serine 80 and 82. Previous reports have shown that phosphorylation of SF1 improves the binding with suboptimal RNA sequences. However, the underlying molecular mechanisms are unclear, considering that the SPSP motif is expected to be remote from the RNA binding interface of SF1.

We study the effects of phosphorylation on the assembly of SF1-U2AF65-RNA ternary complex. Chemical shift perturbations of SF1 vs phosphorylated SF1 show differences near the phosphorylation site. We have previously shown using <sup>15</sup>N R1, R2, and {1H-}-<sup>15</sup>N heteronuclear NOE data that phosphorylation reduces the linker flexibility consistent with the formation of salt bridges between negatively charged phosphates and arginine side chains as seen in a crystal structure of the phosphorylated domain. Interestingly, <sup>31</sup>P NMR spectra of phosphorylated SF1 shows differential line broadening of two <sup>31</sup>P signals upon complex formation with U2AF65, suggesting a long-range effect, that is mirrored by corresponding CSPs in U2AF65. We are characterizing the overall effects of SF1 phosphorylation on the ternary complex using PRE data and SAXS.

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## P212

Structure Based Design of A $\beta$ (1-42) Small Molecule Reporters

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An important pathological hallmark of aggregation related neurodegenerative diseases, such as Alzheimer's or Parkinson's disease, is the formation of highly ordered cross- $\beta$ -sheet containing structures, termed amyloids.[1,2] Taking advantage of these, non-invasive positron emission tomography (PET) in combination with 3D-imaging techniques offers a promising approach for early stage diagnosis. However, detecting the distribution of tracer molecules in the brain, PET is strongly limited by pharmacokinetics and binding selectivity of the applied ligands. Structure based *in silico* screening offers a fast and simple strategy to identify new possible tracer molecules. We generated a pharmacophore model based on the surface properties of the A $\beta$ (1-42) structure (PDB code 2nao).[3] Molecule supplier database screening, followed by additional molecule property related selection, lead to a reasonable small set of molecules exhibiting the ability to bind into the chosen binding pocket. We validated molecular fragment hits *in vitro* for protein interaction, as well as binding selectivity, using STD NMR and HPLC. Following this approach, we were able to identify several new potential tracer molecules showing good A $\beta$ (1-42) selectivity, as well as binding affinities in the micromolar range. Overall, the *in silico* structure based molecule screening in combination with *in vitro* analysis is a time and sample efficient method allowing for more profound ligand investigation.

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**Deep insight into the dynamics of highly flexible domain of delta subunit of RNA polymerase by combination of computational methods, relaxation, and relaxometry measurements**

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Delta subunit of RNA polymerase is exclusively present in Gram positive bacteria. It was proven that it is important for virulence of the bacteria, it ensures the quick and proper response to the changing cell environment conditions, and its role for regulation of RNA polymerase activity was also demonstrated [1]. However, the function of the delta subunit is not fully understood at molecular level. Especially, the intrinsically disordered C-terminal domain of the delta subunit deserves attention. Highly flexible regions of proteins are known to be able to bind various interacting partners with well tuned affinity, which is a prerequisite for regulation of cellular processes. Several transient contacts within the C-terminal domain were detected [2], significantly influencing conformational preferences of the delta subunit. Site directed mutagenesis proved that the conformational balance and dynamics is critical for function of the RNA polymerase. NMR relaxation offers a deep insight into the complex dynamics and energetic landscape of the delta subunit. Here, we present results of a study including (i) analysis of conventional relaxation experiments combined with molecular dynamics simulations following the ABSURD protocol [3] and (ii) analysis of high-resolution relaxometry data [4]. The high-resolution relaxometry allowed us to collect site-specific relaxation rates at 8 magnetic fields as low as 0.1 T providing an unique insight into the dynamics of the delta subunit. The results provide a detailed description of both local and global conformational sampling as well as the time scales of the related dynamics. This work was supported by the Czech Science Foundation grant No. GA18-04197Y.

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### A PHIP Strategy Yielding $^{13}\text{C}$ -Hyperpolarized Unprotected Amino Acids in Aqueous Solutions

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The hyperpolarization of biologically relevant molecules for signal-enhanced magnetic resonance detection holds great promise for studying biological processes *in vitro* and *in vivo*. Being important metabolites as well as the building blocks of peptides, the canonical amino acids are promising targets for hyperpolarization, and a number of parahydrogen based strategies have been devised to hyperpolarize amino acids[1-7]. For biochemical and biomedical applications, however, these approaches suffer from limited biocompatibility of the obtained products, due to the utilization of toxic solvents, such as methanol- $d_4$ , or because chemically modified (e.g. *N*-protected) amino acids are obtained.

Herein we present a PHIP strategy for hyperpolarizing proteinogenic amino acids under conditions of improved biocompatibility. We obtain chemically unmodified proteinogenic amino acids, which are directly produced in aqueous media. This is enabled through the use of a heterogeneous nanocatalyst[7], which allows for PHIP hyperpolarization of the *N*-unprotected amino acids in water and further provides the prospect of easy catalyst separation. Efficient coherent hyperpolarization transfer from  $^1\text{H}$  to  $^{13}\text{C}$  is achieved at high field, using the recently presented ESOTHERIC[8] approach. This allows for polarization storage on the carbonyl- $^{13}\text{C}$  for tens of seconds.

Whereas the strategy presented is demonstrated here for the hyperpolarization of single amino acids, it is likely that the approach can be extended into a general strategy for  $^{13}\text{C}$ -hyperpolarizing small synthetic peptides in aqueous solutions.

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Effects of electron spin dynamics at 7 Tesla on DNP enhancement and NMR spectra

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Dynamic Nuclear Polarization (DNP) is the most widespread and ubiquitous technique for signal enhancement in Nuclear Magnetic Resonance (NMR). Introduction of the ability to arbitrarily modulate the 193 GHz waveform used for excitation of the electron spins at 7 Tesla resulted in up to a factor of 4 improvement in DNP efficiency under varying range of experimental conditions and across different samples. To understand the underlying spin dynamics behind this performance enhancement we utilized the pulsed Electron Paramagnetic Resonance (EPR) capabilities which allowed us to demonstrate that broadband excitation of the inhomogeneously broadened EPR line shifts the DNP mechanism from indirect cross effect to direct-microwave driven cross effect.[1]

Unpaired electron spins are always present in the DNP sample preparations and can sometimes have unwanted, detrimental, effects on the NMR spectra such as shifting the position of the peaks (Paramagnetic Shift) and decreasing resolution by linewidth broadening (Paramagnetic relaxation) collectively referred as paramagnetic effects (PE). We have recently observed the reversal of PE upon microwave irradiation in DNP experiments at liquid helium temperatures which was manifested by significant narrowing of the <sup>7</sup>Li NMR line and reversal of the paramagnetic chemical shift.[2] The extent of the PE was found to decrease with increased saturation of the electron paramagnetic resonance line, modulated as a function of microwave power, frequency, duration of irradiation, and time between microwave irradiation and NMR detection. We suggest that the origin of the observed REversal of PRE by electron Spin SaturatiON (REPRESSION) effect stems from the shortening of the electron phase memory time upon electron spin bath saturation by microwave irradiation.

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Interaction of replication protein A with  
Bloom syndrome protein and Fanconi Anemia Group J  
Jinwoo Kim, Donguk Kang\*, Chin-Ju Park  
*Gwangju Institute of Science and Technology*

Human DNA helicases, BLM (Bloom syndrome protein) and FANCI (Fanconi Anemia Group J) have a common interaction partner, Replication Protein A (RPA). It has been identified that the interaction of BLM and FANCI with RPA stimulate their DNA unwinding ability, but binding properties of both proteins to RPA were not investigated. Here, we present interactions of RPA-BLM and RPA-FANCI by NMR spectroscopy and Fluorescence Polarization anisotropy experiments. We revealed that two acidic peptide regions of BLM specifically bind to RPA70N domain. Also, we first suggested that the acidic region present at the C-terminal of FANCI binds with RPA70N. Our analysis shows that the common binding strategy underlies for maintaining RPA-helicase interactions.

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Ageing effects on the distribution of crosslink densities in elastomer blends as studied by high- and low-resolution solid-state <sup>1</sup>H NMR

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Conventional methods for determining crosslink densities (CLDs) in elastomers, such as equilibrium swelling of vulcanizates (using the Flory-Rehner equation), and stress-strain measurements (using the Mooney-Rivlin equation) lack phase-specific distinction of crosslink densities in elastomer blends [1]. NMR spectroscopy offers better solutions to overcome these limitations, but studies concerning quantitative measurements of CLDs in elastomer blend phases are rare as well as qualitative [2, 3]. In this study, phase-resolved CLDs in the form of residual dipolar couplings, obtained by a homonuclear double-quantum MAS recoupling pulse sequence [4] will be discussed for blends of sulphur-vulcanized natural rubber (NR) and styrene butadiene rubber (SBR). Thermo-oxidative ageing of the blends shows an evolution of CLDs in the blend phases that follow the characteristic ‘reversion’ and ‘marching’ phenomena in NR and SBR, respectively, thus leading to a distribution of properties on the microscopic scale. Further, data from low-field benchtop NMR spectrometer show a formation of a rigid fraction in NR-rich compositions due to oxidation, thus affecting the dynamics.

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Dipolar Relaxation of Water Protons in the Vicinity of a Collagen-like Peptide

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Type II collagen is the most abundant macromolecule in articular cartilage. In the tissue collagen forms anisotropic, fibrillous structures. Longitudinal relaxation is generally thought to be isotropic in articular cartilage [1]. We investigate the conditions needed to observe the anisotropy of the collagen network in the longitudinal relaxation time  $T_1$ .

We have computed longitudinal relaxation rates  $R_{1\text{intra}}$  of water protons due to intramolecular  $^1\text{H}$ - $^1\text{H}$  dipole-dipole coupling from molecular dynamics simulations of a dissolved collagen-like peptide 1QSU [2]. The effects of peptide orientation with respect to the main magnetic field and residual dipolar interaction were included in the analysis. Movements of different spin pairs were assumed to be uncorrelated. Comparisons were made to results from simulations of bulk water.

Overall, the intramolecular dipole-dipole couplings were estimated to be the largest contributor to the proton relaxation. We found that for the water molecules in the first hydration layer of the peptide,  $R_{1\text{intra}}$  has a clear minimum when the long axis of the peptide is oriented perpendicular to the main magnetic field. This anisotropy was mostly lost in the second hydration layer. The intermolecular couplings were estimated to have a smaller, and nearly isotropic contributions to the relaxation, comparable to relaxation rates in bulk water.

Since the anisotropy in relaxation rates was only seen for the intramolecular couplings in the first hydration layer of the peptide, we expect that quite severe confinement of water molecules and uniform alignment of their macromolecular environment would be needed to observe the anisotropy in experimentally determined  $T_1$ .

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Cholera Toxin 58 kDa B-Subunit assignment and dynamics;  
Triple Resonance Back-bone Experiments compared to 15N and 13C Direct Detection  
Göran Karlsson<sup>\*,1</sup>, Ulrika Brath<sup>2</sup>, Jacob Cervin<sup>1</sup>, Ulf Yrlid<sup>2</sup>, Susann Teneberg<sup>1</sup>  
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Cholera is an acute, diarrheal illness caused by infection of the intestine by the bacterium *Vibrio cholerae*. An estimated 2.9 million cases and 95,000 deaths occur each year around the world. During infection, the bacterium releases a toxin. The cholera toxin is an oligomeric complex made up of six protein subunits: one copy of the enzymatic A subunit and five copies of the receptor binding B subunit (CTB). Cholera toxin has been suggested to bind to cells in the human intestine via the GM1 ganglioside. The x-ray structures of cholera toxin in the apo form, with the bound GM1 ligand as well as the structure of the highly homologous heat-labile enterotoxin (LT) are known.

We used NMR as a tool to study the CTB protein-ligand interaction. A prerequisite is backbone and preferably side chain assignments. Some kind of protonated samples are required and we used uniformly {1H,13C,15N} CTB, expressed in *Vibrio cholera*. Direct heteronuclear (13C and 15N) 2D and 3D experiments (2D NH, 2D NCA, 2D NCO, 3D HCACO) (5 mm TXO / 800 MHz) were compared to 1H-detected standard triple resonance experiments (3mm TCI / 800 MHz) when we obtained the complete backbone and methyl group assignment of the pentameric apo-CTB as well as the GM1-CTB. Differential scanning calorimetry shows two transitions at 65° C and 82° C, respectively. At 45° C, the estimated rotational correlation time is sufficiently fast to allow observation of most of the Cb signals in backbone experiments. Chemical shift perturbation on ligand binding indicates an allosteric mechanism in accordance with previously reported ligand binding cooperativity. The backbone dynamics (15N NOE, T1 and T2 relaxation as well as T2 relaxation dispersion) was analyzed for both CTB-apo and CTB-GM1 forms in order to correlate changes in dynamics with allosteric changes on ligand binding.

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## P220

## Pore Structure Analysis of Porous Materials by PFG-NMR Diffusion Measurements

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In literature self-reporting particles, for example with an optical sensing response, are being developed [1-2]. Despite well-reported optical properties and reactions of polyionic liquid inverse opal microspheres (PIL-IOMS), the underlying structural changes in this hierarchically porous material are underexplored. In this work PIL-IOMS were used, whose water content and therefore their pore diameter, was controllable via the anion. The particles were probed by diffusion of water and polyethylene glycol molecular weight standards. PFG-NMR was used to obtain the diffusion coefficients, in combination with spin relaxation experiments. It was possible to identify different species of PEG and water in the samples, depending on the sample's pore diameter and used anion. In general, the reduction of the diffusion coefficients relative to bulk diffusion is of a minor extent, indicating a rather open pore structure. It was found that the shrinking due to water loss in the PIL-IOMS reduced their hindrance to diffusion. This implies that the gates between pores are not closed, but become wider. Samples with smaller pore diameter reduce the diffusion less, indicating a more open structure.

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### New Sample Formulations for DNP Enhanced NMR Spectroscopy at High Magnetic Fields and Elevated Temperatures

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Dynamic Nuclear Polarization (DNP) has recently established itself as a valuable technique to enhance the sensitivity of solid-state NMR spectroscopy. Over the years, sample formulations have been carefully optimized for intermediate magnetic fields (9.4 T), where DNP equipment is commercially and readily available to scientists in the field. An extensive list of polarizing agents has been developed, that can be used in either aqueous media or organic solvents. The best performing polarizing agents at 9.4 T are dinitroxides such as AMUPol<sup>[1]</sup> or TEKPol<sup>[2,3]</sup>, that lead to enhancement factors of around 250 at 100 K. Experiments are usually conducted at cryogenic temperatures, where the saturation of the electron spins is facilitated. The performance of these radicals however plummets at higher magnetic fields and/or higher temperatures. Here, two strategies are proposed to reduce these impediments.

First, we introduce a new series of water-soluble dinitroxide biradicals with a decreased linker length, dubbed TinyPols. These TinyPols yield enhancement factor of up to 90 and 30 at 18.8 and 21.1 T respectively, which surpasses AMUPOL by a factor of 2. The very good performance of TinyPol, compared to AMUPOL at high magnetic field, is attributed to the larger electron-electron couplings, in line with theoretical predictions.<sup>[4,5]</sup> Correlations between the fine structure of TinyPols and their solubility and DNP properties will be presented.

Second, we will discuss the development of new polarizing matrices that ambient temperatures. We have recently shown that by using ortho-terphenyl (OTP), enhancements could be preserved up to 243 K, the glass transition temperature ( $T_g$ ).<sup>[6]</sup> Here we report trehalose glasses as polarizing matrices, whose  $T_g$  is far above ambient temperatures at low water content. Different sample preparation protocols were investigated in combination with various polarizing agents. The DNP performance of these polarizing media will be presented along with their behavior at elevated temperatures.

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Non-stationary 2D NMR  
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 University of Warsaw

Among various NMR tools designed for the analysis of small molecules, two-dimensional (2D) experiments are particularly informative. Unfortunately, they are usually too time-consuming to serve as a “snapshot” technique for reaction monitoring. The process occurring in the sample will not stop just because spectroscopist needs more time to acquire the data. In fact, even if one can control the speed of the process, the spectrometer time is often too precious to allow the measurement of a series of conventional 2D experiments [1]. Although various techniques of fast or non-uniform sampling are helpful, they can still suffer from artifacts caused by *non-stationarity* of an FID signal.

Typical example of *non-stationarity* are resonance frequencies shifting in the course of a studied process. This leads to sensitivity and resolution drop, sometimes hindering the detection of transient products.

Here, I will discuss alternative approaches to acquiring and processing non-stationary 2D NMR signals. The examples will include:

- The application of Radon transform[2] and its variants[3] to deal with non-stationary 2D and pseudo-2D pure-shift [5] data.
- A correction for non-stationarity based on interleaved acquisition of 2D and 1D data. An interesting multi-way reaction involving dynamic transient products will be shown.

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**Phase behavior and ion dynamics of nanoconfined LiBH<sub>4</sub> in silica**  
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The increasing demand for high capacity yet safe storage of renewable energy calls for the development of all-solid-state batteries. To utilize these solid-state batteries to their full potential, a new type of electrolyte has to be developed. Nanoconfined lithium borohydride is a candidate solid-state electrolyte due to its high lithium mobility at ambient temperatures. The origin of the high lithium mobility is not fully understood. We studied nanocomposites of lithium borohydride and nanoporous silica with different pore sizes, using <sup>1</sup>H, <sup>67</sup>Li, and <sup>11</sup>B solid-state NMR at various temperatures, to get an in-depth insight in the phase behavior and ion dynamics of lithium borohydride in the silica pores.

Our results allow us to formulate a detailed dynamic model for lithium borohydride confined in silica; bulk-like LiBH<sub>4</sub> is separated from the pore walls by an amorphous, highly dynamic LiBH<sub>4</sub> fraction displaying both Li<sup>+</sup> and BH<sub>4</sub><sup>-</sup> diffusion, even at ambient temperatures. The dynamic fraction increases as a function of temperature. Li<sup>+</sup> exchange between bulk-like and ‘dynamic’ LiBH<sub>4</sub> is slow, but at elevated temperatures ≥ 90 °C, above the phase transition of the bulk-like fraction, lithium ions rapidly diffuse through both confined LiBH<sub>4</sub> fractions, and exchange between the fractions at rates approaching the MHz time scale.

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Dangling-bond recombination in amorphous silicon studied by multifrequency electrically detected magnetic resonance

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The dangling bond (db) is the most prominent electronic defect in hydrogenated amorphous silicon (a-Si:H). Acting as recombination centers for charge carriers, dbs crucially determine the electronic material properties. Understanding the mechanism of db-related recombination thus constitutes one of the most fundamental issues concerning a-Si:H.

Since a neutral db is paramagnetic, the ideal tool to study its microscopic structure is electron paramagnetic resonance (EPR) and, in particular, electrically detected magnetic resonance (EDMR) spectroscopy, which is selective to current-limiting processes involving paramagnetic species. The common picture that has emerged from this work is that recombination takes place through tunnelling of electrons trapped in disorder-induced localized band-tail states into neutral dbs, which is further promoted by hopping of holes towards the recombination site. Such a process gives rise to the characteristic EPR fingerprints of dbs and conduction- (CBT) and valence-band-tail (VBT) states.

Here, we test these hypotheses by performing room-temperature multifrequency continuous wave (cw) EDMR on a-Si:H solar cells, covering an unprecedented microwave-frequency range from 50 MHz to 263 GHz. Combined with a global spectral-fitting strategy and a statistical bootstrap analysis, this multifrequency approach allows to disentangle the overlapping resonances and determine their line-shape parameters with high accuracy.

Further insights into the recombination dynamics can be obtained from pulsed EDMR spectroscopy. Electrically detected Rabi-nutation experiments provide valuable information by probing the coupling strengths between the electron spins involved in the recombination process. By combining these experiments with numerical simulations, we show that the proposed tunnelling process cannot explain the experimental data. Instead, we propose a direct Shockley-Read-Hall (SRH) recombination mechanism, where a conduction electron (or hole) is directly captured into an excited db state, as previously found for crystalline-silicon surface dbs (so called Pb centers). Consequences of these findings for defect recombination in a-Si:H will be discussed.

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Investigations of allostery and dynamics in the E2:E3 interactions in Ube2g2 and gp78 – advancements to the full 60 kDa complex

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Ubiquitination is a pivotal, multi-step, enzymatic post-translational modification that conjugates ubiquitin into chains onto protein substrates. Ubiquitination triggers a plethora of biological signals, and our focus is on protein homeostasis achieved through degradation of misfolded, unassembled, and highly regulated proteins in the ER. The specificity and mechanism of interaction between ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) is key to the combinatorial diversity in the proteome's regulation pathway. The first prometastatic mammalian E3, gp78, functions with the E2 Ube2g2 in the process of endoplasmic reticulum associated degradation (ERAD). gp78 is a membrane protein that contains a cytosolic tail (comprising the ligase activity) consisting of three domains: the RING domain (responsible for ubiquitin transfer), the CUE domain (a ubiquitin binding domain), and the G2BR domain (a specific binding region to the cognate E2 (Ube2g2)). Previous work established the structures and interfaces of each domain of Ube2g2 and for the RING and G2BR in complex with Ube2g2 [Mol. Cell 2009, 2013; Structure 2012; EMBO J. 2013]. Allosteric and dynamic changes [Structure 2017] propose an overall structural model and mechanistic understanding. In order to advance the concerted mechanism, it is important to examine the entire molecular system consisting of the full cytoplasmic tail (gp78C) in complex with Ube2g2 and the Ub-ligated Ube2g2-Ub. Recent progress in expressing full length gp78C (30 kDa) coupled with flexible labeling protocols (15N/13C/2H and ILV-13CH3) enable triple-resonance NMR experiments for backbone assignments. Utilizing sortase ligation [PNAS 2011; J. Biomol. NMR 2011, 2015; Nature 2016], full length gp78C is segmentally labeled to enable NMR assignment and structural studies. Finally, to mimic the natural membrane environment, gp78C can be tethered onto a nanodisc membrane mimetic, where the mechanism of interactions among the three domains can be extensively studied by both NMR and biophysical techniques in the presence of Ube2g2.

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## P227

**Physicochemical Studies of Rare Earth-Element Phosphates:  
129Xenon and 31P Solid-State NMR Combined with DFT Calculations**

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Anu M. Kantola<sup>2</sup>, Shubhankar Bhattacharyya<sup>3</sup>, Oleg Antzutkin<sup>3</sup>,  
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Fifteen elements of lanthanide group plus Yttrium and Scandium are known as Rare Earth Elements (REEs). Because of their unique physicochemical properties (e.g. fluorescence, phosphorescence, ...), they have a wide range of applications in technology [1-3]. Phosphate minerals are known as potential resources for REEs. Because of low percentage of these elements in phosphate minerals, deeper study of physiochemical properties of REEs from leaching solution systems of these minerals is required [4].

In this study, for the first time the surface chemistry and porosity of rare earth phosphate compounds will be evaluated via <sup>129</sup>Xe NMR. Chemical shift of adsorbed <sup>129</sup>Xe in materials is extremely sensitive to its local environment, and therefore xenon is an ideal probe to study surface and porosity of samples [5,6]. Detailed characterization and structure elucidation of these compounds is carried out with <sup>31</sup>P solid state NMR [7]. The detailed structures of REE-phosphates are optimized and the experimental xenon and phosphorous chemical shift tensors are explained by the first principles Density Functional Theory (DFT) calculations of both periodic and cluster models of the materials [8].

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## P228

## CONFORMATIONAL PREFERENCE OF SMALL MOLECULES BY 2D NOESY SPECTROSCOPY AT SUPERCRITICAL PARAMETERS OF STATE

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2D NMR spectroscopy is an extremely useful method for analyzing the structure and dynamics of **small molecules** in liquids and in solid state. Modern 2D NMR approaches allow obtaining direct information about the geometrical configuration of small molecules, which is very important when solving problems of modern physical chemistry of fluids (e.g. micronization of drug substances, polymorphism in pharmaceutical drugs, and supercritical fluid impregnation of polymeric materials). However, the NMR method is utterly problematic when it touches upon experiments at pressures above 100 bar. This report presents the peculiarities of a high-pressure NMR experiment, as well as the effect of the NMR cell on the accurate determination of the conformer distribution at the supercritical condition. In addition, the latest results obtained using the unique high-pressure NMR spectroscopy instrumentation will be presented and discussed together with the development vistas and possibilities of the 2D NOESY method applied at the supercritical condition of state on the example of ibuprofen. Conformation distribution of ibuprofen was determined in supercritical carbon dioxide (scCO<sub>2</sub>). A good agreement was found between the NMR and MD results for ibuprofen in scCO<sub>2</sub>.

The work was funded by the Russian Science Foundation (project no. 18-73-10088).

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**Overhauser Dynamic Nuclear Polarization as an  
Enabling Technology for Fast Flow NMR Spectroscopy**

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Overhauser Dynamic Nuclear Polarization (ODNP) is employed to solve the premagnetization issue in continuous flow Nuclear Magnetic Resonance (NMR) spectroscopy. This might enable monitoring of very fast chemical reactions and processes, which is important in chemical engineering. NMR detection is performed with a mobile, medium-field NMR spectrometer (benchtop, 43 MHz proton frequency) due to many advantages of the device, e.g. low weight and adequate shim settings.

For ODNP, i.e. transfer of angular momentum between electron and nuclear spins, the use of stable radicals (as a source of unpaired electron spins) is essential. After hyperpolarization is accomplished, the radicals (e.g. TEMPO-derivatives) are disturbing further applications or measurements of the sample, as they considerably lower the longitudinal relaxation time constant ( $T_1$ ) of the nuclei and thus the lifetime of the hyperpolarization. This problem is solved in the present work by immobilizing the radicals in a fixed bed.

For a broad application of ODNP, a versatile inert radical matrix that is stable in many liquids has to be available. In the present work, a larger number of such matrices was synthesized and tested: 4-amino-TEMPO was fixed with different spacers on various solid support materials (e.g. polysaccharide-, polymethacrylate- and glass) of different pore- and particle sizes. The synthesized radical matrices were investigated in ODNP-enhanced NMR measurements in stopped and continuous flow with different solvents, compared with each other and with dissolved TEMPO. Considerable ODNP enhancements up to a factor of 80 in continuous flow with detection in the benchtop device were generated. The project aims for real-time reaction and process monitoring with single scan measurements in highly diluted systems.

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## P230

### Ultrafast Single-Scan TOCSY NMR Detection of a PHIP-Hyperpolarized Protease Inhibitor

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Olga Avrutina<sup>4</sup>, Kolmar Harald<sup>4</sup>, Gerd Buntkowsky<sup>2</sup>

<sup>1</sup>International Tomography Center SB RAS, <sup>2</sup>Eduard-Zintl Institute for Inorganic and Physical Chemistry, Technical University of Darmstadt, <sup>3</sup>International Tomography Center, <sup>4</sup>Clemens Schöpf-Institute of Chemistry and Biochemistry, Technical University of Darmstadt

Two-dimensional NMR spectroscopy is one of the most important spectroscopic tools for the investigation of biological macromolecules. However, due to the low sensitivity of NMR spectroscopy, it takes usually from several minutes to many hours to record such spectra. Here, the possibility of detecting a bioactive derivative of the sunflower trypsin inhibitor-1 (SFTI-1), a tetradecapeptide, by combining parahydrogen-induced polarization (PHIP) and ultrafast 2D NMR spectroscopy is shown. The PHIP activity of the inhibitor was achieved by labeling with O-propargyl-L-tyrosine. In 1D PHIP experiments a signal enhancement of a factor of approximately 1200 compared to standard NMR was found. Antiphase type of hyperpolarization was converted to enhanced net polarization by an adiabatic passage spin order conversion (APSOC) or by applying a so-called "out-of-phase echo". In the case of five spin system (PHIP-polarized O-allyl-L-tyrosine) the out-of-phase echo method was found to be more efficient. Strong net enhancement permits measurement of 2D NMR correlation spectra of low-concentrated SFTI-1 in less than 10 seconds, employing ultrafast single-scan 2D NMR detection. As experimental examples, ultrafast-PHIP TOCSY spectra of low concentrations of the biologically active peptide SFTI-1 labeled with the O-propargyl-L-tyrosine residue as a PHIP hyperpolarization agent were measured in a single scan. These results open new avenues towards the in situ 2D NMR spectroscopy of biomolecules, such as locally resolved 2D NMR spectroscopy, time-resolved detection of conformational changes, or monitoring of the association and dissociation kinetics of a substrate on the time scale of the hyperpolarization lifetime.

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**P231**

**Carbon nitride exfoliation by using  
Bis(trifluoromethane)sulfonimide-based ionic liquid**

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A broad range of compounds utilises carbon nitride sheets C<sub>3</sub>N<sub>4</sub> (CN), which consist of heptazine units. This structural analogue of graphene sheets appears to manifest semiconducting properties with ~2.7 eV energy gap, which allows photochemical reactions in direct sunlight. Other advantages include chemical and thermal stability and non-toxicity - advantages of CN open new applications in the area nanotechnology.[1] CN sheets can be obtained from urea, melamine or dicyandiamide through thermal condensation. Likewise graphene, much effort was made to exfoliate carbon nitride using ultrasounds, strong acids, organic solvents or thermal exfoliation methods. However, these methods lack efficiency in exfoliation and can induce defects in the structure. Accordingly, the search for high-performance exfoliation techniques of CN sheets is still pursued. Among many methods, the liquid-phase exfoliation offers an advantage, especially when simplicity, cost and scaling-up of the entire process are considered. Therefore, we have tried a new approach based on the tailored properties of ionic liquids (ILs). Ionic liquids vary from standard organic solvents in a few ways: low vapour pressure while keeping similar surface tension and the surface energy, which is essential for the efficient solvent-mediated exfoliation of the layered structures.[2] Moreover, imidazolium-based ILs have successfully been used as a dispersion medium for the graphene nanosheets, graphene oxides, reduced graphene oxides and inorganic graphene analogues.

This work aims to provide a better understanding of the interaction between bis(trifluoromethane)sulfonimide ionic liquid and carbon nitride. We focused mainly on the self-diffusion process, measured via nuclear magnetic resonance techniques. Measurements of the IL-carbon nitride systems were studied at 14 T Agilent NMR spectrometer.

#### Acknowledgements

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## P232

**Investigating the catalytic intermediate of an  
artificial heme protein by hyperfine spectroscopy**

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Metalloenzymes catalyze a broad range of chemical reactions. Their catalytic activities and compatibility with different reaction conditions depend on the detailed electronic properties of the metal active site and seemingly small modifications may cause significant changes in catalytic properties. Recently, we exploited this fine-tuning of enzymatic catalysis by incorporating a non-canonical amino acid, *N*<sub>δ</sub>-methylhistidine (NMH), as a ligand axially coordinating heme in a modified myoglobin variant called Mb\*(NMH) [1]. Replacing the native histidine by NMH enhances the catalytic activity of this heme protein for the abiological cyclopropanation reaction of styrene and ethyl diazoacetate (EDA), allowing for aerobic and non-reducing conditions, in contrast to the wild type protein.

Here, we set out to study the influence of NMH on the underlying catalytic mechanism by characterizing the reaction intermediate of EDA-bound Mb\*(NMH) [1]. This intermediate, a low-spin Fe(III) species, forms quantitatively upon addition of EDA. Using the 2D pulsed EPR experiment HYSCORE, we determined a <sup>13</sup>C-EDA hyperfine coupling indicative of a direct Fe-C coordination. We compare Mb\* with Mb\*(NMH) in order to gain insight into the differences in heme electronic structure induced by the axial NMH coordination. We find a clear shift of the rhombic *g* values along with an enhanced *g* anisotropy for Mb\*(NMH). Further investigations by uniform and non-uniform sampled HYSCORE show subtle changes of the <sup>14</sup>N hyperfine couplings that are not readily assigned. In order to achieve the spectral assignment we devised a strategy for biosynthetic isotope labeling of heme. Our approach may help unravel the intriguing influence of NMH on the electronic environment of the Fe(III)-intermediate that is key to the remarkable enhancement of catalytic activity.

**Reference:** [1] T. Hayashi, M. Tinzl, T. Mori, U. Krenzel, J. Proppe, J. Soetbeer, D. Klose, G. Jeschke, M. Reiher, D. Hilvert, *Nat. Catal.*, 2018, 1, 578-584.

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## P233

### The Role of Low-Concentrated Intermediates in Signal Amplification by Reversible Exchange (SABRE) Hyperpolarization

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SABRE[1] is a rapidly developing, parahydrogen based, nuclear spin hyperpolarization technique. Using SABRE, parahydrogen is converted into magnetization of exchanging ligands of an Ir based organometallic complex. While the role of the main SABRE complex in the formation of hyperpolarized molecules has been investigated extensively, little attention has been paid to low concentrated intermediates in this process. Recently, we have shown that an unpredicted change in the spin-order of parahydrogen from the singlet into two-spin order occurs in SABRE systems at high fields, known as singlet-triplet mixing[2,3]. This change of spin-order is not only a key step in the spontaneous formation of hyperpolarization at high fields[4], but has also been shown to limit the efficiency of polarization transfer schemes at high magnetic fields[5]. Here we show that two, low concentrated, intermediates ( $[\text{Ir}(\text{Cl})(\text{IMes})(\text{Py})_2(\text{H})_2]$  and  $[\text{Ir}(\text{CD}_3\text{OD})(\text{IMes})(\text{Py})_2(\text{H})_2]$ ), which we detect, assign and analyse at room temperature[6] using Chemical exchange saturation transfer (CEST) are a source of singlet-triplet mixing. By eliminating these intermediates and manipulating the spin-system by RF-irradiation, the nuclear spin singlet lifetime of the hydride protons was increased by more than an order of magnitude, from  $2.2 \pm 0.1$  s to  $27.2 \pm 1.2$  s, significantly limiting the singlet-triplet mixing in this system. This approach offers a promising way forward to enhance the performance of SABRE at high magnetic fields.

**References:** [1] Adams, Ralph W. et al, Science 323.5922 (2009): 1708-1711 (<https://doi.org/10.1126/science.1168877>). [2] Buntkowsky, Gerd et al. Eds. Wiley-VCH: Weinheim, 2006; Vol. 2 pp 639-682. (<https://doi.org/10.1002/9783527611546.ch21>). [3] Kiryutin, Alexey S., et al. The Journal of Physical Chemistry C 121.18 (2017): 9879-9888. (<https://doi.org/10.1021/acs.jpcc.7b01056>). [4] Knecht, Stephan, et al. Journal of Magnetic Resonance 287 (2018): 74-81. (<https://doi.org/10.1016/j.jmr.2017.12.018>). [5] Knecht, Stephan, et al. Molecular Physics (2018): 1-10. (<https://doi.org/10.1080/00268976.2018.1515999>). [6] Knecht, S. ChemRxiv, preprint 2019 (<https://doi.org/10.26434/chemrxiv.8026049>).

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STD-NMR Spectroscopy Reveals Preferential Binding of  $\beta$ -D-Glucopyranose to Glucose Sensor

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Optical biosensors that quantify specific sugars in small-scale bioreactors are important in biotechnological process development. One established design of recombinant biosensors comprises a periplasmic binding protein (PBP) for the desired metabolite flanked by two genetically encoded fluorescent proteins (FP) that form a FRET-pair. Ligand binding induces a conformational change of the PBP that results in repositioning of the two FPs, ideally accompanied by a large change in the easily detectable FRET ratio R (ratio of acceptor and donor emission intensity). Development of new biosensors with optimized binding and detection features requires production and testing of numerous candidates carrying, e.g., different linkers between the FP and PBP or mutations in the binding pocket.

We use saturation transfer difference (STD) NMR spectroscopy as an orthogonal technique to evaluate and screen biosensor candidates. Employing a previously optimized glucose/galactose sensor (Glc sensor) we explored the potential of STD NMR for sensor development and characterization. Only small amounts (1 to 20  $\mu$ M) of non-isotope-labeled, recombinant Glc sensor were needed. 1D <sup>1</sup>H STD NMR spectra of the excess ligand were recorded. The optimized pulse sequence applied interleaved acquisition of scans with on- and off-resonance saturation, subtraction was achieved by a dedicated phase cycle. STD NMR covers a large affinity range from high nanomolar to millimolar. Five different sugars were tested. Only glucose and galactose showed a pronounced binding signal in agreement with previous fluorescence data. Ten different variants of the Glc sensor were studied. STD NMR confirmed glucose binding in all cases. Surprisingly, STD NMR revealed preferential binding of the  $\beta$ -D-glucopyranose form, a feature that was not known from previous investigations of the Glc sensor with other techniques.

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## P235

## Air-Stable Polarizing Agents for Triplet Dynamic Nuclear Polarization

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The general problem of NMR and MRI is their low sensitivity. The NMR/MRI signal intensity is proportional to the nuclear spin polarization that is on the order of  $10^{-5}$  or less at a common magnetic field of NMR/MRI. On the other hand, triplet dynamic nuclear polarization (triplet-DNP), a method to enhance NMR/MRI sensitivity using photoexcited triplet electrons, has the great potential to hyperpolarize nuclear spins at room temperature. Since the first report of room-temperature triplet-DNP in 1990, pentacene has been the only and best option of triplet polarizing agent. However, the poor air stability of pentacene has severely limited the applicability of triplet-DNP. Here we report the first example of polarizing agents with significant air stability as well as high polarizing ability comparable to pentacene (H. Kouno *et al.*, *J. Phys. Chem. Lett.* **2019**, *10*, 2208-2213). The introduction of electron-withdrawing diaza-substitution to pentacene and tetracene reduces the lowest unoccupied molecular orbital level and endows much improved stability under the ambient conditions. Importantly, the diaza-substituted pentacene and tetracene offer similar, or even slightly better, <sup>1</sup>H NMR signal enhancement compared with pentacene in the prototypical triplet-DNP test using *p*-terphenyl crystals. This work removes one of the largest obstacles toward the application of triplet-DNP for the hyperpolarization of biological molecules.

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# Application of WURST Amplitude-Shaped Pulses in Multiple-Quantum MAS NMR Spectroscopy of Spin-3/2 and Spin-5/2 Nuclei

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Multiple-quantum MAS (MQMAS) NMR spectroscopy is one of the most widely used techniques in solid-state NMR for gaining site-specific resolution of overlapping central-transition lineshapes for half-integer quadrupolar nuclei.[1,2] However, a major drawback of this method is the low MQ excitation and reconversion efficiency, which has led to broad variety of MQMAS sequences with improved sensitivity.[2] Herein we demonstrate that WURST[3] amplitude-shaped pulses (WASP-N) may show intrinsically higher 3Q excitation efficiencies than conventional rectangular pulses. Also, the WURST shape parameter N enables smooth amplitude profiles, which effectively decreases pulse reflections. On this basis, we were able to increase the effective radio-frequency amplitudes in the 3QMAS experiment for spin-3/2 and spin-5/2 nuclei, eventually leading to experimental enhancement factors above 2, corresponding to a reduction in measurement time by a factor of ~4.

WASPs can be incorporated in already available pulse sequences (e.g. the three-pulse z-filter or t1-split shifted-echo) by simply replacing conventional rectangular pulses, making the application of WASPs easy and convenient. Moreover, the additional 3Q enhancement gained by a WASP excitation can be combined with other reconversion schemes like DFS.

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**P237****Bullet-DNP: Rapid sample shuttling with low heat load**Karel Kouřil<sup>\*,1</sup>, Hana Kouřilová<sup>1</sup>, Benno Meier<sup>2</sup><sup>1</sup>University of Southampton, <sup>2</sup>University of Southampton / KIT

We have recently shown that the hyperpolarized material obtained by dynamic nuclear polarization (DNP) at cryogenic temperatures can be transferred from the polarizer magnet to a target magnet in the solid form while maintaining a high level of the nuclear spin polarization - this method is named bullet-DNP [1]. This approach allows for rapid sample transfer and minimal dilution of the hyperpolarized substance. However the (warm) helium gas used for the sample shuttling can cause a substantial heating of the polarizer cryostat leading to longer cycle times and high consumption of liquid helium (one litre of liquid helium can be boiled off in a single sample transfer (shot)).

Here we present a system for heat-efficient sample ejection which reduces the heat load on the cryogenic setup below 100 J per shot. Such heat load will warm up 1 litre of superfluid helium at 1.5 K by less than 300mK and will not cause any significant boil-off. This is achieved by initially using only a small stream of gas to lift the sample out of the cryogenic region. Above the cryogenic region the sample is then picked up by the (much stronger) main stream of helium which rapidly shoots it to the target magnet.

We further present a system for rapid sample loading into the DNP setup. The system allows to maintain low temperature of the frozen sample during the loading procedure. This is required when samples are temperature-sensitive and thawing must be prevented. We use this setup for experiments on samples with UV-induced radicals.

**Reference:** [1] K. Kouřil, H. Kouřilová, S. Bartram, M. H. Levitt & B. Meier, Nature Communications, vol. 10, pp. 1733, 2019.

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## P238

## Bullet-Dynamic Nuclear Polarization and UV Radicals

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In Bullet-Dynamic Nuclear Polarization the sample is transferred from the polarizer to the target magnet as a solid. In the target magnet it is rapidly dissolved – transfer and dissolution take approximately 900\ ms. As we have previously reported we can achieve liquid-state <sup>13</sup>C polarization levels of 30\% at less than 10-fold dilution while using sub-mL solvent volumes as required for NMR spectroscopy [1]. However, the presence of radicals may lead to enhanced relaxation at low fields [2].

UV Radicals [3-7] are created by UV irradiation from precursors at low temperatures and quenched by a temperature jump above 190 K. Thus they can be generated when their presence is required for the DNP polarization step. Prior to the sample transfer they can be quenched to reduce polarization losses. The absence of radicals in the hyperpolarized solution is a further advantage.

Here we present initial results on combining these two approaches. So far 570-fold liquid-state signal enhancement (at 11.7 T) was achieved using 1-<sup>13</sup>C pyruvic acid as both a precursor and a substrate in a D<sub>2</sub>O/glycerol mixture.

**References:** [1] K. Kouřil, H Kouřilová, S Bartram, M H Levitt & B Meier, Nature Communications, vol. 10, 1733, 2019. [2] D. T. Peat et al., Phys. Chem. Chem. Phys., vol. 18, no. 28, 19173, 2016. [3] T. Kumada et al., Journal of Magnetic Resonance, vol. 201, 115, 2009. [4] T. R. Eichhorn et al., Proceedings of the National Academy of Sciences, vol. 110, 18064, 2013. [5] A. Capozzi et al., The Journal of Physical Chemistry C, vol. 119, 22632, 2015. [6] A. Capozzi et al., Nature Communications, vol. 8, 15757, 2017. [7] A. Capozzi et al., Angewandte Chemie, vol. 58, 1334-1339, 2019.

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## P239

## Effects of pyrene moiety incorporation on G-quadruplex structures

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G-quadruplexes are structurally diverse non-canonical secondary structures formed by guanine-rich nucleic acids capable of performing a broad range of cellular functions, most notably regulation of gene expression. As independent aptamer molecules, G-quadruplexes can also bind specific cytosol or extracellular proteins, which makes them promising drug targets for treatment of various diseases. G-quadruplex ligands are usually polyaromatic compounds, which interact with solvent accessible DNA nitrogen bases through aromatic stacking. They can stabilize G-quadruplexes and therefore regulate their functions. Incorporation of various chemical moieties and nucleotide analogs in G-quadruplex aptamers can be used to fine-tune desirable properties of aptamers including structural stability, high selectivity and affinity for target proteins and resistance against nuclease degradation.

In the current study we used a 15-mer DNA oligonucleotide thrombin-binding aptamer (TBA) with the sequence 5'-d[GGTTGGTGTGGTTGG]-3'. [1] In the presence of sodium or potassium cations TBA folds into antiparallel G-quadruplex with a chair-type topology, which is capable of binding and inhibiting thrombin enzyme. [2] TBA's simple and well characterized structure makes it an ideal model for studying effects of various chemical modifications on G-quadruplex structure. We showed that in the presence of potassium ions the individual replacements of T4, T9 and T13 with U<sup>py</sup> (5-(pyrene-1-yl-ethynyl)-dUMP) nucleotides stabilize G-quadruplex while retaining the TBA fold. In the case of T9 substitution, a dynamic equilibrium between unimolecular and bimolecular G-quadruplex structure was observed. The bimolecular structure is comprised of two distinct G-quadruplexes – parallel and antiparallel one. We believe that the effect of pyrene moieties on TBA could potentially be used for optimization of various therapeutic G-quadruplex based aptamers.

**References:** [1] Bock L. C., Griffin L. C., Latham J. A., Vermaas E. H., Toole J. J. *Nature*, 355, 564-6 (1992). [2] Pica A., Russo Krauss I., Merlino A., Nagattoishi S., Sugimoto N., Sica F. *FEBS J.*, 280, 6581-6588 (2013).

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### Silicophosphates – Reaction Monitoring and Studies on the Stability in Aqueous Solutions

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In different crystalline silicophosphates like  $\text{SiP}_2\text{O}_7$  or  $\text{Si}_5\text{P}_6\text{O}_{25}$  the silicon is coordinated to six oxygen atoms.[1] Of particular interest is the role of silicon pyrophosphate phases. Materials with such  $[\text{SiO}_6]$  moieties can be obtained by various reaction routes. We established a “water-free” synthetic route based on the reaction of phosphorous precursors like crystalline phosphoric acid with tetra(alkoxy)silanes in organic solvents.[2,3]

During the reaction of pyrophosphoric acid with different tetra(alkoxy)silanes solids were precipitated.[4] According to  $^{29}\text{Si}$  and  $^{31}\text{P}$  MAS NMR chemical shift data hydrogen phosphate structures are formed.[1] The products were characterized via 2D HETCOR and other solid state NMR experiments.[5] Additionally, a DFT calculated structure model was developed, which was verified by solid state NMR spectroscopy. To monitor the reaction progress and to investigate the mechanism  $^{31}\text{P}$  NMR spectroscopy measurements at different temperatures were used. The studies showed redistribution of the P-O-P linkage during the reaction.[5] Furthermore, the stability of the given silicophosphates in water and other aqueous solutions were analysed by in situ mixing of the solvent and the solid in a Wilmad-LabGlass Reaction Monitoring System.  $^{31}\text{P}$  solution NMR spectroscopy was used to investigate the formation of molecular intermediates.

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## P241

Polarization of low- $\gamma$  nuclei by PHIP transfer at high magnetic fields

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PHIP (Para-Hydrogen Induced Polarization) is one of the most useful and cost-efficient methods of creating nuclear spin hyperpolarization. Signal enhancement in the PHIP method comes from the non-equilibrium nuclear spin order of the parahydrogen molecule. PHIP does not require expensive equipment and allows amplification of NMR signals by approximately a factor of  $10^4$ , making it ideal for studying catalytic hydrogenation reactions, observing intermediate states and creating hyperpolarized contrast agents for MRI. An important application of this method is the transfer of PHIP from protons to “insensitive” magnetic heteronuclei, like  $^{13}\text{C}$ ,  $^{15}\text{N}$ , etc.

In this work we study the transfer of PHIP to  $^{13}\text{C}$  nuclei at high magnetic fields. To convert PHIP into enhanced magnetization of a heteronucleus, we implement a scheme with resonant RF-excitation at the NMR frequencies of protons and carbon. The RF-field at the proton frequency stays constant, while the field at the carbon frequency is adiabatically decreased from the maximum value to zero in order to pass through anti-crossing of spin levels in the doubly rotating reference frame.

To maximize the efficiency of transfer we study the dependence of the polarization transfer efficiency on the frequency of the  $^{13}\text{C}$  RF-field and on the amplitude of proton RF-field. A method is proposed for effective suppression of signals coming from thermally polarized nuclei. The transfer scheme is optimized using “constant adiabacity” passage upon variation of the  $^{13}\text{C}$  RF-field. A comprehensive optimization of the scheme allowed us to achieve 41,000-fold signal enhancement, which corresponds to 33.4% of  $^{13}\text{C}$  polarization.

Implementation of the scheme with double-frequency RF-excitation makes it possible to efficiently transfer PHIP to the heteronuclei, suppressing unwanted background signals. The conversion of PHIP into the magnetization of heteronuclei will possibly contribute to chemical and biological NMR studies exploiting spin hyperpolarization.

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The P-domains of calnexin/calreticulin as adapters for a subset of foldases and chaperones

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Lectin chaperones calreticulin (CRT) and calnexin (CNX) are responsible for folding secreted proteins in the endoplasmic reticulum. To perform this function, they cooperate with ER resident foldases such as protein disulfide isomerase ERp57 and peptidyl prolyl cys-trans isomerase cyclophilin B (CypB). Recently, CRT was shown to interact with putative chaperone ERp29. Here, we use NMR to show that ERp29 directly binds to the P-domain of CRT/CNX. Addition of a sub-stoichiometric amount of ERp29 led to the selective disappearance of signals from the tip of the P domain. Furthermore, titration with the C-terminal D-domain resulted in changes to the same signals that broadened in the presence of full-length ERp29. From a fit of chemical shift changes, the Kd between the ERp29 D domain and CNX P domain was measured to be better than 20 μM. We determined the structures of the ERp29 D-domain in complex with the P-domains from CRT and calmeglin (CMG), a tissue-specific calnexin homologue, to reveal the specificity determinants of the P-domains towards ERp29. Importantly, R223 of ERp29 is positioned in the heart of the binding site and makes key salt bridges with D348 of CMG (D248 of CRT). Unexpectedly, the binding involves the same tip of the CRT/CNX P-domain that was previously demonstrated to be responsible for interactions with ERp57 and CypB. In particular, mutation of D348 in CNX abrogates binding to ERp29, as it does towards ERp57 and CypB. The structures were confirmed using mutagenesis studies and NMR titrations. Striking similarity of binding features to CRT/CNX interactions with ERp57 and CypB underlines converging evolution in development of the ER folding machinery.

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## P243

**Real-time monitoring of calcium phosphate nucleation seed formation**Thomas Kress<sup>\*,1</sup>, Gregory L Olsen<sup>2</sup>, Thierry Azaïs<sup>3</sup>, Dennis Kurzbach<sup>4</sup><sup>1</sup>University of Vienna, <sup>2</sup>Unvirsity of Vienna, <sup>3</sup>Sorbonne Université, <sup>4</sup>University Vienna

Because they follow non-classical crystallization mechanisms, many biomineral nucleation processes remain the subject of intense debated [1]. It has been proposed that in many cases soluble prenucleation inorganic species can evolve through aggregation to form their mineral solid phases. To adequately understand the formation of biominerals, new analytical approaches are needed, to allow real-time access to the structural dynamics of these non-equilibrium processes. A promising route towards this is real-time NMR spectroscopy, due to recent advances in instrumentation and hyperpolarization techniques. [2]

Here, we show that prenucleation inorganic species involved in the formation and precipitation of calcium phosphates can be detected using NMR.

To allow the study of fast precipitation events, we have developed an injection system that allows fast, turbulence-free and reliable mixing of two solutions in-situ in an NMR tube. This system is compatible with D-DNP hyperpolarization techniques, which can further boost NMR signal intensities.

Combined with time-resolved and parallel-detected <sup>1</sup>H and <sup>31</sup>P NMR, we are able to detect transient calcium phosphate prenucleation species, and thereby obtain information about their size and dynamics, their precipitation rates as well as suggestive hints about the importance of hydration water in the nucleation process.

Furthermore, a proof-of-concept of SPatially ENcoded (SPEN) DOSY [3] applied to <sup>31</sup>P can yield further information in real-time to describe the kinetics of calcium phosphate nucleation.

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CELL-FREE EXPRESSION TO INVESTIGATE SITE-SPECIFIC DYNAMICS OF GPCRs

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The high relevance of G protein-coupled receptors (GPCRs) becomes apparent from their diverse distribution in human tissue and their participation in a variety of physiological functions. This makes them highly interesting molecules for basic research and also a major target for pharmaceutical drugs.

Besides the structural architecture of the seven transmembrane proteins and its interaction with ligands and intracellular binding partners, their dynamic properties represent a fundamental role for their function in signal transduction. Recently, we were able to get insight into the dynamics of peptide-binding class A GPCRs by solid-state NMR spectroscopy. It was possible to characterize the dynamics of the receptors in the presence and in the absence of different ligands and embedded in different membranes [1,2,3]. The order parameters that were derived from the Dipshift experiments gave a measure for the dynamic properties of the receptors. However, they were determined for the fully <sup>13</sup>C labeled neuropeptide Y and growth hormone secretagogue receptors, and thus represent average values of all residues. They did not resolve any specific properties of distinct sites. Here we show how to use cell-free expression for selective isotopic labelling of GPCRs. The receptors are expressed in the precipitated form and are reconstituted in lipid bicelles. The resulting NMR spectra show a reduced spectral complexity and allow the investigation of specific amino acid sites, e.g. with respect to receptor activation.

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## P245

### Instruments and methods for NMR PRE relaxivity studies up to 1.4 GHz/33 T of MRI contrast agents based on high-spin lanthanide coordination clusters

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In Nuclear Magnetic Resonance (NMR), magnetic field dependent phenomena attract considerable interest: Paramagnetic relaxation enhancement (PRE) is theoretically, experimentally and from the application point of view an important research area, always looking for new relaxation agents with specific properties especially at high magnetic fields and for improved understanding of the PRE mechanisms. Contrast enhancement by PRE in magnetic resonance imaging (MRI) for medicine and material science results in an improved contrast in an image. Recently, paramagnetic coordination clusters, shortly abbreviated as  $\text{Ln}_{30}\text{Co}_8$ , have been synthesized. They are based on spin-coupled 3d and 4f rare earth paramagnetic centers and exhibit highly interesting PRE properties. An essential point is that the molecular size of the clusters closes the gap between small molecules as nitrides and the larger (super-) paramagnetic nanoparticles applied for example in the context of hyperthermia. The fundamental mechanisms for the observed PRE effect are still under discussion.

The investigation of the PRE properties of these compounds requires - additionally to the conventional NMR fields - ultra-high magnetic fields above the actual limit of superconducting magnets of 23.5 T. These fields are up to now only available at a few specialized high field facilities liking the LNCMI operating resistive magnets up to 37 T at 24 MW electrical power. However, as these magnets exhibit limited stability and homogeneity, their NMR applications are so far mostly limited to solid state physics. In our contribution, we demonstrate the feasibility of PRE studies on liquids in such magnets despite their limitations. We will particularly focus on instruments (broadband tunable NMR probes) and methods (single scan NMR, deconvolution techniques, IR and CPMG sequences) that allow efficient PRE studies from 800 MHz to 1.4 GHz as shown by studies involving new ultrahigh-spin clusters.

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## P246

**Light dynamics of retinal disease relevant G90D mutant of bovine rhodopsin**

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Rhodopsin belongs to the largest GPCRs (G-protein coupled receptor) membrane protein family in the human genome. Light absorption by rhodopsin is the initiation point for photoactivated signaling cascade in retinal rods. Point mutations in the protein sequence cause the disruption of the phototransduction resulting in the impaired visual cycle. G90 is a unique position, depending on the nature of the mutated amino acid it can either lead to human night blindness diseases retinitis pigmentosa (PR) or congenital stationary night blindness (CSNB). G90D is a constitutively active mutation, which affects the retinal binding position, resulting in the constant basal activity of the protein. Although, the crystal structure of light active conformation of G90D mutant exists, the low resolution of the retinal binding pocket does not provide information about the exact retinal conformation at the atomic level. Moreover, the lack of the crystals in the dark (ground) state reflects the crucial impact of the mutant, leaving the molecular mechanism unclear.

In order to fulfil the lacking structural gaps concerning the exact retinal conformation and mutation-induced changes in the binding pocket in the dark and light active state of G90D mutant, we performed a combination of liquid and solid state NMR spectroscopic experiments. The analysis was accomplished by the comparison with the wild type data. The liquid state NMR showed 2 conformations of the mutant, referring to the preactive state in the dark. This structural heterogeneity was confirmed by DNP-enhanced solid state NMR experiments. Additional ssNMR experiments reported that the retinal is in the 11-cis conformation and is covalently bound to the protein. Eventually, kinetics of the light activation process of the CSNB associated G90D mutant performed by flash photolysis experiments showed a unique photo cycle.

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P247

**An Integrative EPR and EM Approach to unveil the mechanics of a Tc toxin**  
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Tc toxins are 1.7 MDa protein complexes that are found in insect- and human-pathogenic bacteria. After endocytosis, Tc penetrates the membrane of the host's cells and translocates a deadly enzyme into the cytosol. The complex consists of three subunits: the 1.4 MDa TcA pentamer, which mediates target cell association, membrane insertion and toxin translocation, and two smaller subunits, TcB and TcC, which form a 250 kDa cocoon that encapsulates the toxic enzyme. TcA contains a ~40 residue long, stretched linker between the outer shell and the toxin translocation channel [1], which contracts upon the pH-triggered shell opening of TcA and drives membrane permeation [2]. However, the exact sequence and the kinetics of the prepore-to-pore transition are unknown. Here we present DEER and cw EPR kinetics, corroborated by ODNP data, on two crucial steps of pore formation: shell opening and linker contraction of TcA. Both steps are triggered by basic pH and proceed with half-lives in the 10 hours range, with no indications of enrichment of an open, non-contracted intermediate. Moreover, we found that mutations in the TcA subunit, which prevent membrane insertion, caused an acceleration of pore formation by at least three orders of magnitudes and modified the pH-dependency of the reaction such that pores are also formed at acidic pH values in vitro. A cryo-EM structure of the membrane-integration-deficient TcA variant shows a slight opening of the outer shell, which indicates that a structural rearrangement that might be caused by receptor binding in vivo, has to precede the prepore-to-pore transition to activate Tc.

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## P248

# Multifrequency EPR, SQUID, and DFT Study of Cupric Ions and Their Magnetic Coupling in the Metal–Organic Framework Compound $\infty^3$ [Cu(prz–trz–ia)]

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Metal-organic frameworks (MOF) are attractive materials for gas storage and separation, catalysis, and sensing. Their high structural diversity and functionality are controlled by the use of a variety of organic linkers and metal ions. The possibility to incorporate paramagnetic metal ions into the framework, in particular, paves the way for future use of MOF in the field of molecular magnets and magnetic sensors. However, utilizing magnetic MOFs in future applications requires a fundamental understanding of their magnetic properties and the involved magnetic coupling phenomena among the magnetic framework ions.

EPR spectroscopy is a powerful tool to explore on one hand the magnetic properties of framework ions and on the other hand the microscopic nature of magnetic defects and extraframework species. In this work, we employ multifrequency EPR spectroscopy at 9 GHz, 34 GHz, and 320 GHz, together with magnetic susceptibility measurements and DFT calculations, to characterize the magnetic properties of these cupric ion pairs in such dinuclear triazole-bound metal ion units of the pure copper  $\infty^3$  [Cu(prz–trz–ia)] MOF.

In the case of the presence of magnetic framework ions together with paramagnetic defects and extraframework species, a multifrequency EPR approach is of particular benefit as it allows for straightforward disentanglement of the complicated superimposed powder spectra. [1,2]

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P249

Unraveling the heme regulatory motifs in the pro-inflammatory IL-36 cytokine family

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The IL-36 family ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) members belong to the interleukin-1 superfamily and are involved in the regulation of immune and inflammatory responses. IL-36 $\alpha$ , IL-36 $\beta$ , IL-36 $\gamma$  are suggested to play a role in psoriasis, rheumatoid arthritis and recently their involvement in pregnancy complications was shown. These members have pro-inflammatory activity and control the induction of other inflammatory mediators like chemokines, cytokines (IL-6, IL-8) and antimicrobial peptides. All members of the IL-36 family bind to the same IL36R receptor and activate downstream NF-kappa-B and MAP kinase pathways during pro-inflammatory response in target cells. Our studies demonstrate the regulatory role of labile heme in the regulation of IL-36 family members. The biophysical studies performed on all three members revealed the binding of heme through different heme-regulatory motifs, coordination geometry and also uncovered novel heme binding amino acids. Docking of IL-36 $\alpha$  on its receptor justified the need of processing the disordered N-terminal pro-peptide of IL-36 ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) for their full activation. In vivo studies showed the heme mediated negative regulation of IL-36 signaling pathway where binding of heme to IL-36 cytokines leads to decreased expression of proinflammatory IL-6 and IL-8 cytokines. This study adds three new proteins in literature which are regulated by heme and can help in understanding of IL-36 mediated pathophysiological diseases.

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## P250

**Dimerization of DNA G-quadruplexes: an EPR investigation**Yury Kutin<sup>\*,1</sup>, Lukas Stratmann<sup>1</sup>, Guido Clever<sup>2</sup>, Müge Kasanmascheff<sup>1</sup><sup>1</sup>TU Dortmund, <sup>2</sup>TU Dortmund

G-quadruplexes are DNA secondary structures containing stacked guanine tetrads stabilized by central cations. They are formed by self-assembly of guanine-rich oligonucleotides by Hoogsteen base pairing. Studies have shown that G-quadruplexes form in vivo in oncogene regulatory regions and at telomeric ends of chromosomes, thus shortening cancer cell lifetimes [1].

Many G-quadruplex species are known to form higher-order structures like dimers, which are believed to play a crucial role in G-quadruplexes biological activity. This makes the understanding of the structure and formation of these structures an important goal, where the EPR spectroscopy can make a substantial contribution. Recently, rigid Cu<sup>II</sup>-based spin labels were successfully incorporated into biomimetic tetramolecular DNA G-quadruplexes [2]. The potential of the DNA-bound metal spin labels for distance measurements was demonstrated by intramolecular pulsed EPR experiments on Cu<sup>II</sup> ions attached at 3' and 5' ends of G-quadruplex monomers [3].

In the present work we use the orientation selective PELDOR (or DEER) and RIDME techniques to derive Cu<sup>II</sup>-Cu<sup>II</sup> distances in various forms of G-quadruplex dimers to gain insight into the topology of these higher-order structures. With the Cu<sup>II</sup>-based spin labels attached at either 3' or 5' ends of several G-quadruplex species, the dimerization at the opposite ends was studied.

EPR was also used to probe the dimerization of G-quadruplexes mediated by organic molecules. This provided information on both the resulting structures and the efficiency of the potential dimerization pathways. Interaction of G-quadruplexes with a variety of transition metal complexes makes EPR the method of choice for distance measurements within G-quadruplex-metal complex adducts, providing valuable information on binding modes.

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## P251

### Studying the photocycle intermediates of Cph1 from *Synechocystis* sp. PCC6803 by low-temperature solid-state NMR

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Phytochromes are red/far-red photoreceptors, containing a covalently linked linear tetrapyrrole cofactor. In canonical phytochromes such as Cph1 from *Synechocystis* sp. PCC 6803 the N-terminal photosensory module comprises a PAS (Period-Arnt-Single-minded domain) – GAF (GTPase-Adenyl cyclase-FhlA domain) – PHY (phytochrome-specific domain) construct followed by a histidine kinase function.

In the last decade several investigations on the reversible photocycle of Cph1 were performed with the result, that the Pfr is characterized by strong chromophore-protein interactions and defined protonic and charge distributions. By contrast, the Pr-state Cph1 assembled with phycocyanobilin (PCB) shows at least two isoforms, Pr-I and Pr-II, and ‘softer’ chromophore-protein interactions.[1] Furthermore, the intermediate states Lumi-F and Meta-F of the photochemically induced backward-reaction from Pfr to Pr were characterized in terms of protein-chromophore interactions. The solid-state NMR data indicates that break and formation of hydrogen bonds between the chromophore and specific amino acid residues are occurring. [2,3]

However, up to date it was not possible to freeze-trap the intermediate states Lumi-R and Meta-R of the photochemically induced conversion from Pr to Pfr. Therefore, the photosensory module Cph1Δ2 assembled with an *u*-<sup>13</sup>C/<sup>15</sup>N – labelled PCB will be investigated with solid-state MAS NMR techniques under illumination. For the complete <sup>13</sup>C and <sup>15</sup>N assignments of these two intermediates, a series of homo- and heteronuclear correlation experiments will be applied.

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## P252

### Interaction between the human prion protein and amyloid beta oligomers as seen by solid-state MAS-NMR

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Both prion protein (PrP) and amyloid beta (A $\beta$ ) play important roles in the pathogenesis of neurodegenerative disorders as they are able to misfold and form toxic assemblies. A $\beta$  oligomers have been identified as neurotoxic agents relevant for the pathogenesis of Alzheimer's disease. Recently, it was shown that human PrP (hPrP) is a receptor for oligomeric A $\beta$ (1-42)[1,2]. We previously biochemically confirmed and further investigated this interaction<sup>3</sup>.

In the present study, we investigated the interaction between hPrP and A $\beta$  oligomers by solid-state MAS-NMR spectroscopy using different samples of A $\beta$  oligomers complexed by recombinant hPrP, where either hPrP or A $\beta$  was uniformly <sup>13</sup>C/<sup>15</sup>N isotope labelled[3]. Although a site-specific resonance assignment was impossible due to a high degree of resonance overlap and poor signal dispersion, we could draw following conclusions:

The N-terminal part of hPrP is immobile and therefore visible in dipolar spectra. Chemical shifts for most of the amino-terminal residues are characteristic for absence of a defined secondary structure. However, some Ala and Val residues around position 115 may have underwent a transition to an  $\alpha$ -helical conformation, as observed already before[4]. Most of the well-defined C-terminal part is immobile. A comparison of the chemical shifts of residues within the carboxy-terminal part of A $\beta$  oligomer bound hPrP with those of free hPrP in solution indicates a loss in secondary structure in the last two  $\alpha$ -helices.

For A $\beta$ (1-42) oligomers, secondary chemical shifts point towards a substantial  $\beta$ -sheet content. Conformational heterogeneity was observed for restricted regions in the amino acid sequence. Nevertheless, a partial resonance assignment was possible.

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Insight into the export cycle of the multidrug  
ATP-Binding Cassette (ABC) transporter BmrA by solid-state NMR

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We here analysed using solid-state NMR different conformational states of the ATP-Binding Cassette (ABC) transporter BmrA reconstituted in lipids by spectral fingerprinting.

ABC transporters convert the energy gained from ATP hydrolysis to make a trans-bilayer movement of substrate. ABC transporters are present in all kingdoms of life, and notably those which transport a large variety of substrates contribute to drug resistance by pumping therapeutic molecules out of cells. The detailed mechanism these proteins use for their function still remains obscure.

Solid-state NMR allows in principle structural investigations of membrane proteins embedded in a lipid bilayer. Still, the large size of ABC transporters needs specific approaches to assign resonances, and we here implemented selective amino acids labelling combined with paramagnetic relaxation enhancements. We analysed the spectral fingerprints of the ABC transporter BmrA from *Bacillus subtilis* (120 kDa) in different nucleotide-bound forms, and using mutants mimicking different conformational states of the transporter, to collect NMR-snapshots of the export cycle. In addition to <sup>15</sup>N/<sup>13</sup>C, we also used <sup>31</sup>P to analyse binding of ATP and its analogues such as ADP-vanadate, which allowed to gain information from the point of view of the substrate.

Using these approaches, we could show that i) the simple binding of two ATP molecules is sufficient to induce the closed state[1]; ii) the structural changes of the nucleotide binding domain transit via stiffening of a subset of residues dependent on a specific motif (X-loop) in order to export a drug[1]; iii) ATP is sequentially hydrolysed, allowing the ADP-release and the ADP/ATP exchange after the hydrolysis of the first ATP.

**References:** [1] Lacabanne D, Orelle C, Lecoq L, Kunert K, Chuilon C, Wiegand T, Ravaut S, Jault J.M., Meier B.H. and Böckmann A. Flexible-to-rigid transition is central for substrate transport in the ABC transporter BmrA from *Bacillus subtilis*. *Communications Biology*. 2019 April.

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## P254

## Atomic Description of Catalytic Sites Using Advanced Solid-State NMR Methods

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ENSCL, UCCS, Institut Chevreul, Lille, France / Institut Univ. de France, Paris, France

The development of improved heterogeneous catalysts can be undertaken in a rational way by a better understanding of their atomic-level structures. Solid-state NMR spectroscopy is very well suited to the study of heterogeneous catalysts because it can give information on the local structure. However, the lack of sensitivity and resolution limits the characterization of surface sites, notably when they are occupied by isotopes with low natural abundance, slow longitudinal relaxation (<sup>29</sup>Si, etc.) or subject to large anisotropic interactions, such as chemical shift anisotropy, CSA (<sup>195</sup>Pt, etc.) or quadrupolar interaction (<sup>27</sup>Al, <sup>47,49</sup>Ti, etc.).[1]

Recently we have developed and applied innovative NMR techniques to circumvent this issue and obtain new insights into the structure of heterogeneous catalysts. We have demonstrated that the sensitivity and the quantification of 1D <sup>29</sup>Si NMR experiments on functionalized mesoporous silica can be improved using UDEFT (Uniform Driven Equilibrium Fourier Transform) technique.[2] Furthermore, various schemes to excite <sup>195</sup>Pt nuclei subject to CSA of 1000s of ppm have been explored and used to correlate <sup>1</sup>H and <sup>195</sup>Pt signals of Pt complexes.[3]

Besides spin-1/2 isotopes, we have also introduced efficient and robust techniques to probe connectivities and proximities involving quadrupolar nuclei with  $I \geq 1$ . Correlation experiments involving <sup>27</sup>Al nuclei at high magnetic field have notably been employed to probe the structure of Brønsted acid sites at the surface of amorphous silica alumina and have demonstrated, for the first time, the presence of Brønsted acid sites based on AlO<sub>5</sub> sites, and their synergy with AlO<sub>4</sub> sites in these widely used solid acid catalysts.[4]

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## P255

**Integral membrane proteins studied by proton-detected solid-state NMR at fast MAS frequencies (60 – 110 kHz): optimizing linewidth and pulse sequences**Nils-Alexander Lakomek<sup>\*1</sup>, Vlastimil Jirasko<sup>2</sup>,Marie-Laure Fogeron<sup>3</sup>, Matthias Ernst<sup>2</sup>, Beat H. Meier<sup>4</sup>, Anja Böckmann<sup>5</sup><sup>1</sup>ETH Zürich, Physical Chemistry, <sup>2</sup>ETH Zurich, Physical Chemistry,<sup>3</sup>Institut de Biologie et Chimie des Proteines, UMR 5086 CNRS, Université de Lyon 1,<sup>4</sup>ETH Zurich, <sup>5</sup>Molecular Microbiology and Structural Biochemistry - CNRS

<sup>1</sup>H-detected fast magic-angle-spinning (MAS) solid-state NMR is emerging as an important analysis method for proteins of which only sub-milligram amounts are available. This makes also integral membrane proteins which can be expressed at low yields only accessible for a structural investigation by solid-state NMR.

On the example of the Hepatitis C viral membrane protein NS4B (Non-Structural membrane protein 4B), which is predicted to be an  $\alpha$ -helical integral membrane protein, we have tested the spectral quality of preparations reconstituted into liposomes of various lipid composition. We find a strong dependence of the spectral resolution on the lipid composition that seems to be connected to the lipid phase transition temperature, with spectra recorded above the lipid phase transition temperature yielding high resolution and those recorded below the lipid phase transition temperature showing broad signals. However, for all lipids investigated, the bulk <sup>1</sup>H and <sup>15</sup>N transverse relaxation rate constants are very similar, pointing to inhomogeneous line broadening as the determining factor for spectral resolution.

Nevertheless, the short transverse relaxation times of NS4B (bulk <sup>1</sup>H R<sub>2</sub>' rate constants ~ 5 ms) put substantial challenges for higher-dimensional spectral backbone assignment experiments. We employ CP (cross-polarization) and DREAM (dipolar recoupling enhanced by amplitude modulation) for coherence transfer. Based on the <sup>1</sup>H, <sup>15</sup>N and <sup>13</sup>C relaxation rate constants which are effective during the different evolution times and coherence-transfer periods, we have optimized HN detected three-dimensional backbone assignment experiments. The high MAS frequencies of 100 kHz needed for resolution require relatively high RF amplitudes of the inverse nuclei during the CP or DREAM mixing periods, leading to non-selective transfers. We have identified several unwanted magnetization-loss mechanisms and have optimized the respective backbone assignment experiments accordingly.

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NMR screening of RNA secondary structure and binding of c-di-GMP (3',5') to the Cd1-Riboswitch  
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Riboswitches are a class of RNA regulatory elements that are located in the 5'-UTR (untranslated region) of genes. Upon binding of small molecules, riboswitches undergo a structural rearrangement preventing the formation of terminator or antiterminator elements regulating the transcription of the downstream genes. The secondary messengers c-di-GMP (3',5') enables terminator formation off the Cd1-riboswitch. In *Clostridium difficile* this "off" signal leads to suppression off flagella protein expression and promotes aggregation of the bacillus. As the flagella are important factors in virulence a deeper understanding of the structural and kinetic features of the Cd1-riboswitch is vital [2].

Utilizing a single nucleotide extension strategy [3] combined with <sup>15</sup>N labeled RNA and 2D NMR experiments like SF-HMQC and NOESY we investigate the formation of the aptamer domain minimal motive and the P1-Helix. Our investigations on the aptamer domain minimal motive could shows that the binding capability for c-di-GMP is already present at length of 83 nucleotides. Showing that the minimum motive of the Aptamer domain is much shorter than currently predicted in literature [4]. With this results and additional conformation by mutation of key nucleotides we are starting to form a model of the Cd1-riboswitchs structural functionality based on ore studies and recent literature [5].

**References:** [1] Sudarsan et al. (2008) Science. 321, 411–414. [2] Purcell et al. (2012) JB. 194, 3307–3316. [3] Helmling et al. (2015) et al. J. Biomol. NMR 63, 67–76. [4] Purcell et al. (2016) FEMS 40, 753-773. [5] Helmling et al. (2018) Nat. Commun. (9), 944.

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**P257**

**Rotational Dynamics of Polyacid Chain Segments in  
Polyelectrolyte Multilayers Studied by Spin-Label EPR Spectroscopy**

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A nitroxide spin label has been covalently attached to the weak polyelectrolyte poly(ethylene-alt-maleic acid) (P(E-alt-MA)) to study the rotational dynamics of the polyacid backbone in swollen polyelectrolyte multilayers (PEMs) formed by P(E-alt-MA) and the oppositely charged weak polycation poly(allylamine hydrochloride) (PAH) by continuous wave (CW) electron paramagnetic resonance (EPR) spectroscopy.

PEMs are interesting objects of fundamental research but also of high practical relevance. Multilayers can be prepared on planar surfaces or colloidal templates by the layer-by-layer technique. Sensor materials, functional coatings, and selective membranes are discussed and explored as applications of PEMs.

In a first series of experiments, the growth of PEM films on the inner surface of glass capillaries has been monitored by quantitative EPR using the SL-P(E-alt-MA) for the preparation of every polyanion layer. A parabolic growth of the multilayer films with up to 16 layers was found. In a second series of experiments, the strength of the spin-label technique was exploited, i.e., the SL-P(E-alt-MA) has been used as a reporter molecule for the study of the dynamics in the PEMs. Multilayers of PAH/P(E-alt-MA) with 16 and 17 layers, respectively, were prepared where the SL-P(E-alt-MA) has been selectively placed in a single layer. The segmental rotational mobility of the spin-labeled polyacid and the internal rotation of the spin label have been determined by simulation of the line shape of experimental CW EPR spectra. A pronounced odd-even effect has been observed, i.e., the rotational dynamics of the P(E-alt-MA) backbone in the PEMs is influenced by the chemical nature of the polyelectrolyte in the terminating layer. It is largest for the layer at or close to the surface and reduced for layers in the bulk of the PEMs. As our results demonstrate, spin-label EPR is a powerful tool for the investigation of the polymer chain dynamics in PEMs.

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Detection of Conformational Dynamics of the Y2 Receptor by Site-Specific Spin Labeling using NMR and EPR measurements

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Site-directed spin labeling (SDSL) is a powerful technique to monitor highly dynamical conformational states of membrane proteins in both NMR and EPR. In this work, the nitroxide spin label MTSL was introduced to single cysteines in the G protein coupled neuropeptide Y2 receptor (Y2R) that has been associated with a number of key physiological functions, including the regulation of appetite and circadian rhythm. So far, nine Y2R mutants containing either artificial or reintroduced single cysteine modifications were expressed successfully in *E.coli* fermentation with a yield of 5-10 mg purified protein per liter expression medium and functionally reconstituted into a phospholipid bicelle environment. Complete MTSL coupling to the free cysteines was confirmed by a CPM fluorescence assay. First continuous wave EPR measurements with spin labels coupled to different extracellular (A202<sup>45,49</sup>C and L300<sup>7,29</sup>C) and intracellular (C151<sup>3,53</sup> and R262<sup>6,29</sup>C) positions, have shown multi-component powder EPR spectra comprising both mobile and immobilized populations, indicating multiple dynamic conformational states of the receptor. Further, a solid-state MAS NMR approach was established to measure distance dependent paramagnetic relaxation enhancement (PRE) effects between the MTSL labeled receptor and its natural ligand, the neuropeptide Y (NPY), site-specifically labeled with <sup>13</sup>C isotopes. Unlabeled receptor was used as reference to compare longitudinal relaxation rates (R1). First measurements were performed with the Y2R-A202<sup>45,49</sup>C mutant, which contain the spin label in close proximity to the ligand binding pocket. An enhanced relaxation rate was observed for the I28-C<sub>delta</sub> site in the NPY from 1.3 s<sup>-1</sup> to 1.9 s<sup>-1</sup>, but not for residue A14, which is in good agreement with our structural model (Kaiser et. al, 2015). Measurements with differently labeled NPY variants, other Y2R mutants, and the complex with intracellularly bound arrestin-3 will be the next steps in order to investigate structural changes upon Y2R activation.

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**Proton-detection >100 kHz MAS NMR for the study of viral capsids**

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Alexander Malär<sup>2</sup>, Morgane Callon<sup>2</sup>, Marie Dujardin<sup>1</sup>, Michael Nassal<sup>3</sup>,

Marie-Laure Fogeron<sup>1</sup>, Beat H. Meier<sup>4</sup>, Anja Böckmann<sup>1</sup>

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We here use the hepatitis B virus capsid made from the core protein as a model to establish approaches allowing to investigate conformation, assembly and interactions in large protein complexes. We have previously demonstrated that HBV capsids produced in *E. coli* can be studied by solid-state NMR at high resolution and sensitivity using <sup>13</sup>C-detection experiments[1,2].

We now use <sup>1</sup>H-detected solid-state NMR spectra recorded at 100 kHz magic-angle spinning (MAS) on few hundreds micrograms of fully protonated capsids to assign the H<sup>N</sup> resonances. We compare sensitivity and assignment completeness to previously obtained <sup>13</sup>C-and <sup>15</sup>N assignments. We also demonstrate that deuteration and H<sup>N</sup> back-protonation improves the proton linewidths, even at 100 kHz MAS, by a factor of 1.5. Yet, several H<sup>N</sup> protons cannot be back-exchanged due to solvent inaccessibility, which results in the loss of 15 % of the amides in the spectra.

<sup>1</sup>H detection unfolds its full potential for proteins available only in small quantities. We show that the core protein synthesized in wheat-germ cell-free reactions[3] autoassembles, and that protonated and deuterated capsids allow to obtain high-quality 2D and 3D <sup>1</sup>H-detected 110 kHz MAS spectra. It is of interest that the cell-free system, in contrast to bacterial expression, allows to obtain 100 % protonation of H<sup>N</sup>. In addition, we demonstrate that antivirals targeting capsid assembly can be added to the cell-free reaction to assess assembly modulation on exit from the ribosome, opening the way to study the structural bases of these events.

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Interfacial Structure and Ion Transport Motion Influenced by Surface Chemistry under Nano-Confinement

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The confinement of materials in nanoporous media greatly affects their thermal and dynamic properties such as melting point, glass transition temperatures etc. Size of the pores and the host-guest interaction at the interfaces play an import role in the confinement effect. Here, we study the effect of the confinement, in particular, surface chemistry on the interfacial structure and the transport behavior of the liquid inside porous system by solid state NMR and PFG NMR methods.

Ionic liquid (IL) based electrolytic solution (LiTFSI in Pyr13TFSI; 1-methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide = Pyr13TFSI) confined in nanoporous polymer hosts were investigated with respect to the pore size/porosity and the surface chemistry of the polymer host. As host material, mesoporous resorcinol-formaldehyde (RF) polymer monoliths with three-dimensionally connected pore structure were prepared. RF polymer monoliths are rich in hydroxyl and ether groups, allowing us to study the influence of surface polarity on the ionic motion of confined electrolyte. Mass transport motions are studied by impedance spectroscopy and PFG NMR. Good ionic conductivity, which is dependent on the porosity (i.e., pore volume) of the confining host material, is obtained. Further pulsed field gradient (PFG) NMR experiments revealed that diffusion coefficient of Pyr13 cation becomes smaller than that of TFSI anion inside RF pores, which is contradictory to the bulk IL system. This change in the ionic motion is due to electrostatic attraction between the pore walls and Pyr13 cations, resulting in layer structure composed of cation-rich layer adsorbed at the pore wall surface and a anion-enriched bulk-like layer at the pore center. Our study demonstrates that the cationic vs. anionic transport motion can be selectively controlled by optimizing surface chemistry of the porous framework.

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MRI characterisation of a microscale parallel bioreactor

Clo   Legrand<sup>\*1</sup>, Mick Mantle<sup>1</sup>, Matthew Cheeks<sup>2</sup>

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A key goal in biopharmaceutical development is to reduce costs and accelerate cell culture process development. Disposable single use bioreactors have aided the rapid development and manufacturing of drug products, but to further reduce costs and timescales, scale-down bioreactor systems have become popular. The ambr<sup>TM</sup> system has gained much attention in recent years through the scalability of cell growth characteristics. The ambr<sup>TM</sup> shape is however dissimilar to large scale bioreactor systems. Using reactors with similar configurations allows for several key assumptions to be carried over during scale up. Additionally, it allows for similar fluid dynamics and flow properties to be achieved, which will influence both mixing and mass transfer. Investigations into the hydrodynamics of the ambr<sup>TM</sup> system are therefore of interest. In this study we present the first use of magnetic resonance imaging (MRI) velocimetry to study the flow field inside the ambr<sup>TM</sup> vessel.

A single 15 mL ambr<sup>TM</sup> vessel was placed inside a 200 MHz spectrometer. MRI experiments were performed using conventional 2D velocity imaging techniques. The effects of varying working volume, impeller speed, impeller flow direction and suspensions viscosity were investigated.

MRI techniques provide a non-invasive method for the highly accurate visualization of hydrodynamics. Quantitative 3D flow fields were obtained for the ambr<sup>TM</sup>, validating earlier CFD predictions (Nienow et al., 2013, Biochemical Engineering Journal, 76, 25–36). The flow regime inside the vessel is found to be transitional rather than laminar. Regions of high velocity are only obtained within the swept volume of the impeller. The proximity of the walls and the sparger effectively act as baffles, creating a flow pattern which is more typical of larger baffled reactors. Volume and flow direction are found to have a greater influence on the flow pattern than impeller speed, with dead zones and surface effects seen at 18 vol% solids.

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# Revisiting the Isotopic Analysis of Aroma molecules by Deuterium NMR in Natural Abundance in Lyotropic Liquid Crystals

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The evaluation of the natural isotopic profile of (bio)-compounds is an ongoing challenge for the authentication/determination of their origins or to understand their metabolic/synthetic pathways. In the field, the 2H SNIF-NMR® protocol has been developed around the quantitative 2H-[1H] 1D-NMR spectroscopy at natural abundance level (NAD NMR) in isotropic solvents to study the Site-specific Natural Isotope Fractionation of molecules [1]. Despite the power of this method, the analysis of economical-interest molecules such as flavour molecules used in the food industry (Vanillin, Frambinone,...) remains incomplete due to peak overlaps or spectral equivalence of some 2H sites.

For instance, the (2H/1H) isotopic profile of aromatic core of vanillin measured is incomplete due to a fortuitous isochrony of two 2H sites [2]. In the case of frambinone, the enantiotopic positions of methylene groups cannot be discriminated in achiral solvents, preventing the study of the isotopic fractionation (2H/1H) of enantiotopic positions [3].

In this work, we show how the use polypeptide-based lyotropic liquid crystals (PBLG) as chiral aligning media and NAD 2D-NMR experiments can be combined to overcome these difficulties [4,5]. Thus, the anisotropic NAD NMR (ANAD NMR) gives access to all order-sensitive NMR interactions such as residual quadrupolar coupling, 2H-RQC, specific to spin  $I > 1/2$  [4], while the tilted  $Q$ -resolved-type 2D-NMR experiments allow the separation of deuterium anisotropic chemical shifts and quadrupolar splittings [4,5].

The relevant experimental conditions for optimal, reliable quantitative measurements in ANAD 2D-NMR, and the most important factors impacting the quality of spectra are examined.

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## P263

## NMR studies towards a better understanding of catalytic processes

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Catalytic processes are ubiquitous appearing in nature and are also important for chemical industry. Understanding these can help to develop better and more efficient catalytic systems. NMR has proven to be a very powerful tool to investigate these processes by detection of intermediate species or by studying reaction kinetics. Recently, we have used PHIP NMR methodologies to observe ruthenium carbenes which were formed by an unusual gem-hydrogenation mechanism[1] and even could follow their fate to understand their role in the catalytic cycle, which helped to discover new reactivities[2] and enabled the determination of reactivity trends.[3]

For Brønsted acid catalysed reactions NMR allowed the observation and characterisation unexpected covalently bound catalyst intermediates,[4] to improve mechanistic understanding by observing dynamic equilibria between reaction intermediates[5] and the support of mechanistic proposals by measuring a <sup>13</sup>C kinetic isotope effect (KIE) at natural abundance.[6]

We will present some of our more recent studies, where we have used NMR techniques to provide deeper insights into underlying chemical mechanisms.

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## P264

## Disentanglement of inner sphere and outer sphere mechanisms in Overhauser DNP

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Within the last 15 years dynamic nuclear polarization (DNP) has emerged as an efficient tool to boost the sensitivity of various magnetic resonance techniques[1]. Dissolution DNP has proven its applicability in magnetic resonance imaging (MRI) as well as solid-state DNP in nuclear magnetic resonance (NMR)[1]. However, liquid state NMR DNP is still in its infancy. Even though the general possibility of large signal enhancements at large magnetic fields has been demonstrated, many significant features of the polarization transfer mechanism are still to be investigated[2-3].

As the polarization transfer in liquids is exclusively driven by relaxation (Overhauser Effect), the interplay of different molecular motions and their impact on relaxation is decisive for the DNP efficiency[3]. Here, we show experimentally how translational diffusion and rotation individually influence the coupling factor. The manipulation of either molecular motion was achieved by changing the temperature or the size of the polarizing agent. <sup>1</sup>H-DNP measurements at 0.34 T were performed in toluene and chloroform doped with nitroxide derivatives. They reveal the impact of motions with significantly different timescales on the DNP efficiency.

Furthermore, systems where scalar relaxation is dominating the polarization transfer were investigated with the same approach by performing <sup>13</sup>C-DNP at 1.2 T. Upon the increase of the polarizing agent size, these systems display an increase of the absolute coupling factor. This peculiar behavior may be connected to an additional contact contribution which is modulated on a timescale suitable for high DNP efficiency at low magnetic field. In the future, DNP efficiency may be boosted even further through rational polarizer design.

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P265

**Structural characterisation of the complex between antibiotic teixobactin and native lipid II by fast magic angle spinning solid-state NMR**

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Teixobactin represents a new class of antibiotics that target cell wall biosynthesis by binding to lipid II and lipid III [1]. It has no detectable resistance thanks to its unique but yet not fully understood mechanism of operation [1]. Recently, we have used combination of solution and solid-state NMR to determine 3D structure of native teixobactin in DPC micelles and characterise its binding to lipid II from Gram-positive and Gram-negative bacteria [2]. We provide direct evidence for binding of the C-terminal ‘cage’ to the pyrophosphate moiety of lipid II. We find that the N-terminal part of teixobactin does not only act as a membrane anchor, as previously thought, but is actively involved in binding. The N-terminal part of the peptide undergoes coil to  $\beta$ -strand conformation upon binding to the partner facilitating aggregation, which likely contributes to the high bactericidal activity of the antibiotic [2]. We show that teixobactin forms a specific complex with lipid II in membrane mimics and assembles into well-structured fibrils that yield high quality solid-state NMR spectra. Here, we describe our progress to high-resolution structure of this intriguing two-component fibril based on combination of <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N and <sup>31</sup>P spectroscopy at 60-100 kHz magic angle spinning. Our results reveal several unexpected features of the interaction between teixobactin and lipid II.

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## P266

## T1/T2 relaxation measurements based on real-time pure shift NMR

Xiaoqing Lin, Yuqing Huang, Zhong Chen

NMR spectroscopy serves as a versatile tool for information regarding “invisible” structure via non-invasive detections. Inversion-Recovery (IR)[1] and Carr-Purcell-Meiboom-Gill (CPMG)[2] NMR experiments reveal T1/T2 relaxation times by exponential curve-fitting, useful for analyses on molecular dynamics and interactions. However, both conventional IR and CPMG methods are generally hindered by poor signal dispersion caused by narrow proton chemical shift ranges and extensive proton-proton J coupling splittings.

In this abstract, an NMR approach based on the combination of real-time Zangger-Sterk (ZS) [3] pure shift technique[4] and IR/CPMG modules is proposed to achieve high-resolution T1/T2 relaxation measurements for complex samples. Periodical repeating occurrence of ZS module is implemented in the acquisition period for real-time broadband-decoupling signals. Then acquired data chunks are reconstructed for resulting pure shift spectra without any special data post processing. The feasibility of the proposed method is verified by experiments on a simple sample of 1M 1-bromobutane dissolved in chloroform-d<sub>6</sub>. In addition, two complex samples of 200 mM quinine and 40mM azithromycin dissolved in chloroform-d<sub>6</sub>, are used to show the practicability of the proposed method. In conclusion, this method presents an effective tool for T1/T2 relaxation measurements on complex samples, with the same acquisition efficiency as conventional IR/CPMG experiments.

This work is supported by NNSF of China (U1632274).

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## P267

**A sensitivity-improved selective refocusing experiment to  
measure scalar coupling constants**

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*Xiamen University*

NMR spectra provides indispensable information such as chemical shift and scalar coupling which plays a pivotal role in molecular structure determination and analysis. Unfortunately, it is often difficult to extract proton-proton scalar coupling since signals are often hidden in overcrowded regions in conventional 1D NMR spectroscopy.

Hence, the gradient-encoded selective refocusing method (G-SERF) [1] and a great number of its variants for measuring proton-proton coupling constants have been proposed. However, the sensitivity is an issue in that experiments, because the signal intensity is determined by the slice thickness of the sample that depends on encoding gradient and the bandwidth of selective pulses which is limited by the smallest chemical shift difference of any two coupled protons [2].

Here, we present a method dubbed PE-SERF (perfect echo selective refocusing) which can determine all JHH values involving a selected proton with improved sensitivity compared to original G-SERF. The module of perfect echo involving selective pulses and gradient-encoded selective refocusing are combined in the method. Instead of single proton, a pair of coupled protons is allowed to share a sample slice, and thus the slice thickness can be increased and the spectral sensitivity can be improved, which benefit molecular structure elucidation.

Recently, PSYCHEDELIC [3] was presented to measure the homonuclear couplings for crowded spectra, which also holds the reduced spectral sensitivity compared with conventional spectra. So that, it is uncertain to compare the sensitivity of PE-SERF with that of the PSYCHEDELIC method, because they are based on different principle.

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Insight into *Bacillus subtilis* biofilm architecture by solid-state NMR

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FMP Berlin

Biofilm formation is a common feature of bacterial colonies enabling them to gain an advantage in environments with high evolutionary pressure. Our research focuses on biofilms of the bacterium *Bacillus subtilis* at the air-liquid interface of standing solutions. The long-term aim is to acquire basic insights on a molecular level through application of solid-state Nuclear Magnetic Resonance Spectroscopy (NMR).

We successfully established a protocol to grow completely labeled (<sup>13</sup>C, <sup>15</sup>N) biofilm which enables us to examine the native state of molecules present in the matrix. We use these samples to probe sugar composition and monitor changes that abolish cell clustering.

Furthermore, we are interested in the essential biofilm protein TasA and its role in contributing to the firm phenotype. Our studies show that TasA fibers are necessary for *Bacillus subtilis* to aggregate and that we can mimic the natural occurring state *in vitro*. Through integration of multiple methods, including cryo-EM and solid-state NMR, we aim to create a reliable model of TasA in its fibril form.

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**Domesticating the Beast: Assignments for 70 kDa  
Tryptophan Synthase via Proton-Detected Solid-State NMR**

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Proton detection under fast magic-angle spinning has been enabling spectroscopists to overcome their hurdles and involve innovative routes to access various information sought for solid proteins. We could develop tools that would grant access to proteins of increasing complexity in the last years, ranging from assignments over structure and dynamics to protein interactions.[1,2] In particular, we could show protons to be versatile reporters for distances and relaxation properties themselves.[3,4] Most importantly, proton-detected, higher-dimensionality spectroscopy has been key for proteins in the 30 kDa range, for which now protein structure and dynamics, as well as information regarding their side-chain chemical moieties are now accessible.[5,6]

Proteins larger than this have so far posed severe limitations even for these methods, and we have been developing methodology to further expand the applicability of solid-state NMR spectroscopy.

In this work, we show that with a new range of complementary, higher-dimensionality fast-MAS approaches, we are able to assign substantial parts of the 650 amino acids enzyme tryptophan synthase, which is the enzyme that finalizes the bio-synthesis of tryptophan in the cell. This system being a heterotetramer and thus much too big for solution NMR, our innovative solid-state NMR approaches are will grant access to a plethora of biology-related questions like water accessibility of the indole tunnel and dynamics within the active site and the cofactor.

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## Frequency-Domain THz-EPR on Ferric Porphyrins

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Iron(III) porphyrins play vital roles as catalysts, protein cofactors, and single-molecule magnets (SMMs). SMMs represent potential candidates for spin-based nanoscopic data storage due to slow relaxation of the magnetization below their blocking temperature. Since the zero-field splitting (ZFS) parameters  $D$  and  $E$  strongly affect the properties of intermediate- and high-spin ferric porphyrins, precise determination is crucial for developing improved compounds. However, their large ZFSs up to several dozens of  $\text{cm}^{-1}$  exceed the frequency/field range accessible by conventional, field-domain EPR spectrometers. Using broadband sources in the THz and FIR range, frequency-domain Fourier-transform (FD-FT) THz-EPR enables probing a much wider range of transition energies.<sup>1</sup> The highly versatile FD-FT THz-EPR spectrometer at BESSY II allows for measurements from  $5\text{--}370\text{ cm}^{-1}$  by employing either synchrotron radiation or a Hg-arc lamp combined with applied fields of  $0\text{--}10\text{ T}$  and in various excitation geometries.

Employing a combination of FD-FT THz-EPR, SQUID magnetometry, Mössbauer and theoretical calculations, we investigated the electronic structure and magnetic properties of the ferric porphyrin  $[\text{Fe}(\text{TPP})(\text{H}_2\text{O})_2]\text{ClO}_4$  and determined a strong ZFS with a large, positive axial anisotropy ( $D = +19.2\text{ cm}^{-1}$ ).<sup>2</sup> This ZFS entails slow magnetic relaxation and is rationalized by a spin-admixture model with a dominant contribution of the  $S = 5/2$  state ( $\approx 85\%$ ).

Furthermore, we have implemented a way to generate circular polarized THz radiation in order to potentially determine spin Hamiltonian parameters with even higher accuracy by exciting different transitions more selectively.<sup>3</sup> A prototype study on the high-spin iron(III) ( $S = 5/2$ ) complex hemin is presented.

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**Exploring the conformational space of the E2-E3-ubiquitin complex**

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Ubiquitination is a post-translational modification of proteins that is mainly involved in signalling the labelled protein for degradation. The E1-E2-E3 cascade is responsible for attaching a ubiquitin molecule to the target protein in the following process. First the E1 activating enzyme attaches one ubiquitin molecule to the E2 ubiquitin conjugating enzyme, and then with the help of E3 ubiquitin ligase this ubiquitin is transferred to a lysine residue of the target protein. This process can be followed by the addition of multiple ubiquitins, resulting in either a single poly-ubiquitin chain or a branched ubiquitin complex. Whereas the genome of any organism usually only contains one E1 enzyme, there are a few E2 enzymes and numerous E3 enzymes encoded.

The E2-ubiquitin conjugate has been shown to be flexible, evidenced also by the multiple crystal structures reported previously (Page et al. 2012 Biochemistry; Wright et al. 2016 Nat Struct Mol Biol), presenting an open, closed, and backbent conformation of the complex. As single crystal structures only allow to explore one snapshot from the dynamic range, all of these structures might be present in the conformational ensemble under various circumstances. By using a high-resolution ensemble method, such as PELDOR (Pulsed Electron-Electron Double Resonance) spectroscopy, we can explore the conformational distribution of the studied complex.

By measuring 4-pulse PELDOR and then analysing the traces by CYANA (combined assignment and dynamics algorithm for NMR applications) modelling we were able to characterize the conformational space of the E2-bound ubiquitin. Furthermore, we also investigated the conformational distribution of the E2-ubiquitin conjugate in the presence of the RING finger domains of the Hrd1 and Doa10 E3 enzymes to discover the preferable conformation for ubiquitin transfer.

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Ultra-Clean Pure Shift 1H-NMR applied to metabolomics profiling.  
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*PUCP*

Even though Pure Shift NMR methods have conveniently been used in the assessment of crowded spectra, they are not commonly applied to the analysis of metabolomics data. This work exploits the recently published SAPPHIRE-PSYCHE methodology in the context of plant metabolome. We compare single pulse, PSYCHE, and SAPPHIRE-PSYCHE spectra obtained from aqueous extracts of *Physalis peruviana* fruits. STOCSY analysis with simplified SAPPHIRE-PSYCHE spectra of six types of Cape gooseberry was carried out and the results attained compared with classical STOCSY data. PLS coefficients analysis combined with 1D-STOCSY was performed in an effort to simplify biomarker identification. Several of the most compromised proton NMR signals associated with critical constituents of the plant mixture, such as amino acids, organic acids, and sugars, were more cleanly depicted and their inter and intra correlation better revealed by the Pure Shift methods. The simplified data allowed the identification of glutamic acid, a metabolite not observed in previous studies of Cape gooseberry due to heavy overlap of its NMR signals. Overall, the results attained indicated that Ultra-Clean Pure Shift spectra increase the performance of metabolomics data analysis such as STOCSY and multivariate coefficients analysis, and therefore represent a feasible and convenient additional tool available to metabolomics.

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Amyloid fold conservation in the twilight zone of sequence similarity

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Abdelmajid Noubhani<sup>1</sup>, Brice Kauffmann<sup>1</sup>, Joseph Wall<sup>1</sup>, Guido Pintacuda<sup>2</sup>,  
Sven Saupe<sup>1</sup>, Birgit Habenstein<sup>1</sup>, Antoine Loquet<sup>\*,1</sup>  
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The amyloid fold is a generic fold characterized by highly repetitive stacking of  $\beta$ -strands into fibrillar polymers. Amyloids are involved in various pathologies but also display different adaptive functional roles in animals, fungi and bacteria. Numerous proteins can adopt the amyloid fold, pointing to its adaptability to high sequence variability; yet amyloid propensity is also highly sequence dependent. The sequence-to-fold relation in amyloids is less well understood than for globular and membrane-proteins and appears to combine at the same time elements of sequence-dependence and independence. The low number of available atomic structures limits the exploration of the amyloid sequence-to-fold interplay for this important class of proteins.

We develop a solid-state NMR-based approach [1] for atomic structure determination in the fibrillar propagative state to establish amyloid sequence-to-fold relation. Here we characterize HELLF, a novel fungal amyloid protein that is functionally homologous to the HET-s model prion yet highly dissimilar in primary sequence. We find that HET-s and HELLF are able to form identical  $\beta$ -solenoid folds despite their very low sequence similarity, but lack cross-seeding ability. Next, we engineered a protein with minimal sequence homology to HET-s that still behaves as a prion while keeping a  $\beta$ -solenoid fold. We finally design a HELLF/HET-s chimeric protein that breaches the seeding barrier between HELLF and HET-s. The comparative study of these natural and artificial prion variants reveals the loose sequence-to-fold relation in  $\beta$ -solenoid amyloids and identifies determinants for heterologous amyloid cross-seeding or the lack thereof.

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NMR spectroscopy of human Hsp90

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Hsp70 and Hsp90, two central chaperones of the heat shock protein (Hsp) family, play an important role in maintaining protein homeostasis [1]. In addition to their discrete chaperone function, they can build up the Hsp70/Hsp90 chaperone machinery where Hsp70 is linked to Hsp90 via the Hsp-organizing protein (Hop) [2, 3, 4]. It is well known that Hop can bind simultaneously to both chaperones and thereby enables the orchestrated interplay between Hsp70 and Hsp90 [5], however, structural data at high resolution is not available.

This project aims to gain detailed insights into the Hsp70/Hsp90 chaperone machinery by NMR targeting in particular the conformational rearrangements within Hsp90 upon its interaction with Hop and during the association of the Hsp90:Hop complex with Hsp70. A major bottleneck to the structural analysis of the Hsp70/Hsp90 chaperone machinery is its highly dynamic nature. To overcome these intrinsic challenges and gain structural information at single-residue resolution, we use a side-specific isotope labeling strategy combined with <sup>1</sup>H-<sup>13</sup>C methyl-TROSY experiments optimized for high-molecular weight proteins [6]. By that we want to provide fundamental knowledge about the mode of protein (mis-)folding regulated by the Hsp70/Hsp90 chaperone machinery.

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An Inversion of NMR Data in 1 and 2 Dimensions Based on  
Regularization Method with Non-negative Constraints  
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The inversion of NMR relaxation time is very important to study object’s molecular dynamics. The inversion is to solve the Fredholm integral equation of the first kind with non-negative constraints, which is known as an ill-posed problem. In this paper, a novel method is presented for NMR inversion based on the regularization method. The proposed objective function can transform the minimization regularization with nonnegative constraints into unconstrained maximization problem, of which the objective function is piecewise, quadratic and differentiable. The generalized Quasi-Newton algorithm is applied to solve the problem and an optimized method to automatically choose regularization parameter is described combining L-curve method with GVS method. The numerical simulations results show that this proposed method is capable of well inverting both 1D and 2D NMR data and obtaining the reliable NMR inversion spectrum even at a low SNR. At last, the proposed method is employed to inverse 2D NMR data from Chinese lacustrine Qingshankou shales with different saturated states. After inversion, the regions on the T1-T2 maps from native, imbibed and dry states chunk samples that are corresponding to water, oil and viscose organic matters can be distinguished accurately.

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**Improved Trityl-Nitroxide biradicals for high field DNP**Alessandra Lucini Paioni<sup>\*,1</sup>, Weixiang Zhai<sup>2</sup>,Siddarth Narasimhan<sup>1</sup>, Yangping Liu<sup>2</sup>, Marc Baldus<sup>3</sup><sup>1</sup>*NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University,*<sup>2</sup>*Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmacy, Tianjin Medical University,* <sup>3</sup>*Utrecht University*

In recent years Dynamic Nuclear Polarization (DNP), a hyperpolarization technique that boosts the NMR sensitivity, has become a powerful tool for structural studies. DNP applications span from the solid-state NMR studies of proteins, to the development of heterogeneous catalysts. However, its use has been mainly limited to low magnetic fields, due to the unfavourable field dependence of the cross-effect (CE) DNP enhancement. The DNP efficiency decreases drastically at high fields, and this behaviour is known to be caused by the intrinsic properties of the biradicals that are currently being used as DNP polarizing agents.

In our contribution we examine a new series of water soluble biradicals, which are promising candidates for DNP enhanced solid-state NMR at high magnetic fields. To achieve a deeper understanding of the different DNP performances, we compare our experimental findings to theoretical studies using quantum mechanical spin system calculations. In particular, we analyse the relative contributions of dipolar and J-coupling interactions between the two electron spins for DNP efficiencies. Furthermore, we investigate DNP build-up times as well as molecular parameters including the hydrophilicity of the biradicals, which together are crucial for obtaining high DNP enhancements without altering sample integrity.

In summary, we present improved DNP agents more suitable for biological studies that can benefit from the increased resolution achievable at high fields. At the same time, we outline the characteristics of the biradicals which are necessary for further optimizing their DNP performance.

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### Investigation of the Enzymatic Mechanism and the Reaction Kinetics of Prokaryotic RNA Methyltransferases Using NMR Spectroscopy

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In our studies, we are focusing on the E. coli rRNA methyltransferases RlmJ and RlmF that methylate the exocyclic amino group of adenine 2030 and adenine 1618 in 23S rRNA during the early stages of ribosome assembly, respectively. In the case of RlmJ, the substrate secondary structure consists of an 18-nucleotide-long hairpin loop that is methylated using S-(5'-Adenosyl)-L-methionine as co-factor. The reaction is characterized by a large conformational shift of the N-terminal motif X upon binding of the co-factor. The second enzyme, RlmF, methylates a 10-nucleotide-long hairpin loop using the same reagent as co-factor. There are far less details known about the enzymatic mechanism of this RNA methyltransferase. The aim of our work is the investigation of the binding characteristics of substrates and co-factor, the elucidation of the reaction mechanism of both enzymes and the determination of kinetic parameters of the methylations using a range of NMR spectroscopic techniques. Dynamic features before and after binding are characterized using NMR titration experiments, ITC and CPMG relaxation dispersion experiments. The latter experiment is carried out either on the backbone amide protons or on labeled side chain methyl groups throughout the protein. The reaction mechanisms are analyzed by using different, chemically modified RNAs as substrates and determining the kinetic parameters using real-time NMR. This opens up the possibility to explore the binding pockets of both enzymes in an atomic fashion. The overall structure of each enzyme is elucidated using triple-resonance NMR-experiments. This will confirm the integrity of the already existing X-ray structure of RlmJ and furthermore, NMR structure determination of RlmF will be attempted to gain insights into the molecular mechanism of this less-studied enzyme.

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### The Concept of Tensorial Constraints in Molecular Dynamics: Conformation and Configuration of Flexible Molecules by RDCs without Alignment Tensor

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Emine SAGER, Thomas Gloge<sup>1</sup>, Armando Navarro-Vazquez<sup>1</sup>, Ina Dix<sup>5</sup>,

Alvar Gossert<sup>6</sup>, Trixie Wagner<sup>5</sup>, Axel Meissner<sup>5</sup>, Ulrich Sternberg<sup>7</sup>

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Residual dipolar couplings (RDCs) and other residual anisotropic NMR parameters provide valuable structural information of high quality and quantity, bringing detailed structural models of flexible molecules in solution in reach. Corresponding data interpretation so far is based on the concept of a so-called alignment tensor, which, however, is ill-defined for flexible molecules. The concept is also only applied to a single or a small set of lowest-energy structures, ignoring the effect of vibrational averaging. Here we introduce a different approach based on time-averaged molecular dynamics with dipolar couplings as orientational restraints that can be used to solve structural problems in molecules of any size without the need of introducing an explicit or approximate molecular alignment tensor in the computations. RDC restraints are represented by their full 3D tensor in the laboratory frame. Enforced rotational averaging of each individual tensorial restraint leads to structural ensembles that best fulfil experimental data. Using one-bond RDCs, the approach has been implemented in the MD program COSMOS and extensively tested. A detailed description of the underlying theory, including the special treatment of force fields for stable and fast MD runs, and applications regarding configurational and conformational analyses of small-to-medium-sized organic molecules with different degrees of flexibility are given.

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**An offset-independent and general sequence for singlet state NMR**

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The ability to select the signal passing through chosen coherence pathways is a powerful feature of NMR. Nuclear singlet states are coherent states that have effective spin 0[1]. Singlet states can be used to sustain hyperpolarized signal [2], to study slow dynamic phenomena[3] and to filter signal originating from specific spin  $\frac{1}{2}$  pairs[4,5].

Despite being NMR silent, singlet states can be accessed indirectly via specially designed pulse sequences. Here we demonstrate a sequence for converting magnetization into singlet states (and viceversa) which is a) offset-independent and b) can be applied in the strong, intermediate and weak coupling regime. Excitation and reconversion of singlet states is achieved by tuning experimental controllable parameters to the spin system of interest.

We show that the proposed sequence can excite selectively singlet states in nuclear spin pairs in model molecular systems as well as in biological relevant systems such as A $\beta$  peptides and brain metabolites.

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Rapid volumetric 3D MRI via simultaneous multi-slab,  
multi-echo spatiotemporal encoding (SMS-ME SPEN)

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2. Centre de RMN à Très Haut Champs, <sup>3</sup>Department of Chemical and Biological Physics,  
Weizmann Institute of Science & Massachusetts General Hospital

SPatiotemporal ENcoding (SPEN) is an ultrafast technique that relies on frequency swept pulses combined with readout gradients to collect 2D images of a sample in a single scan with fewer distortions than EPI. SPEN achieves this at the expense of a higher SAR (power deposition) associated to the use of the widely swept RF pulses. The present study presents a way to extend 2D SPEN scans to 3D volumetric measurements while coping with the SAR complications that would ensue from simply repeating the 2D acquisitions, by introducing two novel additions. The first one is a simultaneous multislab (SMS) procedure exciting and encoding multiple z-slabs simultaneously, and unraveling them by a CAIPIRINHA-like procedure. When combined with the spatial resolution deriving from multiple receivers, a suitable data processing allows 2D images from the various slabs to be resolved with a multiscan-derived gain in sensitivity and in in-plane spatial resolution. In order to resolve multiple z-slices within these resolved slabs, a multi-echo method capable of delivering multiple signals possessing different kz values within each slab, was implemented. Compatibilizing this with the non-idealities deriving from the pulsing of these echo trains, also required collecting a low resolution (navigator) image at kz = 0 immediately following the acquisition of each multi-echo scan. The ensuing 3D SMS-ME SPEN sequence was conducted on human volunteers at 3 T. Six 24 mm-wide slabs were chosen, with three bands spaced 48 mm apart being simultaneously excited in each scan. Three echoes were collected for a total of 24 kz values within 8 scans, with three segments along the ky (SPEN) dimension collected with the CAIPIRINHA scheme. All the data was acquired with 75% partial Fourier sampling along kx. The final spatial resolution for this full brain scan was 1.41x1.41x1 mm, for a total acquisition time of only 1min 36s.

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**<sup>13</sup>C Metabolic Flux Ratio Analysis by ChemAdder - from 2D HSQC to virtual 1D spectra and qQMSA**Hannu Maaheimo<sup>\*,1</sup>, Pekka Laatikainen<sup>2</sup>,Samuli Rantala<sup>3</sup>, Arimo Mertanen<sup>3</sup>, Reino Laatikainen<sup>4</sup><sup>1</sup>VTT Technical Research Centre of Finland Ltd, <sup>2</sup>Spin Discoveries Ltd, <sup>3</sup>VTT Technical Research Centre of Finland Ltd., <sup>4</sup>School of Pharmacy, UEF (Univ. of Eastern Finland)

NMR spectroscopy is an efficient method for obtaining quantitative data on the fractional <sup>13</sup>C enrichment of metabolic intermediates or end-products. In biosynthetically directed fractional <sup>13</sup>C labelling using uniformly <sup>13</sup>C labelled carbon source, the biomass becomes <sup>13</sup>C labelled in a metabolic flux distribution dependent manner and the cleavage and the formation of the covalent bonds of the carbon backbone differing between the alternative pathways is monitored from the <sup>13</sup>C-<sup>13</sup>C scalar coupling fine structure of <sup>13</sup>C NMR spectra. The <sup>13</sup>C-<sup>13</sup>C couplings give rise to from two to four multiplets per carbon. These multiplet components are most conveniently observed and integrated from HSQC spectra. The integrations of these heavily overlapping signals, however, is very difficult using conventional NMR software.

In quantitative Quantum Mechanical Spectral Analysis (qQMSA) it is assumed that an NMR spectrum is a sum of the model spectra of its components and the experimental spectra are replaced by models obtained by fitting the spectra using Quantum Mechanical (QM) theory. This results in spectra pure from impurity signals, noise and other artefacts, but interpreting even the smallest spectral details.

We present a special software ChemAdder capable of <sup>13</sup>C assisted fluxomics. The HSQC spectra of the cell hydrolysate are first converted to virtual <sup>13</sup>C-<sup>13</sup>C coupled 1D <sup>13</sup>C spectra of each amino acid. This opens up the possibility to apply the qQMSA tools of ChemAdder to the HSQC signals and the <sup>13</sup>C isotopomer distribution of each carbon of the 16 amino acids (i.e. the amino acids surviving the acid hydrolysis) can be obtained in less than 10 minutes. The QM models are field independent and the models obtained from the HSQC spectra measured at any field can be used as models for metabolic <sup>13</sup>C labelling spectra measured at any other field.

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## P282

## Ultrafast Diffusion Exchange Measurement

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NMR relaxation and diffusion measurements provide versatile information about dynamics and structures of e.g. porous materials, and reveal interactions of nuclei within their microscopic environment. Since relaxation and diffusion data comprise exponentially decaying components, the processing requires Laplace inversion to extract the diffusion coefficient and relaxation time distributions. Thus, these methods are called as Laplace NMR(LNMR).[1]

Multidimensional approach increases chemical resolution of an NMR experiment. Multidimensional and some 1D-experiments are time consuming, since need of repeating experiment with varying evolution delay or gradient strength to acquire multidimensional data. This restricts the applicability of multidimensional LNMR methods and is considered general problem of multidimensional NMR. Also in many cases it prevents the use of hyperpolarization for signal amplification. The problem can be tackled by introducing spatial encoding of two-dimensional data, as was originally done in ultrafast NMR spectroscopy[2,3] and later in ultrafast LNMR[4-6]. Price to pay is reduced sensitivity. However, single-scan approach enables use of hyperpolarization methods (e.g.PHIP, DNP[3], SEOP[4]), providing much higher sensitivity boost than the loss due to spatial encoding.

In this presentation we introduce, for the first time, single-scan diffusion-diffusion exchange experiment relying on the concepts of ultrafast LNMR. We demonstrate the feasibility of the method with interesting surfactants that have important role in biology and chemistry. The method can also utilize modern hyperpolarization methods increasing sensitivity to study systems such as dilute samples and dynamics of fluids in porous medium. The ultrafast LNMR methods are applicable with mobile NMR, thus widening the application range even further.[4-7]

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Exploring Protein Structures by DNP-Enhanced Methyl Solid-State NMR Spectroscopy  
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The rapid methodology development of sensitivity-enhanced SSNMR spectroscopy has been empowering its success in a broad range of challenging fields in life science. Here I will present a new methyl SSNMR toolkit for exploring the protein structures that offers new types of NMR parameters for decoding the complex molecular systems. These approaches meld DNP-enhancement, heteronuclear NOE, tamed spin diffusion (SD) and strategically designed isotope labeling schemes. First, our method utilizes methyl groups as dynamic sensors for probing the local molecular packing. Second, a new approach, named SIMPLE (Selective Intersite detection via Methyl PoLarization Enhancement), enlightens the molecular interface, e.g. the residues in ligand-binding pocket, with the unprecedented selectivity by combining seamlessly the biochemical stringency, isotope sparseness with spectroscopic selectivity. Third, by taming the <sup>13</sup>C-<sup>13</sup>C spin diffusion, we have been able to determine the <sup>13</sup>C-<sup>13</sup>C distances in the subnanometer range. This covers the gap between the conventional <sup>13</sup>C SSNMR methods and some of the most popular EPR approaches. These methods are developed directly on the light-driven proton-pumping proteorhodopsin (PR), a 7TM membrane protein. Our new data pinpoint that the driving force of the initial proton transfer from the Schiff's base to its counter ion in PR is not identical to that of well-investigated bacteriorhodopsin, therefore unmask a hidden mechanistic diversity of microbial proton pumps. Our approaches are rather user-friendly. They require the minimal NMR knowledge and only the entry-level hand-on training on DNP SSNMR instruments. In particular, all these experiments are casted in the format of 1D spectroscopy and are much easier to analyze compared to many other NMR/EPR methods. We expect these methods will gain high popularity in many fields in near future and will already showcase the immediate applications of our new approaches on some other challenging biomacromolecular systems.

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Structural studies of large RNAs by solid-state NMR based structural biology

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Hepatitis C virus (HCV) RNA genome is ~9.6 kb in length and contains an open reading frame that is flanked by highly organized untranslated regions at 5' and 3' ends (5'- and 3'-UTR). Liver abundant human microRNA miR-122 binds to the 5' UTR of HCV RNA, stimulates HCV replication and translation and leads to changes in architecture of 100 kDa large HCV 5' UTR.[1,2] While several methods have been applied to characterize the interaction of miR-122 with the HCV RNA, precise structural information and the exact mechanism of action are still lacking. Large RNA either alone or in complex with proteins are challenging objects for structural biology. Solid-state NMR is an emerging technique that can provide structural information for large biomolecules at atomic resolution and holds great promises for RNA.[3]

In this project, we aim to develop an integrative approach for characterization of large RNA that utilizes solid-state NMR and small angle scattering (SAXS, SANS) techniques combined with segmental labelling of RNA. While solid-state NMR provides us with local structural information, SAXS and SANS deliver low-resolution long-distance data. We will use our developed approach to decipher the interaction of miR-122 with the 5'UTR to understand the mechanism of microRNA-based viral RNA regulation.

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## P285

**Conformational assignments and dynamic dependence of chitosan with variable degrees of acetylation and molecular weight by high and low-field SSNMR techniques**

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Eduardo Ribeiro deAzevedo<sup>2</sup>, Luiz Alberto Colnago<sup>3</sup>

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The bioactivities and physical-chemical properties of chitosan are strongly dependent on the contents of 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN) units, which define the average degree of acetylation (DA). In this study, high molecular weight chitosans with variable DA grades (5 to 60%) were produced by multistep ultrasound-assisted deacetylation of beta-chitin followed by homogeneous N-acetylation. Chitosan possessing lower molecular weight were prepared through ultrasound depolymerization treatment. The XRD analyses showed that the crystallinity index (CrI) of chitosans increased with DA, showing no significant variation as function of molecular weight. A similar trend was found on C4 and C6 carbon signals resonance in CPMAS spectra that presented a progressive contribution of deconvoluted area as short-range ordered interchain arrangement related with DA. Meanwhile, the molecular weight mainly influenced the C1 signal resonance. These features were cross-validated accordingly to spectrum pattern recognition by singular value decomposition method showing higher correlation with DA and CrI. Such straight dependence was also verified by TD-NMR Magic-sandwich echo (MSE) curves composed by hard/soft hydrogen signal decays, referred to chitosan rigid T2r and mobile T2m fractions in intermediate-to-slow timescale, and high-resolution bidimensional Dipolar Chemical Shift Correlation (DIPSHIFT) curves modulated by H-C dipolar interaction. These techniques confirmed the higher mobility of methyl from GlcNAc moieties that induced a significant decrease of dipolar coupling with the surrounding environment and enhanced the T2m signal decay contribution. The PLS statistical treatment also showed higher correlation among the signal decay profiles with DA and CrI. Therefore, it was possible to simultaneously evaluate the structural and morphological particularities of chitosan through different SSNMR techniques. The authors gratefully acknowledge the support from the Brazilian agencies CNPq (141353/2016-3) and FAPESP (2016/20970-2; 2016/09720-4; 2017/20973-4).

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**P286****Simulations of Novel High Frequency Rapid Scan EPR Experiments****Andriy Marko**<sup>\*,1</sup>, Oleksii Laguta<sup>2</sup>, Petr Neugebauer<sup>3</sup><sup>1</sup>*Central European Institute of Technology (CEITEC)*, <sup>2</sup>*CEITEC Brno University of Technology*, <sup>3</sup>*CEITEC Brno University of Technology, Czech Republic*

Due to the recent development of the microwave (MW) technique, Electron Paramagnetic Resonance (EPR) spectroscopy is currently making a significant progress in the sub-THz frequency range. For example, experiments at frequencies above 0.2 THz were recently performed employing rapid scan methodology to record EPR spectra [1]. In these High Frequency Rapid Scan (HFRS)-EPR experiments the frequency of irradiating microwaves is swept over the EPR spectrum very fast in comparison to the relaxation times of the investigated sample. This enables a great improvement of the experimental sensitivity and investigations of samples with short relaxation times in nanosecond range. HFRS-EPR experiments produce, however, more complicated and less studied EPR signals in comparison to usual EPR spectra which are recorded with very slow sweep rates. In this work computational tools for simulations and interpretations of HFRS-EPR experiments are developed. They are based on the quantum statistical Liouville/von Neumann equation for density matrix in order to determine spin dynamics of paramagnetic system affected by microwaves with a rapidly swept frequency and various spin relaxation processes [2]. We use these computational tools for the feasibility study of novel rapid scan experiments at high frequencies. For instance, a HFRS-EPR experiments for nitroxide radicals in liquids, which are ubiquitously used in EPR and Dynamic Nuclear Polarization (DNP), are simulated [3]. We predict the shape of HFRS-EPR nitroxide spectra and search for the optimal experimental procedures providing the most detailed characterization of molecular dynamics. Furthermore, conditions necessary for the saturation of EPR transitions are analyzed.

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Atomic level investigation of Arkadia’s selectivity and specificity towards E2 enzymes

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Ubiquitination and proteasome-dependent degradation of proteins is a major regulatory mechanism of protein function in eukaryotic cells[1]. The covalent attachment of ubiquitin to substrates occurs through the recruitment of three enzymes: a ubiquitin-activating enzyme E1, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase. The E2 and E3 enzymes are the key components in the ubiquitin pathway, as they are responsible for substrate recognition and ubiquitination[2]. The ubiquitin pathway is very versatile. An E3 ubiquitin ligase may recognize multiple substrates. Even though an E2 enzyme can recognize different E3s, an E3 enzyme can recognize only few E2s. This specific interaction of E2 with E3 enzymes determines the ubiquitination activity of E3[3]. Arkadia and Arkadia2C E3 Ubiquitin ligases act via their C-terminal RING domains as positive regulators of the TGF-β and BMP pathway, respectively[4].

Titration experiments of Arkadia and Arkadia2C with their natural E2 enzyme UbcH5B revealed their interaction properties, thus the investigation of the ability to interact with other E2 enzymes is necessary in order to identify the specificity of Arkadia’s enzymatic functionality. In the present study, UbcH7 was expressed in high yield and its ability to interact with Arkadia and Arkadia2C was studied. NMR titration experiments were performed, as well as biochemical tests, in order to determine the capability of this E2 enzyme to interact with Arkadia and Arkadia2C E3 enzymes. This study provides further insight into the selectivity and specificity of interactions between E2 and E3 enzymes in the ubiquitin pathway.

Acknowledgments:

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- iNEXT EU H2020 Grant No 653706

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Interactions of amyloid peptide AS(71-82) with model membranes:  
structural and morphological study via FTIR and ssNMR

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$\alpha$ -Synuclein (AS) is an amyloid protein involved in Parkinson's disease. In pathological cases, it aggregates in the dopaminergic neuronal network, leading to a progressive degeneration accompanied with a dramatic decrease in dopamine levels. Under physiological conditions, AS is disordered in solution or weakly bound to neuronal membranes, via the formation of  $\alpha$ -helices. The triggers and steps underlying the formation of insoluble  $\beta$ -sheet rich fibrils are still unclear. In our work, we focus on a central 12-residue segment of AS in its amyloidogenic part that is believed to be responsible for the fibrillization of the whole protein: AS<sub>71-82</sub>.

Interactions between AS and neuronal membranes are thought to be the starting point of the fibrillization process, triggering the pathogenic amyloid cascade. In order to investigate and probe the mechanisms responsible for this fibrillization, model membranes composed of different ratios of zwitterionic and anionic phospholipids were used. FTIR allowed the identification of irreversible changes in the  $\beta$ -sheet structure of AS<sub>71-82</sub> upon the gel, fluid phase transition of the lipids, underlining the critical role of peptide/membrane interactions. Furthermore, the <sup>31</sup>P solid-state NMR study of phospholipid polar headgroups is arguably a powerful method to probe the interactions between peptides and model membranes. Recently, a 2D pulse sequence named PROCSA (phosphorus recoupling of chemical shift anisotropy) was developed in order to study model membranes composed of a mixture of phospholipids. This MAS (magic-angle spinning) pulse sequence gives rise to spectra with isotropic chemical shifts in the direct dimension and the powder spectra of each phospholipid in the indirect dimension. This feature is advantageous when compared to standard static <sup>31</sup>P spectra where the powder spectra of all phospholipids are superimposed and hard to separate. Eventually, PROCSA provides insights into the peptide/membrane interactions with structural and dynamical information on the preferentially interacting phospholipid in the mixture composing the membrane.

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**P289****Structural analysis of the multidomain protein TIA-1 and its RNA recognition**Santiago Martinez-Lumbreras<sup>\*,1</sup>, Miriam Sonntag<sup>1</sup>,Maximilia Souza<sup>1</sup>, Charlotte Softley<sup>1</sup>, Michael Sattler<sup>2</sup><sup>1</sup>*Institute of Structural Biology, Helmholtz Zentrum München,*<sup>2</sup>*Biomolecular NMR, Bayerisches NMR Zentrum and Center for Integrated Protein Science Munich at Chemistry Department, Technical University of Munich*

TIA-1 (T-cell Intracellular Antigen-1) is an RNA binding protein involved in alternative splicing of multiple transcripts. It promotes apoptosis by regulating the alternative splicing of FAS pre-mRNA, where inclusion of exon 6 results in the apoptotic form of the FAS death receptor protein [1]. It has been also related to translational regulation of different mRNAs in the cytoplasm by sequestering them in the stress granules and repressing their translation [2]. TIA-1 possess three RNA Recognition Motifs (RRM) connected by flexible linkers and a C-terminal glutamine rich region. Previous studies showed that RRM2 and RRM3 domains dominate the recognition of U/C-rich RNA targets. Although all RRM domains are independent structural modules in absence of RNA, RRM2 and RRM3 cooperatively bind pyrimidine-rich RNA sequences and form a more compact arrangement [3].

Previous attempts of obtaining high resolution structural information of the complex between the TIA-1 RRM2-RRM3 and RNA ligands using X-ray crystallography or NOE-based NMR methods have so far been unsuccessful. Currently high-resolution structures of individual domains and a low resolution model of the multidomain/RNA arrangement (based on SAXS and SANS data) are available [4]. Here, we obtain paramagnetic NMR restraints from covalently labeling the protein with spin labels and lanthanide binding tags at different positions to obtain comprehensive structural restraints to define the structure of the TIA-1 RRM domains bound to a biological relevant RNA target.

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The elastic properties of flexible liposomes as measured using fast field-cycling NMR relaxometry: influence of surfactant concentration and temperature  
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Flexible liposomes have proved to be efficient for transcutaneous administration of drugs. In this context, the bending elastic modulus  $\kappa$  of the membrane turned to be a measurable physical parameter that unambiguously reflects the elastic property of the membrane. The spin-lattice relaxation rate dispersion of protons in unilamellar liposomes has been recently studied using the fast field-cycling (FFC) NMR technique [1-5]. Results were interpreted in terms of a model that considers the lipid dynamics within the membrane. An important feature of the approach is that it allows inferring about its elastic properties, as the elastic constant  $\kappa$  is fully involved in the model. The methodology and the model were validated through measurements of  $\kappa$  in liposomes containing both cholesterol and different surfactants within the membrane, that is, at different elastic conditions of the specimen. In this work we focus in the temperature dependence and the behavior of flexible liposomes formulated with different surfactants. Specifically, we analyzed four different temperatures within the range 291-328K for liposomes of radius of 50nm, composed of SPC (soy phosphatidylcholine) with different additives that are commonly used to enhance the membrane flexibility, at different concentrations. As previously observed in the literature, for the lowest temperatures, the elastic modulus of the membrane decrease with the addition of surfactants. However, the observed behavior is not linear for none the temperature and concentration, nor with the type of surfactant. A correlation analysis reveals that specific properties of the surfactants have a direct influence on the elastic properties as well as in the lipid molecular dynamics.

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The ambivalent role of proline residues in an intrinsically disordered protein:  
from disorder promoters to compaction facilitators

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<sup>1</sup>Max Perutz Labs, <sup>2</sup>CERM

Intrinsically disordered proteins (IDPs) carry out many biological functions. They lack a stable three-dimensional structure, but rather adopt many different conformations in dynamic equilibrium. The interplay between local dynamics and global rearrangements is key for their function. In IDPs, proline residues are significantly enriched. Given their unique physicochemical and structural properties, a more detailed understanding of their potential role in stabilizing partially folded states in IDPs is highly desirable. NMR spectroscopy, and in particular <sup>13</sup>C-detection, is especially suitable to address these questions. We applied a <sup>13</sup>C-detected strategy to study Osteopontin, a largely disordered IDP with a central compact region. By employing the exquisite sensitivity and spectral resolution of these novel techniques we gained unprecedented insight into cis-Pro populations, their local structural dynamics and their role in mediating long-range contacts. Our findings clearly call for a reassessment of the structural and functional role of proline residues in IDPs. The emerging picture shows that proline residues have ambivalent structural roles. They are not simply disorder promoters but rather can, depending on the primary sequence context, act as nucleation sites for structural compaction in IDPs. These unexpected features provide a versatile mechanistic toolbox to enrich the conformational ensembles of IDPs with specific features for adapting to changing molecular and cellular environments.

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**Correlation between p-conjugation network and  
luminescence of fused phenazine derivatives**

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Conjugated organic molecules exhibit unique optical, magnetic and electrochemical properties, and have been used for organic molecular magnets and organic thin-film devices such as solar cells, organic light emitting diodes (OLED), or organic field effect transistors. Their properties are strongly affected by the spin structures, which can be effectively investigated by Electron Spin Resonance (ESR) spectroscopy with the help of quantum chemical calculations.

Organic light-emitting diodes (OLEDs) have attracted significant attention for the application in next generation display technologies. Utilization of triplets is important for preparing organic light-emitting diodes with high efficiency. Very recently, both electrophosphorescence and electrofluorescence could be observed at room temperature for thienyl-substituted phenazines without any heavy metals. It was found that the phosphorescence efficiency depends on the orientation of fused thiophenes. In this work, the excited triplet states of fused phenazine derivatives with pyridines or benzenes were investigated by time-resolved EPR and optical measurements as well as quantum chemical calculations. It was demonstrated that when pyridine is fused to phenazine, the spin density is localized onto the phenazine unit, independently of the orientation of the pyridines.

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PRE based characterization of RNA-protein complexes

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RNA-protein complexes are involved in multiple cellular functions and vary with regard to affinity and specificity. Both double stranded and single stranded RNA chains can be observed in complex with proteins. Here we develop methods based on PRE-measurements to elucidate the process of RNA recognition by the ribosomal protein S1, involved in translation initiation. In the past chemical shift perturbation (CSP) experiments were already performed to characterize the RNA binding of the S1 protein and to identify the amino acids interacting with the RNA. In this project, a paramagnetic nitroxide spinlabel was attached to 14 mer RNAs, which were subsequently added to the protein solution and 15N-HSQC experiments of the complex were performed. Paramagnetic relaxation enhancement (PRE) leads to a faster T1-relaxation of the amino acids close to the spinlabel. This effect is distance dependent and due to its high sensitivity suitable for weak or transient binding events. In 15N-HSQC spectra the change of the signal intensities of all peaks were used to identify the region where the RNA is bound. We varied RNA-protein ratios and used an optimized mixture of diamagnetic and paramagnetic RNA to exploit the potential of the PRE in an intermediate respectively fast regime. To investigate if the RNA has a defined orientation within the complex different positions of the spinlabel were tested.

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**High-Resolution Solid-State NMR of Antibiotics in Native Cellular Membranes**

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The alarming rise of drug-resistant bacteria urgently calls for novel antibiotics. A particularly interesting class of antibiotics target essential Lipid II molecules that are present in bacterial membranes, killing even the most refractory bacteria at nanomolecular concentrations. Unfortunately, the native binding modes of these membrane-active antibiotics are unknown due to the enormous challenge to study these drugs in a native environment. This lack of data critically limits the use of these powerful drugs for antibiotic design. Here, we present a cutting-edge solid-state NMR approach to study antibiotic-Lipid II complexes in liposomes and **directly in native bacterial membranes**.

Using a combination of **high-field 800 MHz DNP-enhanced and 1H-detected solid-state NMR**, we managed to capture the native state of the antibiotic Nisin bound to Lipid II at high resolution. Our data proof that the previous gold-standard model, a nisin – Lipid II complex acquired in artificial DMSO medium, does not represent a physiologically relevant binding mode. Furthermore, for the first time ever, we managed to capture quantitative, well-resolved 2D NMR spectra of an antibiotic directly in native bacterial cell membranes using as little as 5-10 nmol of antibiotic. These cellular experiments unravelled antibiotic domains that are responsible for adapting the Nisin-Lipid II complex to the local properties of different bacterial membranes. Intriguingly, these plastic domains also correspond to pharmaceutical hotspots of the antibiotic.

Overall our solid-state NMR approach enables the study of membrane-active drugs in their native cellular conditions with high resolution. This ultimately leads to a rational understanding of how the local bacterial membrane environment can modulate antibiotic binding and efficiency. Thereby our approach paves the way to study drug-binding and drug-resistance mechanism directly in medically relevant conditions

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**Progress in Bullet-Dynamic Nuclear Polarization**

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We have previously shown that bullet-dynamic nuclear polarization (bullet-DNP) can achieve liquid-state <sup>13</sup>C polarization levels of 30% at less than 10-fold dilution with sub-mL solvent volumes as used in NMR spectroscopy [1].

Low-field thermal mixing [2] has the potential to rapidly polarize low-gamma nuclei, without the need for strong B1 fields at cryogenic temperatures. However, rapid, radical-induced low-field relaxation may hamper a successful polarization transfer. Here we present initial results that suggest that low-field thermal mixing may indeed be combined with DNP.

Furthermore, we are developing bullet-DNP as a fully automated research tool, including sample loading, polarization, dissolution and cleaning/flushing of the detector. We argue that this is a necessary development and report on recent progress.

**References:** [1] K Kouril, H Kourilová, S Bartram, M H Levitt & B Meier, *Nature Communications* (10), 1733 (2019). [2] D T Peat, M L Hirsch, D G Gadian, A J Horsewill, J R Owers-Bradley & J G Kempf, *Phys Chem Chem Phys.* (28), 19173 (2016).

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SAD TI: Singlet Assisted Diffusion Tensor Imaging for porous media investigations

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NMR and MRI techniques enable to derive structural information on porous materials from the study of restricted self-diffusion. The condition to make diffusion a source of such information is to track the displacement of a molecular probe long enough for this to experience the confinements. Since diffusion Magnetic Resonance techniques rely on the acquisition of a signal whose decay constant depends on the spin order that was created, methods based on longitudinal magnetisation have an upper limit to the scope of the technique (cavity size < 100  $\mu\text{m}$ ) given the fact that the relaxation times T1 and T2 associated with longitudinal and transverse magnetisation are typically of the order of a few seconds at best.

Our group has recently demonstrated the advantages of using long-lived spin order in diffusion experiments [1],[2]. In this work we combined the exceptionally long relaxation time TS of singlet order with Diffusion Tensor Imaging (DTI) procedure. By deriving all six components of the diffusion tensor, DTI allows to depict the three-dimensional architecture of cavities and channels where a molecular probe is diffusing.

Although its main field of application is medical MRI, DTI might be usefully applied to a broader class of materials where structural details are required for quality and performance evaluation, including rock samples, anisotropic battery electrodes and cells cultured on 3D-printed scaffoldings for the regeneration of biological tissue, all systems in which current DTI techniques fail to perform because of the short relaxation time of longitudinal spin order.

In this contribution we describe the newly developed singlet-assisted DTI technique and demonstrate its potential to access structural information in 3D phantoms with 1 mm cylindrical channels oriented in different directions of space. These phantoms are used as models of the more interesting structures cited above to which we aim to address our future efforts.

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**Molecular distances using electron and  $^{19}\text{F}$  nuclear spins at high EPR fields (3.4 T/ 94 GHz): from model systems to biological applications**

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One of the main application fields of EPR in biological samples is the investigation of paramagnetic centers (like metal ions or amino acid radicals) in proteins. Electron-nuclear double resonance (ENDOR) allows obtaining information about naturally occurring, nuclear spins ( $^1\text{H}$ ,  $^{14}\text{N}$ , ...) in the immediate neighborhood of such electron spin centers. Interactions with remote nuclei ( $\geq 7 \text{ \AA}$ ) are often poorly resolved owed to the weakness of the interaction. The strongest coupling is typically exerted by  $^1\text{H}$  nuclei. However, their ubiquity in biological macromolecules leads to crowding of  $^1\text{H}$  ENDOR spectra.  $^{19}\text{F}$  nuclei interact almost as strongly with electron spins as  $^1\text{H}$  does, and it is often possible to replace  $^1\text{H}$  by  $^{19}\text{F}$  nuclei due to the bioisosterism of the two atoms. We introduce here  $^{19}\text{F}$  nuclei as spin labels for ENDOR at high EPR fields (3.4 T/94 GHz), where the  $^{19}\text{F}$  resonance frequency is reasonably ( $> 8 \text{ MHz}$ ) separated from all other nuclear resonances. The feasibility of this approach is shown at three stages. First, it is demonstrated that couplings between a nitroxide radical and  $^{19}\text{F}$  nuclear spin are detectable for distances up to  $15 \text{ \AA}$  on small, synthetic models. For  $r \geq 6 \text{ \AA}$  distances are found well consistent with the point-dipole model. In a second set of experiments on spin labelled RNA duplexes, data of comparable quality could be obtained. Finally, first applications of the method are shown on  $^{19}\text{F}$  labelled E. coli ribonucleotide reductase, providing geometrical information about the intermediate tyrosyl radical  $\text{Y}_{350}^\bullet$  in the long range radical transfer pathway of this enzyme. The results indicate that the method of  $^{19}\text{F}$  ENDOR could find application as a general tool in ESR spectroscopy for the intermediate distance range ( $\sim 5 - 15 \text{ \AA}$ ), as did PELDOR for the large distance range ( $\sim 20 - 100 \text{ \AA}$ ).

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Characterization of binding events between cytoplasmic domains of integrin-β1/β3 and the focal adhesion-associated proteins paxillin and kindlin-2  
Marcus Michaelis, Timo Baade, Christof R. Hauck, Heiko M. Möller

Cell migration is of prime importance for physiological processes like immune response, tissue repair, and embryonic morphogenesis [1]. Essential for the attachment and migration of cells across surfaces is the formation of focal adhesions [2, 3]. The cell surface receptor family of the integrins plays a key role as linker proteins between intracellular and extra-cellular environments. The cytoplasmic tails of the integrins α and β subunits connect the complex network of signaling intracellular proteins with the actin cytoskeleton [4, 5]. Two major components in these focal adhesions are the proteins paxillin and kindlin as transducers of integrin clustering and signaling [6].

Therefore, we combined solution NMR spectroscopic and biochemical approaches for the characterization of binding events between these proteins to get better insights into the molecular mechanism in this complex network. We could identify paxillins LIM2/3 domains as binding partner for integrin-β1 as well as integrin-β3. In addition, the LIM2/3 domains also bind to the PH domain of kindlin-2. Furthermore, the key residues responsible for integrin`s affinity to bind paxillin have been validated by mutation studies. These findings will help to understand better the intricate mechanism of the recruitment of focal adhesion associated proteins.

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**Nearest Neighbor Effects in Homopeptide Segments of  
Short Peptides Explored by Circular Dichroism and NMR Spectroscopy**  
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*Magnetic Resonance (BMRZ)*, *Institute for Organic Chemistry and*  
*Chemical Biology*, <sup>3</sup>*Johann Wolfgang Goethe-Universität Frankfurt am Main*

Contrary to the view based on the classical random coil model, it is now clearly established that individual amino acid residues in unfolded peptides and proteins differ with regard to their conformational propensities. The prime example is alanine which shows a very high preference for polyproline II (pPII). Recently, our research groups conducted a conformational analysis of GxyG host-guest peptides with selected mixtures of aliphatic (A,V,L) and more polar/charged residues (S,D,K) as guest residues x and y. We found that while A and D are particularly dependent on their neighbor's conformation, nearest neighbor interactions (NNI) become significant for a larger number of xy-pairs at high temperature owing to entropic effects. In this study we investigate homopeptide segments in GxxG and GxxxG peptides (x= D, K and R). The selection of K and R is motivated by theoretical predictions of Pappu and coworkers who predicted that Coulomb interactions would force such peptides into extended structures [1]. Longer D and K containing segments are prevalent in IDPs and particularly in functionally relevant linear motifs. Preliminary results of circular dichroism experiments indicate an increasing propensity for right-handed helical turn structures (type III) for protonated D in GDDG and GDDDG compared with the conformational distribution of GDG. A pPII-like conformational preference is strongly enhanced in GKKG compared with GKKG and GKG. Currently, we are measuring five different scalar J-coupling constants for each of the non-terminal residues of these peptides that depend differently on the dihedral angles  $\varphi$  and  $\psi$ . We will use an earlier developed Gaussian distribution based conformational model to reproduce these coupling constants. The results will enable us to describe NNIs in the above homopeptides in quantitative and structural terms.

**Reference:** [1] N. Lyle, R. Das, and RV. Pappu, J. Chem. Phys. 139, 121907 (2013); doi: 10.1063/1.4812791

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NMR-based investigation of the altered metabolic response of Bougainvillea spectabilis leaves exposed to air pollution stress during the circadian cycle.

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The metabolism of plants has evolved several different strategies to cope with different types of abiotic stresses, ranging from reconfiguration of central metabolic pathways such as carbon, nitrogen and energy metabolism, to biosynthesis of specialized secondary metabolites. The plant circadian clock is intimately connected with plant response and tolerance to abiotic stress. We performed metabolite fingerprinting of the leaves of Bougainvillea spectabilis (a plant known to be tolerant to several kinds of abiotic stresses) using 1D and 2D NMR spectroscopy. Several of the metabolites identified in our study show a consistent rhythmic pattern during the circadian cycle, indicating that circadian rhythms are a strong influence on plant metabolism. We also used NMR-based metabolomics to identify metabolites that are significantly different in two types of Bougainvillea spectabilis leaves: from plants exposed for prolonged durations to high levels of vehicular emissions and air pollution stress, and from plants grown under controlled conditions with no exposure to vehicular emissions. We used multivariate statistics to understand how air pollution stresses disturb different metabolic pathways in the plant. Our observations of alteration in primary metabolism of Bougainvillea spectabilis in response to air pollution stress include changes in concentrations of aminoacids, TCA cycle intermediates and sugars. These metabolic markers are indicators of photosynthetic dysregulation as well as osmotic readjustment. Sugars such as sucrose and glucose accumulate rapidly in the leaves subjected to pollution stress, which could be a source of protection against oxidative damage via osmotic adjustment. Secondary metabolites produced in abundance in the leaves exposed to air pollution include putrescine,  $\gamma$  aminobutyric acid (GABA), trigonelline and several phenylpropanoids and flavonoids, all of which have been previously noted to be elevated in model plants which are tolerant to abiotic stresses.

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Metabolomic profiling of truffles via NMR spectroscopy

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In order to distinguish the different consumable truffle species and to confirm their geographical origin, the metabolic profile of the fungi from the genus *Tuber* could store the answer within. To accomplish this task the polar extract of the fruiting body from authentic samples were analyzed by <sup>1</sup>H NMR spectroscopy under water suppression.[1] The spectra of the different truffles covering samples from eight species (*Tuber aestivum*, *Tuber albidum*, *Tuber himalayense*, *Tuber indicum*, *Tuber magnatum*, *Tuber melanosporum*, *Tuber sinense*, and *Tuber uncinatum*), from nine countries (Australia, Bulgaria, China, Croatia, France, Hungary, Italy, Romania, and Spain) and the harvest years 2017 – 2019 were compared and evaluated by multivariate statistics. For the data analysis principle component analysis and other machine learning algorithms were applied.

**Reference:** [1] <sup>1</sup>H NMR Spectroscopy for Determination of the Geographical Origin of Hazelnuts, R. Bachmann, S. Klockmann, J. Haerdter, M. Fischer, and T. Hackl, *Journal of Agricultural and Food Chemistry* **2018** 66 (44), 11873-11879.

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New Method for Selective Deuteration of Side-Chains for High-Resolution Protein SSNMR

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We discuss a novel isotope-labeling approach to improve the <sup>1</sup>H<sub>α</sub> resolution and to simplify side-chain <sup>1</sup>H signals for <sup>1</sup>H-detected protein solid-state NMR (SSNMR) with a simple bio-expression method using *E. coli*. SSNMR using <sup>1</sup>H detection is attracting attention as a powerful method for protein structure determination. However, <sup>1</sup>H line broadening due to <sup>1</sup>H-<sup>1</sup>H dipolar interactions cannot be completely eliminated even with 100 kHz ultra-fast MAS, posing an obstacle to signal assignment and structure determination. To improve <sup>1</sup>H resolution for the protein backbone analysis using fast MAS at 60-100 kHz, we developed a method to selectively deuterate side-chains at a high deuteration level with *E. coli* BL21 (DE3) expression system while maintaining the protons at the α-position.

This selective labeling method is based on the transamination reaction in the amino acid biosynthesis pathway. In this reaction, an amine group is transferred from an amino acid to an α-keto acid to convert the keto acid to a new amino acid. In this process, solvent-derived proton is introduced into the α-position of the new amino acid. Using this reaction, we tried to introduce <sup>1</sup>H derived from H<sub>2</sub>O into the α-position of <sup>2</sup>D-labeled α-keto acid derived from [U-<sup>2</sup>D,<sup>13</sup>C]-Glucose in conventional *E. coli* expression system.

To evaluate the quality of the SSNMR spectrum for the selectively deuterated sample, we measured <sup>1</sup>H-detected 2D <sup>13</sup>C/<sup>1</sup>H correlation of a micro-crystal sample of GB1 at a MAS rate of 60 kHz and a <sup>1</sup>H NMR frequency of 900 MHz. The results revealed drastic signal suppression in the side-chain region and 1.3–1.5 times improvements in <sup>1</sup>H SSNMR resolution for the isolated peaks in the α-region, presenting promising prospect for sequential assignment by <sup>1</sup>H-detected SSNMR in the approach. Details of sample preparation, amino-acid selectivity of the deuteration, and some other SSNMR results will be reported.

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Solid-State NMR Studies of Local Structure and Dynamics in  
Proton-Conducting Alginic Acid-Imidazole Composite

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Anhydrous proton-conducting polymers available above 100 °C have been widely developed for their applications in solid electrolyte fuel cells. Several proton-conducting polymer-imidazole composites have been proposed. In these composites, imidazole (Im) molecules are intercalated into a polymer and provide a proton conduction path. When proton conductivity of the polymer-Im composites is dominated by continuous proton transfer in the hydrogen bond network between Im molecules (Grotthuss mechanism), the reorientational motion of Im molecules plays an important role. For alginic acid (AA)-imidazole (Im) composite material (AA-xIm, where x represents the number of moles of Im per mole of carboxyl group of AA), proton conductivity increases with increasing x and becomes in the order of 10<sup>-3</sup> S/cm at around 400 K for AA-2Im. In the present work, we analyzed the local structure and dynamics of the AA-xIm composite using solid-state <sup>2</sup>H and <sup>13</sup>C NMR to elucidate the proton conduction mechanism. The difference between the motions of Im in AA-1Im and AA-2Im was clarified.

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### Assignment and Prediction of NMR Spectra of Paramagnetic Rare-Earth Complexes by First-Principles Methods

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Calculation of NMR parameters helps in the prediction, assignment and analysis of spectra. We present the application of the recently developed methodology for combined DFT and ab initio computation of paramagnetic shifts [1-4] to a series of 7 paramagnetic systems [Ln(III) with Ln = Pr, Nd, Sm, Eu, Er, Tm, Yb] with 1,10-phenanthroline and diethyldithiocarbamate ligands (1:3 ratio). Theory and calculations were used to investigate the paramagnetic effect on the NMR spectra. EPR parameters were calculated to determine the paramagnetic contribution to the isotropic shift. Hyperfine coupling tensor (HFC) was calculated using the four-component relativistic approach [5] with hybrid PBE0 functional and 40% exact exchange. HFC was also calculated using DFT with relativistic pseudopotential on the paramagnetic centre. The g-tensor and (for total spin  $S > 1/2$ ) zero-field splitting tensor were calculated ab initio employing CASSCF and N-electron valence-state perturbation theory [6]. The orbital shieldings were also calculated using DFT. The solution-state NMR spectra were recorded and spectral assignment was independently carried out with the help of 1D and 2D NMR. The total NMR shift calculated with four-component relativistic HFC proved to agree best with the experimental data in most cases. The method is promising for assignment of carbon and proton NMR signals in REE systems.

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Velocity auto-correlation spectra of water-glycerol binary fluid

Ales Mohoric<sup>\*,1</sup>, Janez Stepisnik<sup>1</sup>, Carlos Mattea<sup>2</sup>, Siegfried Stapf<sup>2</sup>

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The molecular dynamics of water in mixtures with 5–66 vol% of glycerol was studied by measuring the spectrum of the velocity auto-correlation (VAS) in the frequency range from 50 Hz to 10 kHz with the NMR method of modulated gradient spin echo (MGSE). The ability of the method to follow the time evolution of VAS reveals the heterogeneity of molecular dynamics in water and its mixtures with low glycerol content attributed to the diffusion in the vortexes of hydrodynamic fluctuations. By increasing the glycerol content, the spectrum exhibits a low-frequency peak that can be attributed to water clustered around a glycerol molecule. By taking into account the generalized Stokes-Einstein formula the form of VAS exposes the shear-thickening viscosity of mixtures, a possible mechanism behind the spontaneous folding of disordered poly-peptides into biologically active protein molecules. Results show that a low glycerol concentration only partially disrupts the hydrogen network of water and its molecular dynamics, but a higher glycerol completely changes the structure and dynamics in mixtures. In glycerol concentrations equal or higher than 10 vol% a new form of VAS develops, which confirms the existence of hydro-clusters formed around hydrophilic glycerol molecules. This strongly influences the dynamics of surrounding water molecules. Viscosity of such mixture inhibits the rapid movement of water molecules and thus protects the macro-molecule against collisions that would prevent the formation of its own structure.

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## P306

## NMR Enantiodifferentiation Study of Spiroglycol Chirality

Eva Monteagudo\*, Albert Virgili, Teodor Parella, Carles Jaime

*Universitat Autònoma de Barcelona*

The 3,9-bis(1,1-dimethyl-2-hydroxyethyl)-2,4,8,10-tetraoxaspiro[5.5]undecane commonly named pentaspiroglycol (PSG) or spiroglycol (SPG) is a high molecular weight rigid alicyclic diol widely used in the chemical industry. SPG has no hazardous classification, it is not mutagenic and is a safe alternative to Bisphenol A, a well-known chemical which is rising concern due to his proved endocrine disruptor activity. Moreover, some of the SPG main applications are focused on epoxy resins, liquid polyester resins, radiation curing resins and in polymer film material field. However, the spiroglycol structure, configuration and conformation have never been deeply studied.

Herein, we perform for the first time a preliminary NMR and computational study of the spiroglycol structure. SPG is a highly symmetrical molecule but it should be chiral due to the presence of a chiral axis. The presence of two enantiomers was demonstrated performing NMR enantiodifferentiation experiments using  $\alpha,\alpha'$ -bis(trifluoromethyl)-9,10-anthracenedimethanol (ABTE) as chiral solvating agent (CSA). The addition of 0.6 equivalents of ABTE allows the differentiation of several spiroglycol proton signals. The lack of resolution observed in the proton spectrum can be tackled through the corresponding  $^{13}\text{C}$  NMR spectrum where a significant enantiodifferentiation at the spirocarbon atom was observed.

In order to physically separate both enantiomers, a SPG derivatization with camphor-sulphonic acid was performed affording the corresponding diastereoisomeric ester mixture.

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## P307

**Average structure parameters of Asphaltenes by Magnetic resonance spectroscopy**  
Chulsoon Moon

Petroleum derived asphaltenes are known to be the most complex fraction of crude oil. They are also known to be coke precursors in refinery process resulting in several problems such as catalyst deactivation and poisoning because of their tendency to flocculate and precipitate during oil upgrading process. It is needed to understand more thoroughly the structures and behaviors of asphaltenes to predict their chemistry during refining process. However, petroleum asphaltenes are very complex aromatic molecules surrounded and linked by aliphatic chains. The inherent complexity of asphaltenes hinders a full identification of their constituents. A great variety of analytical techniques including various types of chromatographic methods and spectroscopic methods have been employed to investigate asphaltene molecular structure and still many things are going on. Among them NMR is a powerful analytical method as it allows to quantify different types of protons and carbons. Average structure parameters such as aromaticity, degree of ring condensation, and alkyl chain length can be evaluated by means of NMR.

Also, asphaltenes contain unpaired electrons and form complexes with vanadium. By EPR analysis organic asphaltene radical and vanadyl porphyrin can be discriminated. EPR can detect these species in order to determine the asphaltene content and can be used to understand the characteristics of asphaltenes. It can be used to predict reactivity of refinery process such as hydrotreating demetalization process.

In this work, we present the magnetic resonance spectroscopic work such as Liquid state NMR, Solid State NMR, Low field NMR, and EPR on petroleum derived asphaltenes in order to evaluate the structural aspects that could be correlated with crude oil behavior in thermal process.

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## P308

**<sup>1</sup>H MQ NMR observations of the segmental orientation autocorrelation function for various polymer systems**Anton Mordvinkin<sup>\*,1</sup>, Marcus Suckow<sup>2</sup>, Diana Döhler<sup>1</sup>, Frank Böhme<sup>2</sup>, Wolfgang Binder<sup>1</sup>, Kay Saalwächter<sup>1</sup><sup>1</sup>*Martin-Luther-Universität Halle-Wittenberg,*<sup>2</sup>*Leibniz-Institut für Polymerforschung Dresden e.V.*

In this contribution, we present a simple analytical fitting approach based upon a power-law model of the segmental orientation autocorrelation function (OACF), by the way of which an effective power-law time scaling exponent and the amplitude of the OACF can be estimated from multiple-quantum (MQ) NMR data at any given temperature [1]. This obviates the use of the time-temperature superposition principle and provides a robust and independent probe of the shape of the OACF, thereby eliminating the systematic errors of previous analytical strategies related to the used shift factors and inhomogeneities distorting the double-quantum (DQ) signal at short times. The approach is validated by application to polymer melts of variable molecular weight as well as elastomers and proved to be suitable for dynamic studies of supramolecular polymer networks with labile cross-links [2,3]. The use of the new approach expands a methodological toolbox for gaining mechanistic insights into the complex supramolecular dynamics.

**References:** [1] A. Mordvinkin and K. Saalwächter, J. Chem. Phys. 146, 094902 (2017); Erratum, J. Chem. Phys. 148, 089901 (2018). [2] M. Suckow, A. Mordvinkin, M. Roy, N. K. Singha, G. Heinrich, B. Voit, K. Saalwächter, and F. Böhme, Macromolecules, 51, 468 (2018). [3] A. Mordvinkin, M. Suckow, F. Böhme, R. H. Colby, C. Creton, and K. Saalwächter, submitted to Macromolecules, 2019.

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Amyloid formation of poly-ubiquitin chains investigated by  
biological rheo-NMR spectroscopy

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Masahiro Shirakawa<sup>1</sup>, Ulrich Scheler<sup>4</sup>, Kenji Sugase<sup>1</sup>

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Abnormal protein aggregation is a common hallmark of Alzheimer’s disease and other neurodegenerative disorders. Despite the growing interest in their pathogenesis, no existing method can capture aggregate nucleation and subsequent growth at atomic resolution in real time. In this study, we have recently established high-sensitivity Rheo-NMR spectroscopy that enables us to detect atomic-level structural changes of a protein during amyloid formation in real time [1]. By using the newly developed Rheo-NMR, we detected site-specific structural information on amyloidogenic proteins during their amyloid formation, gaining insight into the mechanism underlying amyloid nucleation at atomic resolution. Notably, in the formation of polyubiquitin fibrils from the native state structure, we detected the chemical shift changes of the side chains of residues located in flexible regions such as the edges of the alpha-helix and loops. These observations are consistent with our hydrogen-deuterium exchange results of polyubiquitin fibrils that the intrinsically flexible regions became highly solvent-protected in the fibril structure [2]. Thus, inter-molecular associations and secondary structure changes in the identified flexible regions can take place in the course of fibril formation.

**References:** [1] Morimoto, D., et al., Anal. Chem., 2017, 89 (14), 7286-7290. [2] Morimoto, D., et al., Polymers, 2018, 10 (3), 240.

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## P310

**Indirect NMR Detection of Formation and Decay of  
Guanine Cation Radical in Neutral Aqueous Solution**  
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*International Tomography Center*

DNA - the most important intercellular target for UV radiation - can be damaged through the direct absorption by nucleobases, or through photosensitized reactions with endogenous cellular chromophores. Guanine that has the lowest oxidation potential among all DNA components is the main target of one-electron oxidation reactions. Guanine radicals are supposed to be involved in the formation of a whole series of DNA damage products. That is why guanine radicals are of great interest to various research groups studying oxidative DNA damage.

Recently it was reported [1] that transient neutral guanine radical protonates to form a new cation radical,  $(G^{+})'$ , with a proton at position N7 in neutral aqueous solution. The well-characterized cation radical  $G^{+}$  protonated at position N1 has a  $pK_a$  value of 3.9, and the protonation of the neutral radical  $G(-H)'$  was not expected to proceed at neutral pH. Having a spectroscopic tool at hand that is very sensitive to the g-factors of transient radicals, we substantiated the protonation of neutral guanine radical at position N7 in neutral aqueous solution. The tool used was time-resolved chemically induced dynamic nuclear polarization (CIDNP). Primary neutral guanine radical in our experiments was generated in the photo-induced reaction of guanosine-5'-monophosphate (GMP) with triplet excited 3,3',4,4'-tetracarboxy benzophenone (TCBP). The confirmation of the GMP radical protonation was based on the inversion of CIDNP sign for TCBP and GMP protons on the microsecond timescale as a result of the change in magnetic parameters in the pairs of TCBP and GMP radicals due to structural changes of GMP radical. From the analysis of pH-dependent CIDNP kinetics, protonation and deprotonation rate constants were determined, that allowed to obtain  $pK_a=8.0$  of the cation radical  $(G^{+})'$ .

This work was supported by RFBR (project No. 17-03-00656).

**Reference:** [1] J. Choi et al., *J. Phys. Chem. Lett.* 2015, 6, 5045-5050.

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## P311

## REDOR and DIPSHIFT: Two sides of the same coin

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TIFR Hyderabad

REDOR and DIPSHIFT are two of the most commonly used experiments to determine dipole-dipole couplings (DCs) in solid-state MAS-NMR. Their robust performance with respect to experimental settings and the direct dependence of their dephasing profiles on a single variable - the DC, makes them highly attractive for studying a wide range of samples. Both these experiments achieve heteronuclear dipole-dipole recoupling by using rotor-synchronized  $\pi$ -pulses. This overt similarity in their experimental implementation is also reflected in their theoretical descriptions. Further, several modifications have been introduced in DIPSHIFT taking into account the multi-rotor nature of REDOR, further strengthening the link between these two experiments [1].

Here, it will be shown that these two sequences are in fact different implementations of the same master sequence. DIPSHIFT will be shown to be a constant-time version of REDOR. Building on approaches which have allowed us to measure the relatively strong  $^{13}\text{C}$ - $^1\text{H}$  dipole-dipole couplings ( $\sim 21$  kHz) using REDOR at a MAS frequency of 62.5 kHz, we will now show the use of DIPSHIFT to measure the relatively lower  $^{13}\text{C}$ - $^{15}\text{N}$  dipole-dipole couplings ( $\sim 1$  kHz) using a MAS frequency of 20 kHz. This flips the traditionally accepted domains of applicability of these two experiments. These developments shed new light on these old sequences and will further the development and implementation in a wide range of solid samples, to measure distance and dynamics as well. A direct result following these is the implementation of DIPSHIFT at fast MAS frequencies (62.5 kHz) as well as the implementation of REDOR, DIPSHIFT and TEDOR at fast MAS frequencies with RF frequencies lower than the MAS frequency itself.

**References:** [1] Cobo, M. F. et al., (2012). J. Magn. Reson. 221, 85. [2] Jain M. G. et al., (2019). J. Chem. Phys. 150, 134201

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## P312

Methods to improve resolution in  $^1\text{H}$  solid state NMR at ultra-fast MAS

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EPFL

The strong  $^1\text{H}$ - $^1\text{H}$  dipolar coupling network of organic solids yields  $^1\text{H}$  linewidths of few tens of kHz, orders of magnitude larger than those in solution. This homogeneous broadening is far greater than the isotropic chemical shift range and leads to broad and highly unresolved spectra from which very little information can be extracted.

Line broadening can be dramatically reduced by magic angle spinning (MAS).[1] Recently, newly developed technology allows the acquisition of spectra at spinning rates greater than 100 kHz and allows  $^1\text{H}$  linewidths on the order of a few hundred Hz. However, this is still not sufficient for many applications and limits the use of  $^1\text{H}$  in solid state NMR, especially for complex systems.

Here we show that in powdered organic solids in this fast spinning rate regime, homonuclear dipolar couplings are no longer the dominant source of line broadening. We also explore new methods to further improve the spectral resolution, and we show how to obtain experimental linewidths up to a factor of two narrower than those achieved with a conventional experiment at the same MAS rate.

Reference: [1] Andrew, E.R., et al., *Nature*, 1958, **182**, 1659; Lowe, I.J., *Phys. Rev. Lett.*, 1959, **2**, 285.

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## P313

**Theoretical and multinuclear NMR study of the reaction mechanism of diethyl 2,4,6-trimethyl-1,4-dihydropyridine-3,5-dicarboxylate with N-bromosuccinimide.**

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The 1,4-dihydropyridine (1,4-DHP) nucleus is the scaffold for important cardiovascular drugs of calcium antagonist class, such as nifedipine, nitrendipine, amlodipine, and nisoldipine [1].

The increasing interest has been devoted to the synthesis of novel 1,4-DHP derivatives owing biological activities which mainly are not related with their calcium L-channel regulating properties. During the last decade new and efficient gene delivery systems based on cationic self-assembling amphiphilic 1,4-dihydropyridine derivatives were investigated and elaborated [2].

Bromination of the methyl groups at positions 2 and 6 of the 1,4-DHP cycle is one of the most important steps in the synthesis of cationic amphiphilic 1,4-DHP derivatives as potential candidates for the development of new gene delivery systems.

In this work the bromination of diethyl 2,4,6-trimethyl-1,4-dihydropyridine-3,5-dicarboxylate with different amounts (from one to six equivalents) of N-bromosuccinimide (NBS) in methanol was studied by multinuclear NMR spectroscopy and quantum chemistry.

The study revealed previously unknown intermediates which were possible to register only in the reaction mixture. The experimental findings were confirmed by DFT calculations. The mechanism of the bromination in the 2,6-methyl side chains of 1,4-DHPs was estimated and the structures of the major products and intermediates were confirmed by <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N 1D and 2D-NMR experiments.

An increase in the temperature from 25 to 40°C speeds up the reaction rate and does not have an effect on its mechanism.

**References:** [1] Triggie, D. J. The 1,4-dihydropyridine nucleus: a pharmacophoric template part 1. Actions at ion channels. *Mini Rev. Med. Chem.* 3, 215–23 (2003). [2] Hyvönen, Z. et al. Novel cationic amphiphilic 1,4-dihydropyridine derivatives for DNA delivery. *Biochim. Biophys. Acta - Biomembr.* 1509, 451–466 (2000).

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Urinary NMR profiling in pediatric acute kidney injury - a pilot study

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The incidence of acute kidney injury (AKI) in pediatric patients is increasing. Depending on the underlying etiology, AKI mortality is still as high as 60%. Therapeutic interventions that improve renal regeneration following kidney damage do not exist. In addition, the current diagnostic gold standard for AKI, the serum creatinine, is only a quite unspecific late measure of kidney function. Hence, there is an unmet need for novel biomarkers of AKI as diagnostic tools for early detection, differential diagnosis and prognosis.

In this study we investigated whether analysis of the urine metabolite pattern could fill this gap and reliably diagnose AKI in neonates and children. A cohort of 74 pediatric AKI patients (0-18 years) was compared to a control group comprising both healthy children and critically ill children without AKI. This allowed us to distinguish between general traits of sickness like markers of increased catabolic pathways or drug metabolism and those specific for AKI. Multivariate analysis identified a series of metabolites that allowed prediction of AKI with an area under the curve receiver operating characteristics (AUC-ROC) of 0.94 and a confidence level of 0.88 – 0.99. Especially citrate and formate levels were significantly reduced whereas lactate, leucine and valine levels were elevated throughout the samples. Attempts to further distinguish the different causes leading to AKI appeared promising but due to the low cohort number, results need to be validated in subsequent larger studies.

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## P315

 **$^{19}\text{F}$  solid-state NMR of  $\text{TiF}_4$ : Anomalous  $^{19}\text{F}$  chemical shift**Kiyonori Takegoshi<sup>1</sup>, Miwa Murakami<sup>\*,1</sup>, Yasuto Noda<sup>2</sup><sup>1</sup>Kyoto Univ., <sup>2</sup>Kyoyo Univ.

To examine bonding nature of fluorine ligands in a metal coordinated system,  $^{19}\text{F}$  high-resolution solid-state NMR has been applied to  $\text{TiF}_4$ , which bears both bridging and terminal fluorines. By referring to the calculated isotropic shifts using DFT, the observed 12 isotropic signals were assigned to 12 crystallographically different fluorines (6 terminal ( $\text{F}_\text{T}$ ) and 6 bridging ( $\text{F}_\text{B}$ ) fluorines) in  $\text{TiF}_4$ . The isotropic chemical shift ( $\delta_\text{iso}$ ) for  $\text{F}_\text{T}$  appears at high frequency (420~480 ppm from  $\delta(\text{CCl}_3\text{F}) = 0$  ppm) with large chemical shielding anisotropy of  $\Delta\sigma \sim 850$  ppm. Whereas the  $\delta_\text{iso}$  and  $\Delta\sigma$  values for  $\text{F}_\text{B}$  are “moderate”;  $\delta_\text{iso} \sim 0\sim 25$  ppm and  $\Delta\sigma \sim 350$  ppm. The origin of the seemingly anomalous high-frequency shift and large  $\Delta\sigma$  for  $\text{F}_\text{T}$  is ascribed to the second-order paramagnetic shift with increased covalency, shorter Ti-F bonds, and smaller energy difference between the occupied and vacant orbitals on the basis of the Ramsey’s theory. Examination of the orientation of the chemical-shielding shows that the most deshielded component of the shielding tensor is oriented along the Ti-F bond. This characteristic orientation is ascribable to a Ti-F  $\sigma$  bond formed by  $d_{yz}$  of Ti and  $p_z$  of F. Further, we selectively observed  $\Delta\sigma$  and  $\eta$  (shielding asymmetry) for each signals by the rotation-synchronized DANTE and compare with the calculated values. The calculated  $\Delta\sigma$  values are ca. 40~50 % larger than those observed for  $\text{F}_\text{B}$ , indicating deficiency of the DFT calculation in predicting the value of shielding anisotropy.

Acknowledgment: This work was supported by R&D Initiative for Scientific Innovation on New Generation Batteries 2 (RISING2) Project administrated by New Energy and Industrial Technology Development Organization (NEDO).

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## P316

## A Functional Interaction Study Between Two IDPs

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Direct interaction between intrinsically disordered proteins is often difficult to be characterized hampering the elucidation of their binding mechanism. Particularly challenging are the cases of extreme fuzziness of the complex, which is now appearing as a common interaction mode, requiring new models for their description.[1]

So far, nuclear magnetic resonance spectroscopy has proven to be one of the most powerful techniques to characterize at atomic level intrinsically disordered proteins and their interactions, including those cases where the formed complexes are highly dynamics.[2]

Here we present the characterization of the interaction between a viral protein, the Early region 1A protein from Adenovirus,[3] and a disordered region of the human CREB-binding protein, namely the fourth intrinsically disordered linker CBP-ID4.[4]

E1A has been widely studied as a prototypical viral oncogene. Its interaction with folded domains of CBP has been mapped before, proving its functional interaction with this transcriptional factor.[5] However, the role of the linkers of CBP in this interaction has never been investigated before.

**References:** [1] M. Fuxreiter, P. Tompa, 2012, *Advances in Experimental Medicine and Biology*, vol 725. Springer, New York, NY. [2] a) S. Contreras-Martos *et al.*, *Sci Rep.* 2017, 7(1),4676 ; b) A. Borgia, K. Bugge *et al. Nature*, 2018, 555(7694):61-66. c) R. Schneider *et al. Curr Opin Struct Biol.* 2018, 54,10-18. [3] T. Hosek *et al. Chem. Eur. J.* 2016, 22, 13010–13013. [4] A. Piaia *et al. Biophys. J.* 2016, 110, 372–381. [5] a) J. C. Ferreon *et al. Proc.Natl.Acad.Sci.USA* 2009, 106, 13260-13265;b) P. Haberk *et al. Protein Sci.*, 2016, 25,2256-2257.

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## P317

### Combinatorial Selective Labeling Facilitates Mapping of Binding Interfaces in Complex of Spider Toxin with Human Voltage-Gated Sodium Channel

Mikhail Myshkin<sup>\*1</sup>, Maxim Dubinniy<sup>2</sup>, Dmitrii Kulbatskii<sup>2</sup>, Alexander Paramonov<sup>2</sup>, Antonina Berkut<sup>2</sup>, Alexander Vassilevski<sup>2</sup>, Ekaterina Lyukmanova<sup>2</sup>, Zakhar Shenkarev<sup>2</sup>

<sup>1</sup>*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS,*

<sup>2</sup>*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry*

Gating modifier toxins (GMTs) from spider venoms may serve as pharmacological hits for treatment of hereditary diseases caused by mutations in genes coding voltage-gated sodium channels (Nav). Nav channels are transmembrane proteins, consisting of one pore domain and four surrounding voltage-sensing domains (VSDs). In particular, mutations in the S4 transmembrane helical segments of the VSDs of the skeletal muscle channel Nav1.4 lead to leak (gating pore) currents through the VSDs and cause hypokalemic and normokalemic periodic paralyses (PPs). Solution NMR studies of membrane proteins like isolated VSDs of Nav channels may be hampered by limited stability of their samples or low quality of NMR spectra in membrane-mimicking media. In these cases, standard techniques for protein backbone resonance assignment could not provide enough assignment information. Here we used Combinatorial Selective Labeling (CSL) approach and our new CombLabel software [1] to facilitate backbone resonance assignment of VSD-I and VSD-II of human Nav1.4 channel. The extent of obtained backbone assignment (~50%) was sufficient for mapping of the binding interfaces in the complexes of both VSDs with Hm-3 toxin from crab spider *Heriades mellotei*. The binding interfaces of the Hm-3 molecule, responsible for interaction with the membrane, VSD-I and VSD-II, were also determined. Subsequent protein-protein docking driven by NMR restraints revealed two different Hm-3 binding modes. In case of VSD-I the toxin binds to the S3-S4 extracellular loop, while in VSD-II it binds to the S1-S2 loop. We have shown that Hm-3 inhibits leak (gating pore) currents through the Nav1.4 channel with mutations in S4 segments of both VSD-I and VSD-II and represents a useful hit for PP therapy [2]. This work was funded by the Russian Science Foundation (#16-14-10338).

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Characterization of Protein-Polymer conjugates

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<sup>3</sup>Tel Aviv University, <sup>4</sup>University of Potsdam / Fraunhofer IAP

To decorate biotechnologically prepared proteins with chemically synthesized polymers opens the route to hybrid molecules and materials with novel and unique properties.

The protein component may provide highly specific biochemical activity whereas the polymer part may improve long-term stability, modulate bioavailability and enable the integration of biological function into bulk (polymer-)materials. These hybrid molecules are attractive for a broad range of applications, e.g. in separation/chromatography, analytics/biosensing, or medical technology.

As a model system, we conjugated the cellulose-binding CBM3b protein from *C. thermocellum* with poly(N-isopropylacrylamide) (PNIPAm). The polymer chains were either attached to the lysine residues and to the N-terminal amino group via the “grafting-to” approach, or the polymer was grown on the protein following a “grafting-from” scheme.

The protein polymer conjugates were then analyzed by NMR spectroscopy, SDS PAGE and gelfiltration chromatography.

We will present how attaching polymer chains to or growing polymer chains from a globular protein affects the structural integrity and the ligand binding affinity of the protein. Our studies provide a deeper understanding of the protein polymer interactions and provide critical insight on the way to improved functional materials.

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**P319**

**Spin noise, radiation damping, and maser emissions in NMR**

Maria Theresia Zich (née Pöschko), Matthias Bechmann,

Norbert Müller\*, Stephan Ginhör, Victor Rodin

*Johannes Kepler University*

Spin noise and maser emissions in NMR are two phenomena, which are linked, if not caused by radiation damping. [1-3] Our research group has been investigating spin noise in imaging and spectroscopy for some time. [4,5] NMR maser phenomena have been observed initially for hyper-polarized spin ensembles [6] but recently have also been reported in samples with inverted thermal polarization [7-9].

We are presenting our first results aiming at exploiting maser emissions from thermally polarised samples in coherence transfer and imaging experiments, which are based on experience with spin noise detected 2D NMR [10]. The main challenges to overcome are deterministic triggering of the maser emissions and the high non-linearity.

**References:** [1] Bloembergen N, Pound RV. Phys. Rev. (1954) 95: 8. [2] Vlassenbroek A, Jeener J, Broekaert P. J. Chem. Phys. (1995) 103: 5886. [3] Ferrand G, Huber G, Luong M, Desvaux H. J. Chem. Phys. (2015) 143: 094201. [4] Pöschko MT, Vuichoud B, Milani J, Bornet A, Bechmann M, Bodenhausen G, Jannin S, Müller N. ChemPhysChem (2015) 16: 3859. [5] Pöschko MT, Rodin VV, Schlagnitweit J, Müller N, Desvaux H. Nat. Comm. (2017) 8:13914. [6] Chen HY, Lee Y, Bowen S, Hilty C. J. Magn. Reson. (2011) 208: 204. [7] Jurkiewicz A. Applied Magnetic Resonance (2013) 44: 1181. [8] Jurkiewicz A. Chem. Phys. Lett. (2015) 623: 55. [9] Jurkiewicz A. Applied Magnetic Resonance (2019) 50: 709. [10] Chandra K, Schlagnitweit J, Wohlschlager C, Jerschow A, Müller N. J. Phys. Chem. Lett. (2013) 4: 3853.

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### Irreversible conjugation of lanthanide chelating tags for in-vitro and in-cellulo protein PCS NMR spectroscopy

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Understanding the function of a protein relies on the precise knowledge of its structure, dynamics and interactions. Pseudocontact shifts (PCS) have proven to be a valuable source of distance and angle-dependent restraints to monitor structural changes. DOTA-based lanthanide chelating tags (LCTs) have found numerous applications for the measurement of PCS on cysteine mutant proteins. A major drawback of most modern lanthanide chelating tags (LCT) is their dependence on disulfide bonds as chemical linkage combined with long and flexible linkers. However, the inherent instability of disulfide bonds towards strongly reducing conditions as they are found e. g. in living cells renders these LCTs unsuitable for in-cellulo applications. In contrast, thioether bonds are chemically very inert towards nucleophilic substitutions, strongly reducing as well as highly acidic or basic conditions. We have used a seven-fold methylated DOTA scaffold with a pyridine sulfone-based linker to exploit nucleophilic aromatic substitution. However, conjugation rates were low and tagging required long reaction times or more extreme conditions like high pH or high temperature. Here we wish to present a modified LCT which uses a pyridine thiazole sulfone-based linker and offers largely increased tagging rates at physiological conditions. The short and rigid linkage in combination with a new attachment point results in a superior relative orientation of the tag compared to its pyridine-based analogues. This novel orientation further reduces mobility and thus considerably increases the overall tensor size. In conclusion, due to the high stability of the linkage and its novel orientation, large PCS (>10 ppm) and RDC (>30 Hz) can be measured irrespective of the composition of the buffer.

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Observation of high-resolution NMR with a cryogen-free  
high-temperature superconducting bulk magnet

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RIKEN

Helium prices are skyrocketing as the supply of helium from the United States is decreasing. For this reason, bench-top NMR of permanent magnets that do not require helium is beginning to spread. Since a permanent magnet is difficult to generate a magnetic field of 2 T or more, a cryogen-free superconducting magnet will be required in the future. Since we have developed a cryogen-free high-temperature superconducting bulk magnet using a refrigerator, we installed a room-temperature shim and sample spinner on this magnet and observed NMR spectra.

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Study on the Growth Behavior and the Dynamics of  
Polyelectrolyte Multilayers using EPR Spectroscopy

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Polyelectrolyte multilayers (PEMs) have been prepared by using the layer-by-layer (LbL) technique first introduced by Decher.[1] Adsorbing alternatingly the polycation poly(diallyldimethylammonium chloride) (PDADMAC) and the spin-labeled polyanion poly(ethylene-*alt*-maleic acid) (SL-P(E-*alt*-MA)) on the inner surface of a glass capillary multilayer up to 31 layers could be realized. Using the spin-label allows the acquisition of electron paramagnetic resonance (EPR) spectra to get insight into the growth behavior as well as into the dynamics of the PEMs.[2,3] For monitoring the growth of the PEM films with quantitative EPR the spin-labeled polyanion has been used in every second layer during preparation. If the spin-labeled polyanion has been selectively placed in different layers in the PEM film selective information on the dynamics, in particular the chain segment mobility in this specific layer can be obtained. The study has been focused on the results of the EPR measurements at pH values 3 to 6 using citrate buffer for keeping the pH constant. For all multilayers in the pH range 3 to 5 a transition from a parabolic to a linear growth has been observed. The pH has a strong impact on the dynamics of the polyanion in the PEMs.

**References:** [1] G. Decher, J. D. Hong, J. Schmitt, *Thin Solid Films* **1992**, 210 (1-2), 831-835. [2] U. Lappan, B. Wiesner, S. Zschoche, U. Scheler, *Applied Magnetic Resonance* **2013**, 44, 181. [3] U. Lappan, C. Rau, C. Naas, U. Scheler, *Macromolecules* **2019**, 52, 2384.

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The fuzzy  $\beta$ -domain of MEF2D: NMR and computational studies

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The MEF2 transcription factors play crucial roles in muscle cell myogenesis and morphogenesis[1].

Bioinformatical analysis indicates that the alternatively spliced  $\beta$ -domain of MEF2D does not fold into a well-defined structure and likely remains conformationally heterogeneous upon interactions. To study the role of protein dynamics in the biological function, a series dynamical variants of the of 15-37 AA of MEF2D  $\beta$ -domain were designed, affecting the conformational transition upon binding.

The dynamics of the free peptides has been studied using 15N relaxation measurements (T1, T2, heteronuclear NOE) from which reduced spectral density functions were calculated. Random Coil Indexes (RCI) were determined from the chemical shifts (CA, CB, N) to derive model-free order parameters[3]. Diffusion NMR-spectroscopy (DOSY) showed the differences between the degree of structural compactness and aggregation properties. Experimental NMR parameters were comparable to those obtained from 3x100 ns long molecular dynamics simulations (MD). Order parameters of the backbone amide bond vectors, the propensity of transient secondary structure elements, radius of gyration and other descriptors of disorder were calculated. Detailed analysis of the dynamical and structural properties of the peptides will give insights into the altered interaction patterns of the MEF2D  $\beta$ -domain with altered biological outcomes.

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# Photointermediates and Photoreaction Pathways of Sensory Rhodopsins as Revealed by In Situ Photoirradiation Solid-State NMR Spectroscopy

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Photoirradiation solid-state NMR spectroscopy is a powerful means to study photoreceptor retinal-binding membrane proteins by the detection of short-lived photointermediates to elucidate the photoreaction pathways and photoactivated intermediates. An in situ photoirradiation solid-state NMR apparatus has been developed for the irradiation of samples from inside a sample tube with extremely high efficiency to enable observation of photointermediates [1,2]. In situ photoirradiation solid-state NMR is particularly useful to study photoreaction pathways of sensory rhodopsin I (SRI) and sensory rhodopsin II (SRII) because functional photointermediates have relatively longer half-lives than bacteriorhodopsin [1-5].

Pharaonis phoborhodopsin (ppR or SRII) is a transmembrane photoreceptor protein involved in negative phototaxis. We could observe M, N' and O-intermediates by illumination with green light (520 nm). Under UV light (365 nm) irradiation of the M-intermediate, <sup>13</sup>C NMR signal of the O-intermediate increased, indicating that the M-intermediate transformed to the O-intermediate. Subsequently, O-intermediate transformed to N'-intermediate to establish the equilibrium state between the N'- and O-intermediates. N'-intermediate was revealed to be transformed from O-intermediate [2,3].

Sensory rhodopsin I (SRI) can discriminate multiple colors for the attractant and repellent phototaxis. A M-intermediate was trapped at -40 °C by irradiation with green light (520 nm). The M-intermediate was transformed to the P-intermediate by subsequent irradiation with UV light (365 nm). Thus, stationary trapped M- and P-intermediate by irradiation with green and UV lights are responsible for positive and negative phototaxis, respectively [4].

**References:** [1] Naito et al., *Biophys. Reviews*, **2019**, 11, 167-181. [2] Tomonaga et al., *Biophys. J.*, **2011**, 101, L50-L52. [3] Makino et al., *Biophys. J.*, **2018**, 115, 72-83. [4] Yomoda et al., *Angew. Chem. Int. Ed.*, **2014**, 53, 6960-6964. [5] Oshima et al., *Photochem. Photobiol. Sci.*, **2015**, 14, 1694-1702.

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## P325

**Probing transmembrane protein environment by solid-state NMR spectroscopy**Eszter Najbauer<sup>\*1</sup>, Kumar Tekwani Movellan<sup>1</sup>, Tobias Schubeis<sup>2</sup>, Tom Schwarzer<sup>3</sup>,Kathrin Castiglione<sup>4</sup>, Karin Giller<sup>1</sup>, Guido Pintacuda<sup>5</sup>, Stefan Becker<sup>1</sup>, Loren B. Andreas<sup>6</sup>

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A protein's environment is often key to understanding its structure and function. However determining the environment of a membrane protein at atomic resolution is difficult by most techniques used in structural biology. We present a method to site-specifically probe water- and lipid proximities in membrane proteins at a resolution of ~3 Å using magic-angle spinning (MAS) NMR spectroscopy under conditions of high magnetic fields, fast spinning and perdeuteration. We demonstrate the applicability of this method on two trans-membrane proteins: the alkane transporter AlkL from *Pseudomonas putida* and the human voltage-dependent anion channel (hVDAC). By using a combination of sensitive 3D and well-resolved 4D experiments, it was possible to obtain a comprehensive picture of water- and lipid exposed surfaces in both proteins.[1] Site specificity is explained by determining lipid-protein and protein-protein transfer rates. The method can not only be used to explore a membrane protein's environment, but also to facilitate assignments and follow structural rearrangements.

The method was further extended to investigate binding of small molecules to a membrane protein. Cholesterol has been shown to be essential for membrane insertion and gating of hVDAC. Previous methods for studying small molecular binding by NMR either required modified cholesterol or relied on artifact-prone chemical shift perturbations. We used our method to directly identify cholesterol contact sites in VDAC and showed that our results agree well with contacts previously measured in solution[2], and predicted by docking and molecular dynamics simulations.[3]

**References:** [1] Najbauer, E. E.; Tekwani Movellan, K.; Schubeis, T.; Schwarzer, T.; Castiglione, K.; Giller, K.; Pintacuda, G.; Becker, S.; Andreas, L. B., *ChemPhysChem*, 2019, 20, 302-310. [2] Hiller, S.; Garces, R. G.; Malia, T. J.; Orekhov, V. Y.; Colombini, M.; Wagner, G. *Science*, 2008, 321, 1206-1210. [3] Weiser, B.P.; Salari, R.; Eckenhoff, R. G.; Brannigan, G. *J Phys Chem B*, 2014, 118, 9852-9860

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## P326

## Electronic Structure Investigation of Self-doped type Organic Conductors

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So far, we investigated the electronic structure of novel type of organic conductors, ammonium tetrathiafulvalene carboxylate (TTFCOO) and its and tetrathiapentalene derivative (TTPCOO) by high-field ESR and NMR measurements. The pristine TTFCOOH and TTPCOOH molecules are closed-shell. Kobayashi and coworkers (NIMS, Japan) found that self-doped type carrier was generated by substitution of the end group of ( $\text{NH}_3^0$ ) with ( $\text{NH}_4^{+1}$ ), which is regarded as a charge-reservoir. Because of sample limitation (powder), the detailed electronic state (anisotropic g-tensor and linewidth) was not clarified within the framework of conventional X-band ESR spectroscopy. By using W-band, however, a clear powder pattern structure could be found. We can evaluate the principal values of the g-tensor for these salts, assuming anisotropic g-values. We found that TTFCOO system shows 1D column structure. On the other hand, the TTPCOO derivative system seems to be isotropic structure within 2D layer. As a result, TTFCOO system is a narrow-gap semiconductor because of 1D instability, while TTPCOO shows a stable metallic state down to 2K. We also performed Detailed discussion  $^1\text{H}$ -NMR measurements down to 2K and clarified electronic states of these systems.

Recently, Kobayashi developed new family of single component electroactive molecules, zwitterionic tetrathiafulvalene extended dicarboxylate radical (TED-X). They show very high conductivity even at room temperature. The most characteristic feature of TED-X is that the carrier concentration of the system can be controlled within the same framework. It could not be achieved with conventional organic conductors. In this paper, we perform a series of detailed magnetic investigation to understand the competed electronic phases. We discuss the mechanism of the competed electronic phases.

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Baseline Correction Method by Improved Polynomial  
Fitting using Lp-norm Minimization  
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Various baseline correction methods for Nuclear Magnetic Resonance (NMR) spectra, such as polynomial fitting, smoothing, and Asymmetric Least Square (ALS), have been proposed. ALS is a highly accurate and semi-automated method, but this has heavy dependence on hyper-parameter  $\lambda$ . Smoothing is a classical method, but Automated Iterative Moving Average (AIMA) which is one representative of this approach is a fully automatic method. However, the accuracy of the baseline correction using AIMA is lower in comparison to the other methods. Polynomial fitting is a classical approach too, but this has high accuracy of baseline correction. On the other hand, this method requires suitable settings of the fitting functions to exercise sufficient performance, and it is often difficult to set. This requirement reduces user friendliness of the method.

In this report, a new baseline correction algorithm is proposed. This is an improved version of the polynomial fitting. In particular, this attempts to solve

$$\text{minimize } \|c\|_p \text{ subject to } y_I = B_I c,$$

where  $\|\cdot\|_p$  is Lp-norm,  $c$  is the weight vector,  $I$  is the clustered index corresponding to the number of baseline points,  $y_I$  and  $B_I$  are the observed vector and fitting matrix corresponding to  $I$ . The matrix  $B_I$  is designed based on fitting functions that are specified by user. The row vectors of the matrix  $B_I$  are equivalent to the bases that compose an observed vector  $y_p$ , and the elements of vector  $c$  are the weights of each basis. The proposed algorithm can remove the unnecessary bases from the bases specified by the user. Having this ability means that numbers and shapes of fitting functions may be roughly set. The proposed algorithm is less dependent on parameters without sacrificing the advantage of the traditional polynomial fitting.

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P328

Double micro-resonators for orientation-free MR-catheter tracking during interventional MRI, enabled by inductive coupling

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Accurate geometrical tracking of the tip of a catheter during minimal invasive surgery using intervention MRI provides a surgeon with orientation relative to the body. The tip can be visualized in the MR environment by locating a micro-resonator at this position. If the resonator is tuned to the Larmor frequency of abundant nuclei, and subject to a radio frequency excitation pulse, it's motion can be tracked under direct image guidance. The conductive cable connecting the detector to the spectrometer, for signal transfer and matching and tuning, cause dangerous tissue heating and has prevented this technology to find widespread use. An alternative is to couple the resonator wirelessly through inductive coupling to a wired resonator outside of the body. Tuning and matching is then confined to the external resonator. However, as the catheter orientation changes, the coupling between the detector and the external coil will also change, which leads to a change in the visibility of the catheter. Visibility can be totally lost if the wireless resonator and external coil's  $B_1$  fields are orthogonal. Also, when the coupling changes, the entire system will need to be re-matched and tuned. Here we present a novel detector design based on two perpendicular miniaturized saddle coils that maintain constant coupling strength between the MR-catheter detector and the external coil, for all axial orientations. We also present a multi-layer fabrication process for the conductive structure of the resonator, on a flexible and low aspect ratio substrate that is suitable for wrapping around catheters without significantly modifying either its stiffness or diameter. A setup is presented for characterizing the coupling strength of the two coils for all possible orientations. The results for two different detectors (single and double saddle coil) were, compared showing significant enhancement in the coupling in the case of the proposed double coil.

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The Effect of Spin Diffusion on the Decay of Dipolar Order in Spinal Cord

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Tissues contain semi-solids whose spectra are determined by distribution of dipolar interactions. Double quantum filtered (DQF) sequences enable to select species with particular dipolar interaction. Thus, by combining the DQF pulse sequence with magnetization transfer, measuring the rate of spin diffusion among the different species and orientations is possible. This was previously demonstrated for nervous systems such as spinal cord. In the present work, we modify the DQF-MT pulse sequence in a way that follows the time evolution of the dipolar order and spin diffusion.

Spectra were obtained using a 9.4T Bruker spectrometer with an Avance III HD console. Porcine spinal cords were obtained from freshly sacrificed animals and soaked in saline.

The spectrum at shortest magnetization transfer (MT) time consists of two peaks separated by ~20kHz, typical of dipolar interaction between protons in the CH<sub>2</sub> group with their internuclear vector parallel to the magnetic field. At longer MT time of 0.1ms, the splitting collapses while its integral is unchanged, indicating spin diffusion. For even longer MT times of 1-5 ms there is a buildup of a peak with resonance frequency typical of CH<sub>3</sub> groups, indicating MT between CH<sub>2</sub> and CH<sub>3</sub> groups. The slower rate of the latter process is probably the result of CH<sub>3</sub> rotation around its axis.

The decay of dipolar order was fitted to three exponentials. The shortest decay time T<sub>D1</sub> is compatible with spin diffusion and is therefore consistent with the interpretation of the role that spin diffusion plays in reducing the dipolar order. T<sub>D2</sub> is compatible with the magnetization transfer from the CH<sub>2</sub> protons to those of the CH<sub>3</sub>. The decay time T<sub>D3</sub> is compatible with the decay time of dipolar order measured for CH<sub>3</sub> peak. These values are consistent with the values of the dipolar decay time used to interpret the ihMT imaging sequence

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### NON-STATIONARY 2D FIDs FROM REACTION MONITORING - HOW TO DEAL WITH THEM?

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Nuclear Magnetic Resonance is widely used for qualitative as well as quantitative evaluation of chemical compounds. For chemists, it is particularly valuable tool providing an insight into reactions and allowing to study their mechanisms. An interesting case is the observation of intermediate products that can not be observed in the post-reaction mixture. Often, changes in their chemical shifts during the reaction make the observation difficult, leading to the broadening of 2D peaks and their disappearance under the noise.

Most often, in order to monitor chemical reaction, 1D spectra are acquired. However, 2D spectra make analysis more complete. Unfortunately, sampling of a 2D FID is time consuming and can take too long comparing to the rate of studied changes, which can lead to several distortions in a spectrum.

We show two approaches, how to deal with non-stationary 2D FIDs. First, with the use of TReNDS software[3], we have been able to implement interleaved non-uniform sampling of 2D and 1D spectra and use the latter to correct the variations affecting the former. Secondly, we show that the correction can be also applied to a conventionally sampled 2D ROESY spectrum. Our non-standard measurement methods allow to increasing the quality of the spectrum and provide information, e.g. about reaction kinetics and how it is influenced by various conditions, such as change in temperature or pH. For us, correction algorithms helped to reveal the mechanism of AgSO<sub>4</sub> induced synthesis of diphenylmethane-type compounds.

**References:** [1] D. A. Foley et al., NMR Flow Tube for Online NMR Reaction Monitoring; Anal. Chem., 2014, 86 (24), 12008-12013. [2] Q. N. Van et al., Comparison of 1D and 2D NMR Spectroscopy for Metabolic Profiling; J. Proteome Res., 2008, 7 (2), 630-639. [3] M. Urbańczyk et al., Magn. Reson. Chem. 2019, 57 (1), 4-12, (<http://trends.spektrino.com/>).

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## P331

### Interaction of Na<sup>+</sup>-translocating NADH:quinone oxidoreductase with quinones characterized by NMR spectroscopy

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The Na<sup>+</sup>-translocating NADH:quinone oxidoreductase (Na<sup>+</sup>-NQR) is a large protein complex found in *V. cholerae*. It is a part of the respiratory chain involved in energy metabolism and Na<sup>+</sup>-homeostasis. The Na<sup>+</sup>-NQR consists of six structurally heterogeneous subunits (NqrA-F) and at least six redox-active cofactors coupling the free energy liberated during oxidation of NADH and reduction of ubiquinone to pump sodium ions across the cytoplasmic membrane. The sodium gradient is used e.g. for movement of the bacterium and for maintaining constant pH in the cytoplasm. Although the structure of Na<sup>+</sup>-NQR was published [1], it is still not clear how the redox process is coupled to sodium ion translocation and what are the molecular details of the final electron transfer steps.

Saturation transfer difference (STD) NMR spectroscopy in combination with other methods revealed that the NqrA subunit of Na<sup>+</sup>-NQR (50 kDa) harbors the catalytic quinone binding site. Furthermore, STD NMR, SPR experiments, and Trp fluorescence quenching titrations indicated that NqrA can bind two quinone ligands. Interligand NOEs between ubiquinone-1 and the inhibitors 2,5-dibromo-3-methyl-6-isopropylbenzoquinone (DBMIB) and 2-n-heptyl-4-hydroxyquinoline N-oxide (HQNO) showed that NqrA simultaneously interacts with two quinone-type molecules in an extended binding site providing, for the first time, direct experimental evidence for the long-standing double occupancy hypothesis. The NqrA subunit was prepared in <sup>2</sup>H, <sup>13</sup>C, <sup>15</sup>N-labelled form for backbone assignment of the protein and the DBMIB binding site was located by chemical shift perturbation mapping. Detailed analysis by mass spectrometry combined with mutation studies revealed that – in addition to reversible binding – DBMIB modifies the NqrA subunit also covalently at a separate site shedding new light on the inhibitory mechanism of this molecule regarding the Na<sup>+</sup>-NQR and several other oxidoreductases.

**Reference:** [1] Steuber, J., Vohl, G., Casutt, M., Vorburger, T., Diederichs, K., Fritz, G., *Nature* 516(7529), 62-67 (2014).

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Importance of  $t_0$  selection in FID signal deconvolution-based SFC determination  
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In the food industry, low-field NMR has become the standard for solid fat content (SFC) determination of edible fats. The  $T_2$ -relaxation behaviour of a semi-solid sample distinguishes a fast and slow relaxing part after a  $90^\circ$ -pulse, hence an FID experiment, related to the solid fat and liquid oil, respectively. Yet, data acquisition only starts after the  $90^\circ$ -pulse and the dead time. Deconvolution-based quantification of the SFC, however, requires the initial, total signal intensity ( $I_0$ ) before relaxation occurred. The start of the solid fat relaxation is referred to as  $t_0$ . The most common practice relies on extrapolation of the signal intensity to the end of the  $90^\circ$ -pulse to bridge the dead time in order to estimate  $I_0$ . In this study, we investigated whether this  $t_0$  selection yielded an accurate estimation of  $I_0$ , and thus an accurate SFC.

FID-relaxation curves of liquid oil, solid fat and blends with various SFC were acquired using a benchtop 23.4 MHz Maran-Ultra spectrometer at  $5^\circ\text{C}$ . First, all fat blends were analysed using a fixed  $90^\circ$ -pulse length (of 8  $\mu\text{s}$ ). Fixing  $t_0$  between 62.5% and 75.0% of the imposed  $90^\circ$ -pulse length yielded similar  $I_0$  using a bi-Gaussian model fit, when taking into account the proton density of the fat blends. Second, FID curves of a semi-solid sample and a nearly 100% solid sample were collected using different values of the  $90^\circ$ -pulse length. The FID-curves coincided after subtracting 61.0% of the pulse length from the time values (recorded from the start of the  $90^\circ$  pulse), after a correction for the signal intensity of the liquid part. In a next step, the SFC was determined by a Gaussian deconvolution approach based on CONTIN-analysis. The results indicated that the SFC was largely underestimated when an extrapolation to the end of the  $90^\circ$ -pulse was carried out for the investigated fat systems.

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**P333**

**Integrated NMR, Fluorescence, and Molecular Dynamics Benchmark Study of Protein Mechanics and Hydrodynamics**

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Understanding the function of a protein requires not only knowledge of its tertiary structure but also an understanding of its conformational dynamics. Nuclear magnetic resonance (NMR) spectroscopy, polarization-resolved fluorescence spectroscopy and molecular dynamics (MD) simulations are powerful methods to provide detailed insight into protein dynamics on multiple timescales by monitoring global rotational diffusion and local flexibility (order parameters) that are sensitive to inter- and intramolecular interactions, respectively. We present an integrated approach where data from these techniques are analyzed and interpreted within a joint theoretical description of depolarization and diffusion, demonstrating their conceptual similarities [1]. This integrated approach is then applied to the autophagy-related protein GABARAP in its cytosolic form, elucidating its dynamics on the pico- to nanosecond timescale and its rotational and translational diffusion for protein concentrations spanning nine orders of magnitude. We compare the dynamics of GABARAP as monitored by <sup>15</sup>N spin relaxation of the backbone amide groups, fluorescence anisotropy decays and fluorescence correlation spectroscopy of side chains labeled with BODIPY FL, and molecular movies of the protein from MD simulations. The recovered parameters agree very well between the distinct techniques if the different measurement conditions (probe localization, sample concentration) are taken into account. Moreover, we propose a method that compares the order parameters of the backbone and side chains to identify potential hinges for large-scale, functionally relevant intra-domain motions, such as residues 27/28 at the interface between the two sub-domains of GABARAP. In conclusion, the integrated concept of cross-fertilizing techniques presented here is fundamental to obtaining a comprehensive quantitative picture of multi-scale protein dynamics and solvation. The possibility to employ these validated techniques under cellular conditions and combine them with fluorescence imaging opens up the perspective of studying the functional dynamics of GABARAP or other proteins in live cells.

Reference: [1] Möckel et al., *J.Phys.Chem. B* **123**, 1453-1480 (2019).

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Multi-frequency rapid-scan HFEPR spectroscopy

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The development of the rapid scan technique was historically connected to the problem of the enhancement of the signal-to-noise ratio in NMR but it did not find wide application in NMR or EPR due to the rapid development of high power radio-frequency and microwave sources for pulse methods. However, the past decade is marked by the intense development of solid-state THz instruments, which has made it possible to perform EPR spectroscopy at very high frequencies and fields. Unfortunately, the output power of such tunable THz sources is not sufficient for the implementation of pulse methods. Consequently, the rapid scan is the only affordable technique for multi-frequency investigation of spin dynamics at THz frequencies. To our best knowledge, this EPR technique was demonstrated at frequencies up to 94 GHz only. Here, we present results of the first successful implementation of multi-frequency rapid-scan EPR in the (sub)millimeter frequency range with access to extremely short relaxation times (several nanoseconds). The experiments were performed using a home built HFEPR spectrometer operated in induction mode. The spectrometer does not require any resonator, and therefore, we are able to use frequency sweeps instead of magnetic field sweeps as it is done in the majority of experiments. The main advantages of the frequency domain are the extremely high sweep rates (thousands of THz/s) and absence of eddy currents in the sample holder and/or resonator. Furthermore, a new HFEPR spectrometer, which we are recently constructing in the Central European Institute of Technology (Brno) will further extend the frequency range and sensitivity of the rapid scan technique.

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## P335

**Enamine derivatives of pyrrolo[1,2-a]quinoxalines  
generated by 1,3-dipolar cycloadditions**

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Pyrrolo[1,2-a]benzimidazole and pyrrolo[1,2-a]quinoxaline skeletons are present in various bioactive compounds. Thus, pyrrolo[1,2-a]benzimidazole scaffold is present in a range of DNA cross-linkers mimicking the mitomycin antitumor activity against a range of human cell lines, and many pyrrolo[1,2-a]benzimidazole derivatives showed beneficial properties on central nervous system disorders. Pyrrolo[1,2-a]quinoxaline skeleton is present in numerous compounds that proved potent inhibitors of the human protein kinase CK2 while other derivatives shown anti-tuberculosis, antiparasitic, antiallergic, and central dopamine antagonist activities.

Synthetic routes towards pyrrolo[1,2-a]benzimidazoles and pyrrolo[1,2-a]quinoxalines, as well as their chemical and biological properties, have been extensively reviewed [1-3].

We report here on the reactions of *N*-acetylbenzimidazolium chlorides with various activated alkynes, in the presence of bases, leading under mild conditions to a mixture of pyrrolo[1,2-a]benzimidazoles, 4-methylene-pyrrolo[1,2-a]quinoxalines and pyrrolo[1,2-a]quinoxalin-4-one derivatives.

The exocyclic enamine derivatives of 4-methylene-pyrrolo[1,2-a]quinoxaline have been isolated and fully characterized by multinuclear NMR spectroscopy. A mechanism rationalizing the formation of the enamine derivatives is proposed.

**Acknowledgements:** This work was supported by the Ministry of Research and Innovation, CNCS – UEFISCDI within the PNCDI III and EU H2020 programmes, through the projects ERANET-INCOMERA-2018-BENDIS.

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## P336

**Six years NMR monitoring of a galactosemia case**Alina Nicolescu<sup>\*,1</sup>, Natalia Usurelu<sup>2</sup>, Daniela Blanita<sup>2</sup>,Chiril Boiciuc<sup>2</sup>, Victoria Hlistun<sup>2</sup>, Calin Deleanu<sup>3</sup><sup>1</sup>*"Petru Poni" Institute of Macromolecular Chemistry*, <sup>2</sup>*Institute of Mother and Child*, <sup>3</sup>*"C. D. Nenitescu" Centre of Organic Chemistry*

NMR spectroscopy is a quick and efficient tool for diagnosis and follow up of many inborn errors of metabolism. In many suspicious cases, particularly in countries where only a few inborn errors are included in national screenings, NMR may provide the first indication for a rare metabolic disease.

We report on a case of a five months old girl diagnosed with galactosemia which was followed up by NMR over six years. In galactosemia a diet without lactose is mandatory. Before diagnosis, when normal milk is administrated, galactose is building up in organism as the main metabolite of lactose. When the patient is under specific diet the galactose can hardly be detected. However, even when the products based on normal milk are completely excluded, small amounts of galactose are finding their ways into organism, e.g. through fruits and vegetables which are allowed in the diet, or through other metabolic pathways.

In the absence of galactose as main marker of galactosemia, in order to indirectly follow up its presence in the organism, we have been monitoring by NMR the presence of galactitol. Assignments of various signals of metabolites in urine and their concentration ranges over a period of six years are presented. Galactitol proved to be the main marker indicating the efficiency of the specific diet in galactosemia.

**Acknowledgements:** This work was supported by the Ministry of Research and Innovation, CNCS – UEFISCDI through the project PN-III-P4-ID-PCCF-2016-0050 (5DnanoP).

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## P337

### Assignment of solid- state NMR spectra of paramagnetic inorganic materials and complexes sometimes aided by first principles calculations

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Inorganic materials and compounds with transition metals find numerous applications within catalysis, environmental remediation as well as for energy storage and conversion due to their flexible oxidation states. However, their characterization is challenging due to the absence of long-range order, structural defects and/or the multi-phase nature of such materials. Solid state NMR (SSNMR) is poised to probe the atomic level structure, but the presence of unpaired electron spin density often require ultra-fast MAS and interpretation of the spectra is complicated by the additional interactions introduced by the unpaired electrons.

Catalytic materials are obtained by doping paramagnetic Ni(II) into MgAl layered double hydroxides resulting in trimetallic MgNiAl-LDH. However, the distribution of Ni(II) in the LDH layer is nearly impossible to assess with diffraction based techniques due to frequent stacking faults, low crystallinity and almost identical unit cell parameters. Further complications arise when 2D and nanomaterials derived from these are synthesized. <sup>27</sup>Al SSNMR allowed for quantitative determination of the Ni(II) distribution as well as the local magnetic interactions in the materials as a function of interlayer spacing and Ni(II) content using empirical correlations between paramagnetic shifts[1].

A series of transition metal acetyl acetonates complexes,  $[M(acac)_xL]_y$  was investigated by <sup>1,2</sup>H and <sup>13</sup>C MAS NMR spectroscopy and first principles calculations to understand how the d-electron configurations affects the paramagnetic shifts in structurally related complexes. First principles calculations provided detailed insight into the different factors contributing to the paramagnetic shifts and allowed for spectral assignment of these complexes, whereas the different empirical assignment strategies proved ambiguous.

**Reference:** [1] Jensen ND, Forano C, Pushparaj SSC, Nishiyama Y, Bekele B, Nielsen UG. Phys. Chem. Chem. Phys. 20 (2018) 25335-42.

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## P338

## Triplet dynamic nuclear polarization of nanocrystals in water at room temperature

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Dynamic nuclear polarization (DNP) is one of the most powerful methods allowing various nuclear spins to be hyperpolarized. In the common DNP, organic free radicals are employed as polarizing agents for the hyperpolarization of small molecules such as pyruvic acid. However, it is not suitable for large biomolecules like proteins since the low-temperature condition tends to cause their denaturation. Therefore, the development of a method to increase nuclear polarization in water at room temperature is strongly desired.

Room-temperature DNP using photo-excited triplet electrons (triplet-DNP) has been achieved by utilizing the non-equilibrated polarization of triplet electron spins. However, the nuclear polarization process using the photo-excited triplets has been limited to the solid state. This is mainly because the effective integrated solid effect and the accumulation of spin polarization through spin diffusion require the solid crystals with long spin-lattice relaxation time  $T_1$ . In addition, paramagnetic oxygen molecules severely quench the photo-excited triplet state. Therefore, it has not been clear whether it is possible to achieve triplet-DNP in water containing dissolved oxygen molecules.

Here, we show the first demonstration of triplet-DNP in water by downsizing the conventional bulk crystals to nanocrystals. This is also the first example of triplet-DNP of nanosized crystals. As a proof-of-concept, we employ pentacene and *p*-terphenyl as benchmark polarizing agent and crystalline matrix, respectively. A stable aqueous dispersion of pentacene-doped *p*-terphenyl nanocrystals is obtained by simple ball-milling of the bulk crystals in a surfactant solution. Interestingly, the photo-excited triplet state of pentacene is protected against oxygen quenching in dense *p*-terphenyl nanocrystals. The triplet-DNP process of the nanocrystals in water achieves a high polarization enhancement over 360 times. This work provides the important first step towards hyperpolarization of proteins through proton exchange at the ambient condition. (K. Nishimura *et al.*, *Phys. Chem. Chem. Phys.*, 2019, Accepted)

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P339

weak interactions between ATP and proteins

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Neurodegenerative disorders, including Alzheimer’s disease, are thought to be caused by abnormal accumulation of amyloid fibrils in neurons. Recently, high concentrations of adenosine triphosphate (ATP) have been found to inhibit protein fibril formation (Patel, et al., Science, 2017). ATP is known to store, supply and transport energy in living cells. In addition, a decrease in the intracellular ATP levels may be associated with neurodegenerative diseases (Pathak et al., J Biol Chem, 2015). However, it is not yet known how ATP inhibits protein fibril formation, in other words, the general effect of ATP on proteins remains unclear. Therefore, it is important to analyze the inhibition mechanism at the molecular level. Patel et al. demonstrated that ATP inhibits fibril formation at ATP concentrations greater than 1 mM. In cells, ATP is present at very high concentrations of 1 -10 mM. Therefore, ATP might inhibit aggregation of almost any protein in cells. If ATP can noncovalently interact with target proteins, that would suggest that ATP can inhibit protein aggregation and fibril formation. However, it is not clear if it does and whether these interactions are selective or specific. In this study, we analyze the interaction of ATP with target proteins at the atomic level using solution NMR, a method which can quantify weak intermolecular interactions. We also quantified the binding of ATP to proteins in a concentration-dependent manner and checked the effect of ATP on protein-protein interactions. As a result, the suspected intermolecular interaction was observed for two folded proteins and one intrinsically disordered protein, indicating that ATP is a general binder of proteins.

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P340

### Rotational Motion of Cysteine Ligated to CdSe Magic-Sized Clusters

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Structure and dynamics of organic molecules-ligating clusters significantly influence the optoelectronic property and function of the clusters as well as their stabilization. Recently, we revealed that, cysteine capping on  $(\text{CdSe})_{34}$  magic-sized cluster (CdSe-Cys) has two capping structures: monodentate ligand-cysteine with sulfur-cadmium bond and bidentate one with sulfur- and nitrogen-cadmium bonds.[1,2] In this work, we examine motion of ligand-cysteine capping on  $(\text{CdSe})_{34}$  by performing solid-state  $^2\text{H}$  nuclear magnetic resonance (NMR) spectroscopy in CdSe-Cys with deuterated methanediyl group ( $-\text{CD}_2-$ ).  $^2\text{H}$  quadrupole Carr-Purcell-Meiboom-Gill (QCPMG) and quadrupolar-echo spectra of CdSe-Cys were measured. Temperature dependence on spikelets appeared in the QCPMG spectra suggested that monodentate ligand-cysteine undergoes molecular motion. The quadrupolar-echo spectra were a sum of a motionally-narrowed peak at the center of the spectra and a typical  $^2\text{H}$  Pake pattern, which are assigned to monodentate and bidentate ligand-cysteine, respectively. The line shape analysis for the  $^2\text{H}$  quadrupolar-echo spectra obtained at 0–40°C revealed that the  $\text{CH}_2$  group in monodentate ligand-cysteine undergoes pseudoisotropic rotation by the combination of two intramolecular rotations around the Cd–S and S–C bonds with the activation energy of 19 kJ/mol. This result suggests that the  $(\text{CdSe})_{34}$  core in CdSe-Cys has surface structure where ligand-cysteine can have the mobility, which would affect the optical property of  $(\text{CdSe})_{34}$ .

**Acknowledgment:** This work was supported by JSPS Grant-in-Aid for Scientific Research on Innovation Areas “Mixed anion” (grant number 16H06440) and Grant-in-Aid for JSPS Fellows (grant number 18J11973).

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## P341

## Reducing experimental time using Multiple Fid Acquisition (MFA) strategy

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Speeding-up NMR molecular analysis is an important research field which has been continuously advancing since NMR early days. The relevant benefits are clear and evident:

- Reduce the time per analysis directly reduce its cost
- Gaining spectrometer time to analyze new samples

Many interesting tools and concepts have been appearing in last decades. Concretely, our experience focuses on the development of new NMR experiments using TS[1] (Time-Shared), SA[2] (Spectral Aliasing) and MFA[3] (Multiple Fid Acquisition).

MFA strategy is an interesting strategy that allows the acquisition of different structural information in a single experiment. Basically, MFA experiments consist in the design of pulse sequence experiments which accommodate several acquisition windows per experiment, each registering different relevant information for the structural molecular characterization. The methodology brings a corresponding important time benefit.

Last year, we have reported several new NMR experiments designed with MFA strategy[4,5,6,7] and herein we would present the most relevant achievements. The overall discussion will be mainly focused on the sensitivity gains per time unit of the presented experiments.

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P342

Elucidation of degradation of poly (urea-urethane) with spin-spin relaxation time

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We have demonstrated that <sup>1</sup>H spin-spin relaxation time ( $T_2$ ), which is measured by using low field NMR techniques, is an effective parameter to elucidate the degradation behavior of poly (urea-urethane). It is difficult to reveal the degradation behavior of poly (urea-urethane), when the degradation proceeds in urea or urethane bond units.

We prepared two types of poly (urea-urethane), which were Urethane-A and B, and aged them at 100 °C for up to 10,000 h. After that, we measured tensile strength, elongation at break and  $T_2$ . We also observed <sup>13</sup>C DP/MAS and <sup>15</sup>N CP/MAS NMR spectra.

The tensile strength of Urethane-A decreased, increased and decreased again, and that of Urethane-B decreased slowly. <sup>13</sup>C and <sup>15</sup>N NMR spectra revealed the degradation behavior of Urethane-A and B. The degradation of Urethane-A was caused by molecular chain scission and cross-linking in urea bond units, and that of Urethane-B proceeded with molecular chain scission in urethane bond units.

$T_2$  had three components ( $T_2^S$ ,  $T_2^I$  and  $T_2^L$ ). The results showed that  $T_2^S$  and  $T_2^I$  described the molecular mobility of urea and urethane bond units, respectively. Molecular chain scission in urea bond units reduced the fraction of  $T_2^S$  ( $x_S$ ), and the cross-linking reaction in them increased  $x_S$ . Molecular chain scission in urethane bond units decreased  $T_2^I$  value itself, not the fraction of  $T_2^I$ . Molecular chain scission in urea bond units causes the destruction of hard segment phase. It is because molecular mobility of fragments in-between the scission points becomes high so that it contributes to the fraction of  $T_2^L$ . In contrast, molecular chain scission in urethane bond units increases  $T_2^I$  value itself, but the increase does not become significantly large owing to the remaining urea bond units. Therefore, the degradation sites can be specified by measuring  $T_2$ , when it proceeds in-between urea or urethane bond units.

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## P343

THz ESR study of  $S=1/2$  frustrated  $J_1$ - $J_2$  chain  
 $\text{NaCuMoO}_4(\text{OH})$  as candidate substance for spin nematic

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Recently, low-dimensional frustrated spin systems have attracted much attention to have novel ground states. The existence of a spin nematic phases, analogous to nematic liquid crystal, is expected theoretically in  $S=1/2$  ferromagnetic and antiferromagnetic frustrated chain at slightly below the saturation field. New candidate substance  $\text{NaCuMoO}_4(\text{OH})$  shows relatively low saturation field of 25 T and no random substitution. Superexchange interactions are estimated to be  $J_1=-51$  K and  $J_2=36$  K from the magnetic susceptibility measurements. The specific heat does not show long range order down to 0.6 K. Furthermore, relaxation time from NMR measurements behave a spin density wave (SDW), which is expected blow the spin nematic phase. Previously, feature of a spin nematic phase could not observe due to powder samples. In this work, we treat a three-dimensional magnetically aligned powder sample as quasi large single crystal. This treatment is innovative sample treatments to obtain information of magnetic or other properties of anisotropy. High-frequency ESR measurements of  $\text{NaCuMoO}_4(\text{OH})$  for parallel and perpendicular fields to chain have been performed at 1.8 K using the pulsed magnetic fields up to 55 T and in the frequency region to 1.56 THz. The linewidth for  $\text{H}_\perp$  chain is wider than that for  $\text{H}_\parallel$  chain. Frequency dependency of resonance field and linewidths does not change drastically at around the saturation field. Although to observe coupled nematic spins, which is expected to have  $2\omega$  mode, twice time higher frequency measurements have been performed using FIR laser. Detailed results of frequency dependence of resonance field and linewidths at spin nematic phase will be discussed.

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## P345

## Carbon-carbon J-coupling measurements at natural abundance enabled by dDNP

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Incredible Natural Abundance Double QUAntum Transfer Experiment (INADEQUATE) is arguably one of the most attractive tools for establishing molecular structure. When applied to organic molecules it usually relies on J coupled <sup>13</sup>C spin pairs. While this results in a spectrum that is easy to interpret, it also comes at a cost of very low sensitivity considering that only ca. 0.01% of all carbons present in the sample contribute to the detected signal, explaining rather limited usage of INADEQUATE experiment even in situations where it is an ultimate recourse. 1D version of INADEQUATE when acquired in a manner that conserves splittings originating from J couplings, in principle, yields the same information as its 2D counterpart provided that J couplings are sufficiently distinct and signal overlap does not prevent their measurement for carbons under consideration. Herein, we show that dissolution dynamic nuclear polarization (dDNP) can provide sufficient sensitivity enhancements to acquire 1D INADEQUATE spectra for compounds with natural <sup>13</sup>C content including such natural products as  $\alpha$ -pinene, menthol and limonene, even within the limits imposed by technicalities of dDNP stemming from single-scan nature of the experiment and flow effects associated with sample transfer. Not only we achieve sub-Hertz linewidths, but we are able to nearly perfectly suppress signals associated with isolated carbon-13 nuclei, revealing long-range carbon-carbon couplings that are on the order of few Hertz. Our approach is particularly valuable for correlating quaternary carbons which have longest T<sub>1</sub>s and hence highest signal enhancement in dDNP, but often impose biggest challenge for characterization due to absence of adjacent protons.

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P346

**Structural and aggregation features of a human  
κ-casein fragment with antitumor and cell-penetrating properties**

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Elena Kuligina<sup>2</sup>, Vladimir Richter<sup>2</sup>, Elena Bagryanskaya<sup>3</sup>

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Intrinsically disordered proteins (IDPs) are involved in a wide range of essential biological processes, especially signaling and regulation, as well as pathological conditions such as cancer and neurodegenerative and autoimmune diseases. Recently a human κ-casein proteolytic fragment called lactaptin (8.6 kDa) was found to induce apoptosis of human breast adenocarcinoma MCF-7 and MDA-MB-231 cells with no cytotoxic activity toward normal cells. Earlier, we have designed some recombinant analogs of lactaptin and compared their biological activities. Among these analogs, RL2 has the highest antitumor activity, but the amino acid residues and secondary structures that are responsible for RL2's activity remain unclear. To elucidate the structure–activity relations of RL2, we studied structural and aggregation features of this fairly large intrinsically disordered fragment of human milk κ-casein by a combination of physicochemical methods: NMR, paramagnetic relaxation enhancement (PRE), EPR, circular dichroism, dynamic light scattering, atomic force microscopy, and a cytotoxic activity assay. It was found that in solution, RL2 exists as stand-alone monomeric particles and large aggregates. Whereas the covalent S-S homodimer of RL2 turned out to be more prone to assembly into large aggregates, the monomer predominantly forms single particles. It was shown that the single cysteine of RL2 is an essential amino acid residue for RL2 activity. NMR relaxation analysis of spin-labeled RL2 showed that the RL2 N-terminal region containing cysteine is more ordered than its C-terminal counterpart and also contains a site with a propensity for α-helical secondary structure.

This work is supported by the Ministry of Education and Science of the Russian Federation (state contract no. 14. W03.31.0034).

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Structure and Dynamics of Exoribonucleases  
Jan Overbeck, Remco Sprangers

Xrn1 and Xrn2 are the main 5'-exoribonucleases in the cytoplasm, respectively the nucleus. These enzymes play a major role in the degradation of long RNA molecules and have been subject to a number of functional and structural studies (Xiang 2009, Chang 2011, Jinek 2011, Tesina 2019). To fully understand the underlying molecular mechanism of these essential enzymes, we here solved the crystal structure of the Xrn2 protein core. The Xrn2 zinc finger that is located next to the active site of the core domain of the enzyme was not visible in the crystallographic data and was structurally characterized using NMR spectroscopy. We show that this zinc finger binds RNA in vitro and contributes to the overall RNA affinity of Xrn2. We use methyl TROSY NMR experiments on the 100 kDa protein core and used site directed mutagenesis to obtain a resonance assignment for most of the 48 Ile- $\delta$ 1 methyl groups. These methyl probes were then used to record 1H13C-MQ and 13C-SQ CPMG experiments that display extensive dynamics on the  $\mu$ s-ms timescale in a region of the protein that surrounds the active site. These methyl relaxation data were complemented and confirmed with 19F-based CPMG experiments that we recorded on a number of samples that contained site specific fluor labels. In addition, the methyl group assignments were used to probe the interaction of Xrn2 with substrate RNA and with its main activator Rai1. Overall, our results combine static structures from X-ray crystallography with the state-of-the-art NMR methodology to obtain detailed insights into enzyme function.

**References:** [1] Chang et al., Nat. Struct. Mol. Biol. 18, 270–276 (2011). [2] Jinek et al., Mol. Cell 41, 600–608 (2011). [3] Xiang et al., Nat. Lett. 458, 784-788 (2009). [4] Tesina et al., Nat. Struct. Mol. Biol. 26, 275-280 (2019).

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A new approach to interpret non-negative least squares (NNLS) T2 relaxation results  
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*AgroResonance, INRA*

Fitting a weighted sum of exponentials to a noisy exponential decay is an ill-posed problem. In the  $T_2$  relaxation context, it leads to non-unique solutions because different sets of relaxation times and amplitudes give identical least-square distance between the model and the experimental data. Conventional data analysis combine a non-negative least squares (NNLS) algorithm with a regularization to select a solution. While the hypotheses made for the NNLS algorithm are undisputable, the choice of the regularization (both types and parameters) is prior-knowledge dependent.

In this work, we introduce a new method to analyze NNLS results without regularization avoiding introducing a prior-knowledge dependent step. Instead of analyzing the NNLS outputs as probability density functions (pdf), we focused on the cumulative density functions (cdf). To validate our approach, three models describing  $T_2$  distributions were simulated: a purely discrete one, a purely continuous one and a third one containing three Gaussian distributions. Measuring the  $T_2$  amplitudes at a cdf plateau is unbiased and independent of the decomposition basis. Depending on the SNR, edge-artefacts, i.e. noise adjustment at the beginning of the signal, can be observed. To remove these artefacts, we developed a simple and quick way to determine the shortest  $T_2$  value in the decomposition basis based only on the SNR.

In sum, the proposed method consists in resolving the amplitudes at specific position (i.e.  $T_2$ ) of the cdf distribution. The major assets of this approach are to not introduce prior-knowledge and to precisely define the left bound of the decomposition basis, currently a dead angle of most  $T_2$  inversions.

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Profiling the temperature-dependent frequency of an open-magnet for outdoor applications

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<sup>1</sup>AgroResonance, INRA, <sup>2</sup>AgroScans-PRISM / IRSTEA, <sup>3</sup>AgroResonance / INRA

Low-field NMR relaxometers are powerful instruments as they can be moved outside the laboratory to be closer to the sample (in fields or next to a production line), for on-line quality control for example. Open-magnets present the advantages of (i) being slice selective and (ii) able to record 1D NMR profile if the magnet is mounted on a high-precision lift. The NMR-MOUSE is an example of a device combining both features. This magnet can measure the NMR signal at various depths (NMR profile) with a resolution as low as few tens of micrometers. However, the magnet frequency distribution of such instrument is dependent on the magnet temperature, which is currently nor regulated nor controlled. Hence, its performance might be questioned in case of temperature fluctuations (e.g. outdoor applications), inducing unintended variations in the position of the slice.

In this work, we aim at characterizing the NMR profile shift as a function of the magnet temperature. For this, we measured the profile of a doped water sample while the surrounding temperature of the magnet was varied. We clearly observed a linear variation of the water profile position for temperatures between 10 and 35°C. This variation was measured to be around 43 µm/°C, i.e. roughly one slice thickness (50 µm) per degree. We also observed an important thermal inertia of the magnet, i.e. the slowness with which it reaches and stabilizes at the temperature of the surrounding environment.

This work quantifies a linear temperature dependence of the slice position and demonstrates that this instrument can be used without any constraints in a roughly temperature controlled environment thanks to the thermal inertia of the magnet. However, it is mandatory to correct the measurement depth for magnet temperature variation in case the temperature is not regulated (sample heating, outdoor conditions).

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Site-Specific Dynamics of the Growth Hormone  
Secretagogue Receptor investigated by Solid-State NMR

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The Growth Hormone Secretagogue Receptor (GHSR) belongs to the family of G protein-coupled receptors. It is a seven transmembrane (TM) domain protein which undergoes a conformational change to an active state upon ligand binding and is part of the signal transduction pathways into the cells. It represents an attractive target due to its role in food intake. Besides the knowledge of its structure, its dynamic properties are considered highly relevant for its function.

Previously, the uniformly <sup>13</sup>C-labelled GHSR was characterized with respect to its dynamics and was shown to be highly flexible [1]. However, these results represent an average of the dynamics of all sites of the receptor. Therefore, we aim to investigate the site-specific and single residue dynamics of GHSR.

In order to characterize the dynamics of specific domains, amino acids clustering in the TM helices, the loops and the C-terminus were <sup>13</sup>C-labelled using a cell-free expression strategy. The labeled GHSR was expressed in the precipitated form with a yield of up to 1.2 mg per 1 mL reaction volume and reconstituted in DMPC bicelles. Using DIPSHIFT NMR, the mobility of the C-H bonds of the <sup>13</sup>C-labelled amino acids has been investigated to determine the order parameter of motion for each domain.

As expected, the TM domain is the most rigid part of the receptor, the loops and ends of helices are slightly more mobile while the C-terminus showed the highest mobility.

In the next steps, the dynamics of single key residues involved in the ligand binding or the signal transduction on the intracellular side will be investigated. Histidines were chosen due to the low number of only three native residues and the expected low spectral complexity. Furthermore, Tryptophans will be considered due to their role in ligand binding, e.g. Trp<sup>648</sup>.

Reference: [1] Schrottke et al. (2017). Sci. Rep. 7:46128.

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Solid-State NMR Study of Surface Ligands on Nanocrystals

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Typical colloidal nanoparticle can be viewed as a nanocrystal-ligands complex with an inorganic core bonded with a monolayer of organic ligands. Surface ligands are an essential part of nanocrystal-ligands complexes and dramatically affect their properties, such as stability, biological compatibility and solution processability. Solid-state nuclear magnetic resonance (SSNMR) is one of the best characterization techniques for deciphering the molecular pictures of the nonperiodic and dynamic organic-inorganic interlayer. Here we performed a full set of SSNMR investigations to depict the ligand patterns on nanocrystals and evaluate the ligand-ligand interactions. Centerband-only detection of exchange (CODEX) experiments based on <sup>1</sup>H-driven spin diffusion of <sup>13</sup>C are used to uncover the ligand distribution patterns of the mixed ligand nanocrystal-ligands complexes. <sup>2</sup>H lineshapes and <sup>1</sup>H-<sup>13</sup>C dipolar chemical-shift correlation (DIPSHIFT) are used to study the ligand dynamics and ligand-ligand interactions quantitatively. Finally, a mathematical model based on the NMR-derived ligand arrangement and dynamics successfully predicts the unusual solubility of nanocrystal-ligands complexes with mixed ligands, which is several orders of magnitude higher than that of nanocrystal-ligands complexes with pure ligands.

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**Mechanistic insight in the role of SUMO in  $\alpha$ -Synuclein aggregation**  
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*I.I.T Bombay*

Intrinsically disordered presynaptic neuronal protein,  $\alpha$ -Synuclein plays a critical role in synaptic vesicle trafficking, though the exact mechanism still remains unknown. It is also amyloidogenic in nature and deposits of higher aggregates of  $\alpha$ -Synuclein in the Lewy bodies are the histopathological hallmark of second most prevalent neurodegenerative disorder, Parkinson's disease. Posttranslational modifications  $\alpha$ -synuclein such as ubiquitination, phosphorylation and nitrosylation, play a crucial role in modulation of  $\alpha$ -synuclein aggregation kinetics. SUMOylation, a post-translational modification involving covalent attachment of SUMO to the target protein has been shown to abolish  $\alpha$ -synuclein fibril formation. In our study, we showed that SUMO-1 interacts non-covalently with  $\alpha$ -synuclein and alters the aggregation kinetics of  $\alpha$ -Synuclein by using ThT fluorescence, circular dichroism, nuclear magnetic resonance spectroscopy and atomic force microscopy. Interaction with SUMO results in the delayed aggregation rate of  $\alpha$ -Synuclein. The residues specific interaction studied by solution state NMR reveals that, SUMO1 interacts to the protein in the N-terminal region which has been previously shown to form a  $\beta$ -hairpin loop structure which upon sequestration completely inhibits  $\alpha$ -Synuclein aggregation. This study gives a better insight about the SUMO and Synuclein interaction and may suggest a new signaling or regulatory mechanism of SUMO in Parkinson's disease.

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## P353

## Methyl labelling applications in drug discovery

Stanislava Panova\*, Reto Walser

Astex Pharmaceuticals

NMR is a powerful tool in early stage drug discovery. Its most prominent roles in the pharmaceutical industry are as a sensitive screening method for finding *de novo* hit matter or as an orthogonal biophysical method for confirming hits. Primary hit finding, and validation is most efficiently performed using ligand-observed experiments. The corollary of this approach is protein-observed NMR, where chemical shift changes in a protein are measured upon addition of a hit molecule and which can be used to fully validate a protein-ligand interaction as far NMR is concerned. Ligand titration and structure determination of the protein-ligand complex can further characterize the complex in terms of affinity and binding mode.

While ligand observed methods are generally and broadly applicable, protein observed methods suffer from the usual size limitations encountered in NMR. Exploiting the TROSY effect in selective methyl protonated and methyl- $^{13}\text{C}$ -labelled proteins extends the MW limit for observing  $^{13}\text{C}$ - $^1\text{H}$ -correlations to well above 100 kDa<sup>1)</sup>. Methyl TROSY applications<sup>2)</sup> can be used to map protein-ligand interaction sites, monitor changes in protein conformation, stability and oxidation status, and have proven to be invaluable for 1D ligand-observed affinity assay development, when no appropriate tool compounds are available. We show here a couple of examples where  $^{13}\text{C}$ ,  $^1\text{H}$ -methyl labelling (with or without background deuteration) has enabled us to characterize proteins and protein-ligand interactions in systems with MWs greater than 50 kDa on our 500MHz spectrometer.

**References:** [1] Sprangers, Nature, 445, 618-622, 2007. [2] Tugarinov, J Am Chem Soc, 125, 10420-10428, 2003.

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An approach to the Liquid- Liquid Phase Separation (LLPS) phenomenon from the NMR point of view

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Recently the phenomenon of Liquid-Liquid Phase Separation (LLPS) has gained great interest among the scientific community given its participation in the formation of membraneless organelles that play crucial roles in the cellular environment 1. Great efforts are currently devoted to its structural and functional characterization. However, obtaining structural information at the molecular level of this phenomenon remains elusive given the challenges presented by the dynamics of the system. One of the most powerful analytical techniques in obtaining information at the atomic level is Nuclear Magnetic Resonance (NMR). Given its versatility and the wide variety of experiments available, this technique is presented as a key element in the understanding and characterization of this phenomenon in deep detail. In the present work we show a broad study of the different physical parameters accessible from NMR (T1, T2, Chemical Exchange) for the characterization of the phenomenon of LLPS. The strengths and challenges of current NMR methodologies are evaluated on a model system composed of triethylamine/water. Direct detection in <sup>13</sup>C is shown as a plausible alternative in the study of this process given the favorable relaxation properties of this nucleus.

Reference: [1] Shin, Y., & Brangwynne, C. P. (2017). Science, 357(6357).

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## P355

**Structural and dynamic NMR study of “three-finger” protein Lynx2**Alexander Paramonov\*, Milita Kocharovskaya, Andrey Alenkin,

Mikhail Shulepko, Zakhar Shenkarev, Ekaterina Lyukmanova

*Shemyakin-Ovchinnikov Institute of**Bioorganic Chemistry of the Russian Academy of Sciences*

Human endogenous modulators of nicotinic acetylcholine receptors (nAChRs), the proteins from the Ly6/uPAR family, demonstrate high structural homology with snake  $\alpha$ -neurotoxins. The main structural feature for these proteins is a presence of characteristic LU-domain with “three-finger” fold, consisting of four disulfide bonds stabilizing the core and three  $\beta$ -structured loops. Despite on structural similarities, these proteins demonstrate functional diversity and can play various roles in the biological processes. For different members of the Ly6/uPAR family the ability of regulation of cognitive processes, anti-proliferative activity and participation in the number of regulatory pathways, such as Wnt/ $\beta$ -catenin signalling were shown. Lynx2 is one of the poorly studied members of the Ly6/uPAR family. It is known that Lynx2 is membrane-tethered by GPI anchor protein, binds specifically to  $\alpha 4\beta 2$ -nAChRs and is connected with anxiety-related behavior.

Here we perform the NMR study of structure and dynamics of the water-soluble domain (lacking GPI anchor) of human Lynx2 (ws-Lynx2) in the aqueous solution. We developed for the first time the effective expression system in the form of *E. coli* cytoplasmic inclusion bodies. Using 1D NMR, the screening of conditions for purification and renaturation was conducted. It allows to produce milligram quantities of ws-Lynx2 and its  $^{13}\text{C}$ ,  $^{15}\text{N}$  isotope-labeled analogue. Using heteronuclear NMR in solution, the ws-Lynx2 structure was determined and intramolecular dynamics was characterized.

The study was supported by the Russian Science Foundation (Project № 19-74-20176).

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## P356

### Heteronuclear Cross-relaxation effect modulated by the dynamics of N-functional groups in the solid state under $^{15}\text{N}$ DP-MAS DNP

Heeyong Park<sup>\*,1</sup>, Boran Uluca-Yazgi<sup>2</sup>, Saskia Heumann<sup>1</sup>, Robert Schlögl<sup>1</sup>, Rüdiger-A Eichel<sup>2</sup>, Josef Granwehr<sup>2</sup>, Henrike Heise<sup>2</sup>, P. Philipp M. Schleker<sup>1</sup>

<sup>1</sup>Max Planck Institute for Chemical Energy Conversion, <sup>2</sup>Forschungszentrum Jülich

Dynamic Nuclear Polarization (DNP) is an emerging magnetic resonance technique to enhance the sensitivity of MAS NMR. In a typical MAS DNP experiment, several mechanisms are involved simultaneously when transferring the relatively large polarization of paramagnetic electrons to NMR active nuclei of interest. Recently SCREAM-DNP (Specific Cross Relaxation Enhancement by Active Motions under DNP), which is one of the mechanisms, has been reported through a negative enhanced  $^{13}\text{C}$  DP (Direct Polarization)-MAS DNP by reorientation dynamics of methyl under the DNP condition of 100K.<sup>1-3</sup> This effect is similar to the NOE (Nuclear Overhauser Effect) in that it utilizes the heteronuclear cross-relaxation induced by motional processes, but is distinguished with NOE in that it contributes to the spectrum with a different aspect without  $^1\text{H}$  saturation pulse under DNP condition. Based on the Solomon equation,  $^{15}\text{N}$  is expected to show an opposite contribution (positive) to signal enhancement compared to  $^{13}\text{C}$  in the cross-relaxation effect because  $^{15}\text{N}$  ( $\gamma = -4.3$ ) has the opposite sign to  $^{13}\text{C}$  ( $\gamma = 10.7$ ) for gyromagnetic ratio. In this work, DP and DP<sub>sat</sub> (DP with  $^1\text{H}$  saturation) were employed w/ and w/o microwave to investigate the cross-relaxation effect in  $^{15}\text{N}$  DP-MAS DNP. We provide experimental evidence for the cross-relaxation effect by the spontaneous polarization transfer from hyperpolarized  $^1\text{H}$  to primary both amine and ammonium nitrogen due to the reorientation dynamics during  $^{15}\text{N}$  DP-MAS DNP. The influence on the cross-relaxation effect by the dynamics of amines depending on temperature was also confirmed. Finally, we discuss potential applications of SCREAM-DNP to analyze surface signals with DNP-SENS (Surface Enhanced NMR Spectroscopy) in material science.

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Sequence-selective minor groove recognition of a  
DNA duplex containing synthetic genetic components  
Giacomo Padroni, Jamie M. Withers, Andrea Taladriz Sender,  
Linus F. Reichenbach, John A. Parkinson\*, Glenn A. Burley  
*University of Strathclyde*

The structural basis of minor groove recognition of a DNA duplex containing synthetic genetic information by hairpin pyrrole-imidazole polyamides is described. Hairpin polyamides induce a higher melting stabilization of a DNA duplex containing the unnatural P·Z base-pair when an imidazole unit is aligned with a P nucleotide. An NMR structural study showed that the incorporation of two isolated P·Z pairs enlarges the minor groove and slightly narrows the major groove at the site of this synthetic genetic information, relative to a DNA duplex consisting entirely of Watson-Crick base-pairs. Pyrrole-imidazole polyamides bind to a P·Z-containing DNA duplex to form a stable complex, effectively mimicking a G·C pair. A structural hallmark of minor groove recognition of a P·Z pair by a polyamide is the reduced level of allosteric distortion induced by binding of a polyamide to a DNA duplex. Understanding the molecular determinants that influence minor groove recognition of DNA containing synthetic genetic components provides the basis to further develop unnatural base-pairs for synthetic biology applications. <https://doi.org/10/1021/jacs.8b12444>

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## P358

### Rapid structure determination of molecular solids using chemical shifts directed by unambiguous prior constraints

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The combination of solid-state NMR and crystal structure prediction has shown to be a powerful method to elucidate the structure of amorphous materials and microcrystalline solids: one of the key challenges in chemistry today. However, two main downsides currently prevent efficient high throughput structure elucidation using this approach: (i) the high computational cost associated with CSP methods and (ii) the required density functional theory (DFT) chemical shift calculations.

Here, we introduce the use of unbiased prior constraints from solid-state NMR to accelerate and guide the CSP procedure. Specifically, the constraints extracted from 2D NMR correlation experiments allow to restrict the CSP search space to the correct single molecule conformer regions, guiding from the first step of CSP towards the correct crystal structure without the need for any assumptions. We show that this new approach correctly determines the crystal structures of cocaine, flutamide, and flufenamic acid with significant acceleration in computational times. The CSP computational cost for these structures is reduced to half of the cost associated to CSP without unbiased constraints, and the combination with machine learning methods to predict chemical shifts allows the determination of the crystal structure in record time. Furthermore and most significantly, we correctly determine the crystal structure of powdered ampicillin, which had not been possible using conventional CSP-NMRX methods.

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Generation of Highly Polarized Transportable Pure 2-Ketoisocaproate Sample using Endogenous UV-generated Labile Radicals

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Hyperpolarization via dissolution DNP (dDNP) is a highly promising technique that has advanced into a powerful modality for non-invasive real time monitoring of normal and abnormal cellular physiology with the potential to unravel diseases, develop novel treatment regimes, and quantify enzymatic processes. [1, 2] An important enzyme in this context is branched chain amino acid transaminase (BCAT) that has an important role in nitrogen shuttling and glutamate metabolism in the brain. BCAT has been successfully shown to metabolize hyperpolarized (HP) [1-<sup>13</sup>C]ketoisocaproate (KIC) to leucine in an enzymatic transformation that simultaneously converts glutamate to  $\alpha$ -ketoglutarate. HP [1-<sup>13</sup>C]KIC has been suggested to be a suitable hyperpolarized marker for profiling of tumors and brain diseases. [3, 4]

One limitation of the dDNP technique short nuclear spin relaxation time (T1) requiring the dDNP equipment in the vicinity of the (N)MR magnet for fast transfer. An alternative approach is to use thermally labile UV-generated radicals that annihilates above ~ 190 K, and provide storage/transport of HP samples. [5, 6]

Herein, we demonstrate the preparation of HP KIC aimed at generating highly polarized transportable samples. UV-irradiation of 400 s with a broadband UV source (Dymax BlueWave 75; 19 W/cm<sup>2</sup>C) yielded ~55±5 mM radical (X-band ESR measurement on Magnettech MiniScope 5000). DNP studies and LOD-ESR (longitudinal detection ESR) were performed at 6.7 T and 1.1 K. Electron relaxation time, sample formulation, and radical concentration has been optimized to achieve high polarization. The preliminary studies show very promising results with 42% <sup>13</sup>C polarization in the liquid state after solid state polarization of 1.3 h.

**References:** [1] Ardenkjaer-Larsen et al. *PNAS*, 2003. [2] Kurhanewicz et al. *Neoplasia*, 2019. [3] Karlsson et. al. *Int J. Cancer*, 2010. [4] Butt et. al. *J Cereb Blood Flow Metab*. 2012. [5] Capozzi et. al. *Nat. Commun*. 2017. [6] Patel et. al. *ACIE*, 2018.

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## P360

**G-rich oligonucleotides with GC ends form unusual G-quadruplex structures in the presence of various monovalent cations**

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Guanine-rich DNA oligonucleotides can adopt non-canonical, four-stranded secondary structures, termed G-quadruplexes. The formation of G-quadruplexes requires the presence of cations. In fact, cation nature is one of the major factors contributing to the structural diversity of G-quadruplexes. Their self-assembling ability, electrochemical properties and programmable control of their shape and size make them attractive candidates for nanotechnological applications. One of the proposed ways for programming self-assembly is designing G-quadruplex forming DNA sequences with complementary GC ends, which would act as 'sticky ends' and form linkages between two successive G-quadruplexes via inter-quadruplex GCGC-quartet formation[1].

In the present NMR study we analyzed influence of GC-ends within oligonucleotides S3 d(GCC<sub>2</sub>AG<sub>4</sub>AG<sub>2</sub>) and S4 d(GCC<sub>2</sub>AG<sub>4</sub>AG<sub>2</sub>CG) on resulting G-quadruplex structures. Additionally, effect of different monovalent cations on fold was evaluated. Na<sup>+</sup>, <sup>15</sup>NH<sub>4</sub><sup>+</sup> and K<sup>+</sup> ions promote formation of symmetric, bimolecular G-quadruplexes, which can be considered, as structures composed of two blocks – the 5'-antiparallel and the 3'-parallel block. Although the global fold of G-quadruplexes, which includes A(GGGG)A hexad sandwiched between two G-quartets is preserved, some structural elements were cation dependent. Furthermore, <sup>15</sup>NH<sub>4</sub><sup>+</sup> and K<sup>+</sup> ions promote dimerization of a single G-quadruplex unit through 3'-3' stacking interactions of terminal G-quartets in case of S3.

Interestingly, in all S3 and S4 structures, no dimerization through GC ends was observed. 5'-GC ends are located in the middle of the G-quadruplex structures, where G1 and C2 are part of different structural motifs. Even though the 3'-GC ends present free overhangs in G-quadruplex of S4, no interlocking was observed. In fact, they behave as steric hindrance and prevent dimerization as observed for S3.

**Reference:** [1] Ilc T., Šket P., Plavec J., Webba da Silva M., Drevenšek-Olenik I. and Spindler L. (2013) *J. Phys. Chem. C*, 117, 23208-23215.

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Homogenisation in high-level radioactive waste bentonites probed at sub-microscopic length-scales using deuterium micro-MRI.

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Geological disposal of high-level radioactive waste usually includes bentonite buffer materials. However, the long-term performance of the bentonites for the containment of the nuclear waste is being increasingly questioned due to specific issues associated with “homogenisation” occurring in these materials. Homogenisation is driven by erosion, variations in density and development of swelling pressure. However, the role of pore water pressure, it’s spatial development and impact on swelling potential, self-sealing and permeability have yet to be adequately explored. We have developed novel <sup>2</sup>H micro-MRI methods to demonstrate that these governing hydration processes are indeed both complex and non-uniform in bentonites and natural materials homogenised in the laboratory setting and used for containment of radioactive waste in many European countries.

Bentonites and natural materials used in this study were homogenized with D<sub>2</sub>O. <sup>2</sup>H NMR imaging, combined with <sup>2</sup>H spin-echo T<sub>2</sub> spectroscopy was used to map out T<sub>2</sub> relaxation in these samples. Surface relaxivity of deuterium oxide was determined using beads with different diameters soaked in D<sub>2</sub>O.

We have shown that T<sub>2</sub>s in these materials are non-uniform and the distributions change as deuterium oxide progress toward the end of a core during homogenization. Obtained values of T<sub>2</sub> were indicative of high structural heterogeneity. We further converted T<sub>2</sub> maps to pore size distribution maps to probe length scales of structural heterogeneities that occur in bentonites and natural materials during homogenization. The maps demonstrated that the method captures structural irregularities that are lower than 2500nm. These structural irregularities are not visible in high resolution X-CT images of these cores.

We conclude that <sup>2</sup>H MRI has a great potential to assess heterogeneity in man-made and natural clays. The information is vital for a proper choice of materials used in containment of the nuclear waste.

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## P362

**Thermal behaviours of an API examined by means of an  
NMR Crystallography approach - Case Study of Safinamide mesylate**

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Deep structural studies of drugs and their Active Pharmaceutical Ingredients (APIs) are nowadays needed more than in the past. The importance and usefulness of the knowledge concerning the differences in the APIs physical forms has been growing. Currently the interest focuses not only on the scientific side but for every dosage form has to be taken into consideration as well.

The aim of this work is to systematize the knowledge about Safinamide mesylate crystal structures as well as to investigate thermal stability of their crystal forms. Safinamide is the third-generation reversible MAO-B inhibitor and has recently been introduced to the market for the treatment of Parkinson disease. A complementary approach that combines NMR measurements, analysis of powder and single crystal X-ray diffraction data as well as advanced quantum mechanical calculations was employed in this study. Such a strategy, which is known as NMR crystallography seems to be well suited in this case. It was revealed that the safinamide mesylate (SM) salt was highly unstable and underwent a temperature transformation. We applied various experimental and theoretical techniques to analyse the dynamics and obtain structural constraints for the SM model before and after the thermal treatment. Additionally, the study was supported by Very Fast MAS NMR measurements performed on The UK 850 MHz Solid-State NMR Facility.

Acknowledgment: The project no. Homing/2017-4/37 is carried out within the HOMING programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. The UK 850 MHz solid-state NMR Facility used in this research was funded by EPSRC and BBSRC, as well as the University of Warwick including via part funding through Birmingham Science City Advanced Materials Projects 1 and 2 supported by Advantage West Midlands (AWM) and the European Regional Development Fund (ERDF).

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The Influence of D-Valine Substitution on Membrane Binding and Secondary Structure of the Amphipathic Peptide WLBU2

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WLBU2 (RRWVR RVRRW VRRVV RVVRR WVRR) is a rationally designed, cationic amphipathic peptide consisting of 24 amino acids. While it has a simplistic construction from only three amino acid types, it's a potent antimicrobial peptide (AMP). Unfortunately, AMPs suffer as do all peptides from low proteolytic stability. One efficient way to increase the stability against protease is the partial or full substitution of amino acids with their D-enantiomer. Due to the importance of helical content for antimicrobial activity and the fact that antimicrobial peptides directly target the membrane, we focused our investigations on the peptide's secondary structure as well as membrane binding of WLBU2 and the corresponding peptide with all valines substituted with D-valines (D-Val-WLBU2). We compared the properties of the pure membrane mimetics, either POPC/POPE/cholesterol (5:1:6 molar ratio), which resembles the red blood cell, or POPE:POPG:TOCL (7:2:1 molar ratio), which resembles the G(-) inner membrane, by NMR to the membrane with inclusion of the peptides (P/L ratio: 1/30). <sup>13</sup>C-<sup>13</sup>C MAS NMR spectra of the peptides with <sup>13</sup>C labelled Arg<sup>5</sup> and Trp<sup>10</sup> allowed the assessment of the secondary structure in the presence of this membrane mimetics. The difference between the <sup>13</sup>Cα and <sup>13</sup>Cβ chemical shifts of WLBU2 is indicative for a helical secondary structure, while for the D-Val-WLBU2 a random coil structure is observed. This is in agreement with solution NMR studies, showing only minor changes for D-Val-WLBU2 upon addition of TFE. Static <sup>31</sup>P and <sup>2</sup>H NMR spectra NMR demonstrated that WLBU2 and D-Val-WLBU2 bind the membranes in a similar fashion. We observed decreased averaged quadrupolar splitting of the lipid acyl chain CD-segments when the peptides bind to either membrane, which is explained by a reduced projected chain length of the lipid acyl chains.

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A disorder-to-order transition in phytochrome photoswitching

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Phytochromes sense red/far-red light and control many biological processes in plants, fungi, and bacteria. Using crystallography, information about the two meta-stable states Pr and Pfr, has been gained. This series of discrete structural changes is believed to be responsible for phytochrome intracellular signaling. However, the structural characteristics of these meta-stable states remains to be investigated. We present solution NMR data of a complete 57 kDa photosensory module of the *Deinococcus radiodurans* phytochrome in both the resting and the photoactivated states.

Absorption of red light by the phytochrome dark resting state (Pr) leads to formation of the light-activated state (Pfr). From the light state, the protein spontaneously reverts to dark state by thermal reversion, a naturally occurring process which prevents lengthy experiments from being performed on any sample in the light state. By pulsed red light illumination inside the magnet, one and the same U-2H/13C/15N phytochrome sample could be used to record backbone assignment spectra using the targeted acquisition (TA) approach which offers interleaved acquisition of a series of assignment spectra combined with coprocessing of NUS data. In-magnet illumination for 5 seconds every 20 minutes was performed during data acquisition to continuously keep the sample in the light state for the entire time used to record the assignment spectra.

Phytochromes with a PAS-GAF-PHY photosensory module have a characteristic hairpin loop, called the tongue, which extends from the PHY domain to the chromophore binding pocket in the GAF domain. Crystallographic analysis has indicated that this tongue changes its secondary structure from  $\beta$ -sheet configuration in the dark to  $\alpha$ -helical in light state. The obtained phytochrome assignment has allowed further, detailed studies of this conformational change, and also made possible the investigations of the full photosensory module by NMR relaxation experiments, enabling detailed reports of several additional features of the mechanism for this protein.

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Effect of three different Flow Diverter stents on flow quantification by MRI

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Purpose: 4D flow magnetic resonance imaging (MRI) allows to measure and visualize blood flow in vivo . This is in particular interesting for the investigation of the effect of flow diverter stents used for treating aneurysms. However, metallic stents induce artifacts on MRI like other metal objects. It was realized before that the effect of these artifacts on the measured flow is important but not well known. The goal of this work was to evaluate how these artifacts affect the flow quantification by MRI.

Methods: To measure the flow with and without stents, several setups were constructed, composed of 3D-printed models of human anatomy or straight tubes in agarose. Stents (models) were placed outside of the tubes, so that the flow was not directly affected. 2D-Phase-Contrast (2D-PC), Time-of-Flight (TOF) and 4D-Flow were implemented on a 3T human (Philips) and 7T small-animal MRI (Bruker). Physical flow measurements were used as a reference to validate the MRI flow measurements, ultrasound may be applied later on.

Results:

Routine MRI contrasts were successfully acquired and different artifacts were observed for each stent. 2D PC, 3D TOF and 4D flow MRI were successfully acquired using the developed flow setup. No artifacts were obvious; a details evaluation is in progress and will be presented at the conference.

Conclusions and Outlook:

As expected, all stents induced MRI artifacts, although to a different extend. These artifacts were not obvious on processed 4D flow data; thus, the effect on the flow is unclear and may lead to misinterpretation. Because of these findings, we developed a simplified setup to elucidate this effect in detail. This way we expect to be able to identify the impact of the metal artifacts on the flow quantification. Once identified, the sequence parameters will be optimized to reduce the effect of the artifacts.

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## P366

**Intramolecular hydrogen bonds in 1,4-dihydropyridine derivatives.**  
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Since the original discovery of amlodipine, the 2-substituted 1,4-dihydropyridines have attracted considerable attention due to their various biological activities [1]. For the aimed synthesis of novel therapeutic agents, it is important to establish what structural factors influence their biological activity. For this purpose the original synthetic method was elaborated and the modification of 2,6-Me groups was performed.

Hydrogen bond interactions are principal forces, which determine the molecular structure and self-assembly processes as well as the structure of a great variety of chemical and biological systems [2].

Novel 1,4-Dihydropyridine (1,4-DHP) derivatives with intramolecular hydrogen bonds of the types  $\text{NH}\cdots\text{O}=\text{C}$  and  $\text{CH}\cdots\text{O}=\text{C}$  have been synthesized and characterized by  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$  nuclear magnetic resonance (NMR) spectroscopy, secondary H/D  $^{13}\text{C}$  isotope shifts, variable temperature  $^1\text{H}$  NMR experiments and quantum-chemical calculations.

The DFT calculations revealed that the conformers with the lowest energy are stabilized by weak intramolecular hydrogen bonds of the  $\text{CH}\cdots\text{O}$  type with the 3,5-carbonyl groups in *s-cis*, *s-cis* position relative to the double bonds of the 1,4-DHP cycle.

The diastereotopic protons of the methylene groups at positions  $\text{C}_2$  and  $\text{C}_6$  show unusual temperature dependence due to the changes in the populations of the optimal conformations.

On the base of the NMR and IR spectral data the shortening of the N–H bond length upon intramolecular  $\text{NH}\cdots\text{O}=\text{C}$  hydrogen bond formation was proposed.

**References:** [1] Hyvönen, Z. et al. Novel cationic amphiphilic 1,4-dihydropyridine derivatives for DNA delivery. *Biochim. Biophys. Acta - Biomembr.* 1509, 451–466 (2000). [2] Kuhn, B., Mohr, P. & Stahl, M. Intramolecular Hydrogen Bonding in Medicinal Chemistry. *J. Med. Chem.* 53, 2601–2611 (2010).

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## P367

### Study of the folding of the cold denatured barstar by temperature jump NMR spectroscopy

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Protein folding mechanism and kinetics can be studied with several experimental methods. NMR has a unique advantage with its atomic resolution information in comparison to other spectroscopical methods. One of the downside in studying fast processes is the dead-time of the device. There are several ways to initiate the folding of proteins inside the NMR spectrometer e.g. pressure jump, pH jump, dilution of denaturing agent or temperature jump. The latter has not yet been applied to study protein folding by NMR spectroscopy.

There is a well-known phenomenon called cold denaturation, which is an intrinsic propensity of globular proteins that at low temperature they are denatured. Usually this is below the freezing point, although it can be increased by the addition of chaotropic agent.

We have chosen barstar as a model protein. Barstar has been shown to undergo cold denaturation at 275 K by the addition of 2-3 M urea. First we optimized the denaturation and refolding conditions. We could show that the wild-type like mutant - C40/82A - could be reversibly refolded from its cold denatured state in buffer suitable for optimal radio frequency heating.

The reversibility of the system not just allows more scans to be acquired but also the acquisition of two dimensional spectra with high time-resolution. Previously limiting factor of resolution and the line broadening of the proton signals could be overcome by introducing a new heteronuclei dimension. Detailed analysis revealed a slow kinetic term which is related to the \_cis-trans\_ proline isomerization. Furthermore we designed pulse sequences to correlate the unfolded and folded state by introducing the temperature jump element into the pulse sequence. The latter could allow the study of the earlier steps along the folding pathway.

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Interactions of Edema Factor from *Bacillus cereus* with  
small inhibitors Studied by Ligand-Observed NMR Techniques

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Edema factor (EF) is a major virulence factor of *Bacillus anthracis*, the bacteria that causes of anthrax. After activation by intracellular calmodulin (CaM), EF catalyzes the production of supraphysiological levels of cyclic AMP (cAMP), thus interfering with several host essential intracellular processes. The activation by CaM induces the folding of a disordered switch region, *in silico* studies (Laine, 2010) complemented with experimental data, showed that thiophen ureidoacids (TUA) inhibit cAMP production. However, information on the mechanism of inhibition and on the binding site is lacking. Here, we present a study of the interaction of two TUA compounds with the protein EF from *Bacillus cereus*, a very close homologue of the *B. anthracis* protein. We analyze the interactions with EF, CaM and the complex EF/CaM using various nuclear magnetic resonance experiments including STD (saturation transfer difference), Waterlogsy and TRNOESY (transferred NOESY). The TUA compounds interact with isolated EF, isolated CaM and with the EF/CaM complex. Both compounds bind to the same specific site on EF. Both compounds bind to CaM only in the presence of calcium, which is required to form the  $\beta$ -sheets that are included in the TUA binding site. Competition experiments between TUAs and adefovir, a compound that binds to the catalytic site of EF with high affinity (Shen, 2004), indicates that both TUA molecules are allosteric inhibitors of EF.

**References:** [1] Laine et al. Use of allostery to identify inhibitors of calmodulin-induced activation of *Bacillus anthracis* edema factor. Proc Natl Acad Sci USA 2010. [2] Shen et al. Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus infection. Proc Natl Acad Sci USA 2004.

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**Interleaved spatial/spectral encoding in ultrafast 2D NMR spectroscopy**Bertrand Plainchont<sup>\*</sup>, Patrick Giraudeau, Jean-Nicolas Dumez*CEISAM, CNRS UMR6230, Université de Nantes*

Multidimensional NMR is a central tool in many fields but suffers from long experimental durations limiting its use for many applications such as reaction monitoring or high-throughput metabolomics. The duration of 2D NMR experiments can be reduced to less than a second with the “ultrafast” 2D NMR approach introduced in 2002 by Frydman et al. This technique makes it possible to acquire 2D spectra in a single scan by spatial encoding of interactions along one of the sample’s dimensions. Thanks to successive methodological developments, ultrafast 2D NMR has demonstrated its potential for a variety of analytical problems such as reaction monitoring, quantification of analytes and more generally the analysis of complex mixtures. Limitations exist, however, because of the capabilities of pulsed-field gradients on high-resolution NMR probes. In particular, a compromise between resolution and spectral width has to be made concurrently in both dimensions of the spectra. This restriction of ultrafast 2D NMR is due to the linear relation between spectral frequency and spatial frequency, which exists for commonly used encoding methods.

To overcome this limitation, we will describe an approach to arbitrarily assign a spatial frequency to each spectral frequency, allowing a controlled positioning of spectral regions. As suggested by Shrot et al, frequencies can be encoded in a nonlinear fashion with spatial/spectral (SPSP) pulses. The excitation profile of these pulses however presents undesirable harmonic sidebands in the spectral dimension. We will present a new tailored encoding scheme based on a series of interleaved excitations leading to an efficient suppression of sidebands. We will describe the key features of bidimensional SPSP pulses using numerical simulations performed with the Spinach program. This new approach to record ultrafast 2D NMR spectra will be illustrated experimentally with results obtained in homo- and hetero-nuclear 2D NMR with small molecules mixtures.

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## P370

## NMR study of the acid-promoted hydrolysis of 2-fluoropyridine derivatives

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Pyridine derivatives are of high interest in medicinal chemistry as well as SABRE hyperpolarization studies. Therefore, the stability is very important. Here, we have investigated the stability of several pyridine derivatives fluorinated in 2-position. Some of the substrates were examined previously but without NMR spectroscopic measurements.[1-4] It is well known that e.g. 2-fluoropyridine reacts in presence of  $\text{H}_3\text{O}^+$  to form 2-pyridone.[3,4] The possibility of the hydrolysis of the carbon-fluorine bond and the formation of HF is now clarified by  $^1\text{H}$ ,  $^{11}\text{B}$ ,  $^{13}\text{C}$ , as well as  $^{19}\text{F}$  NMR spectra. A 2 ml  $\text{D}_2\text{O}$ -solution containing e.g. 100  $\mu\text{l}$  2-fluoropyridine was acidified with DCl (30  $\mu\text{l}$ , respectively 50  $\mu\text{l}$ ) and NMR spectra were measured on a Bruker WB-300 (7T) NMR spectrometer. At the beginning, only small chemical shift changes were observed. Spectra were recorded at regular intervals. After around 12 hours new signals raised up. These signals increased the next days and the signals from 2-fluoropyridine decreased.  $^{13}\text{C}$  NMR spectra confirm that the  $^{13}\text{C}$ - $^{19}\text{F}$  coupling get lost and among other signals, a signal at 161.5 ppm is formed.  $^{19}\text{F}$  NMR spectra confirm this observation. After addition of DCl, the pyridine signal at -71.5 ppm decreases and a DF signal was formed at -166.2 ppm for a short time. Two further signals could be detected at -129.3 ppm [ $\text{SiF}_6$ ] $^{2-}$  and -150.6 ppm  $\text{BF}_4^-$ . The DF reacted with the borosilicate of the NMR tube. An  $^{11}\text{B}$  NMR spectrum clarifies the existence of  $\text{BF}_4^-$  (-1.54 ppm).

The evaluation of all NMR spectra allows the confirmation of the previously postulated reaction mechanism[4], which explains the formation of 2-pyridones in acidic solution.

**References:** [1] Bradlow et al., J. Org. Chem., 1949, 14, 509-515. [2] Bradlow et al., J. Org. Chem., 1951, 16, 1143-1152. [3] Clark et al., J. Org. Chem., 1981, 46, 4363-4369. [4] Cantrell, Tetrahedron Letters, 2006, 47, 4249-4251.

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## P371

**Hyperpolarized propane and Xe for heterogeneous catalytic reaction studies**Ekaterina V. Pokochueva<sup>\*,1</sup>, Dudari B. Burueva<sup>1</sup>, Alexandra I. Svyatova<sup>1</sup>, Kirill V.Kovtunov<sup>1</sup>, Thomas Meersmann<sup>2</sup>, Galina Pavlovskaya<sup>2</sup>, Igor V. Koptug<sup>1</sup><sup>1</sup>International Tomography Center, SB RAS and Novosibirsk State University,<sup>2</sup>Sir Peter Mansfield Imaging Centre, School of Medicine, University of Nottingham

Magnetic Resonance Imaging (MRI) was proven to be a powerful tool for studying catalytic reactions *in operando* due to its non-invasive nature and versatility. However, MRI application for gas-phase reactions studies is particularly challenging because of low spin density of reactants and products, and it's further hampered by inhomogeneity of the magnetic field, associated with the presence of solid catalyst beads or even catalytic reactors inside the scanner. The problem of low signal sensitivity can be solved by the implementation of hyperpolarization techniques, such as parahydrogen-induced polarization (PHIP) or spin-exchange optical pumping (SEOP).

In this work we've studied a new type of model catalytic reactors that minimize the perturbation of the magnetic field homogeneity and are thus suitable for MRI investigations of a working catalytic reactor. These reactors are glass tubes with a thin layer of titania, that was impregnated with the Rh precursor solution, and *in situ* reductive treatment of impregnated reactors yields catalytically active porous Rh/TiO<sub>2</sub> coating. The reactors were found to be active in hydrogenation of propylene, and, moreover, they were also selective to the pairwise addition of hydrogen. Developed MRI procedure allowed to achieve the maximum possible separation of signals from hyperpolarized and thermally polarized propane molecules, and <sup>1</sup>H MRI visualization of a working catalytic reactor during propylene hydrogenation with parahydrogen was performed. It was shown that the developed protocol can be applied in case of normal hydrogen. Moreover, we explored hyperpolarized Xe as a sensitive internal temperature probe for propylene hydrogenation reaction. It's expected that combination of two hyperpolarization methods would provide new useful information about catalytic reactions *in situ*.

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## P372

**DNP-enhanced solid state NMR studies of  
amyloid-beta interaction with cellular membrane mimics.**

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Enhancement of NMR signals using dynamic nuclear polarization (DNP) enables studying systems that are difficult to tackle with conventional NMR due to low concentrations or low overall quantities of material. One particular example is amyloid-beta peptide, which self-assembles in human brain tissue leads to neurodegeneration in Alzheimer's disease (AD). Previously, various intermediates along the aggregation pathway of amyloid-beta were captured in frozen solutions, and their structural details were revealed using DNP-enhanced solid state NMR (ssNMR) (Potapov et al. JACS 2015).

The amyloid-beta aggregates have been hypothesized to cause the disruption of cellular membrane leading to neuronal death in the course of AD. In this work, we aim to extend the ssNMR/DNP approach to study the interaction of the amyloid-beta peptide with the cellular membrane mimics in order to elucidate the mechanism by which cellular membrane gets disrupted (in collaboration with Prof. Wei Qiang, SUNY Binghamton, USA). Using model systems consisting of synthetic lipid bilayers the conditions for DNP have been optimized, with typical signal enhancements of about 40-50 obtained using Bruker DNP system at 14 T (600 MHz). We demonstrate that good quality double-quantum-single-quantum (DQ-SQ) one-bond correlation spectra can be obtained with as low as 1:200 protein:lipid ratio.

In contrast to studies with *synthetic* lipids, the main challenge for structural studies of amyloid-beta in *synaptic* membranes is a rather limited amount of the latter. We applied DNP-enhanced ssNMR experiments to reveal structural details of amyloid-beta peptide interacting with synaptic membranes obtained from mice of different ages. There, only 0.5 mg of peptide material was used at 1:10 protein:lipid ratio (1 mg of lipids), thus demonstrating the applicability of this approach for further studies.

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## P373

**Joint use of mechanochemistry and solid-state NMR in the  
synthesis and structural characterization of pharmaceutical cocrystals**

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Mehrnaz Khajali, Aneta Wróblewska, Justyna Śniechowska

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Mechanochemistry offers an efficient and ecologically friendly way of preparation of new pharmaceutical cocrystals. On the other hand, structural analysis of the obtained crystal-line forms can be troublesome, as the procedure does not yield crystals of sufficient quality for single crystal X-Ray diffraction experiments. In such cases solid-state NMR can offer a rich set of tools, which allow not only for following the progress of mechanochemical processes and quick determination of the reaction outcome, but also for structural determination of new cocrystals at a molecular level. In this work we demonstrate different fields of applications of a joint use of various solid-state NMR experiments and mechanochemical grinding in a ball mill. Basing on this strategy the following goals have been achieved: (i) a new mechanochemical protocol for the synthesis of pharmaceutically relevant cocrystals of apremilast, an anti-psoriatic agent, and linezolid, a broad-spectrum antibiotic has been developed; (ii) new cocrystals of apremilast with catechol have been described at a molecular level; (iii) new cocrystals of linezolid have been obtained; (iv) factors influencing the mechanochemical formation of linezolid cocrystals have been identified; and finally (v) 2-unsubstituted imidazol-3-oxide has been identified as a new possible coformer for pharmaceutical cocrystals. As an example, in very fast MAS NMR spectra of newly obtained cocrystals of apremilast with four different coformers slightly different interactions of the studied coformers with apremilast molecule are noticeable, despite the cocrystals being isostructural.

This study was financially supported by the National Science Center, Poland (Grant No. UMO-2017/25/B/ST4/02684). PL-GRID is gratefully acknowledged for providing computational resources.

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**Using in cell 2D  $^{13}\text{C}$ - $^{13}\text{C}$  solid-state NMR as a biophysical tool to characterize cell-wall and starch from the microalga *C. reinhardtii***

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Saccharides, in addition to their important energetic role, are used in the cell as building blocks for complex molecules. Glycosidic bonds between them can create different types of molecules, with linear or complex branched assemblies.

The main storage molecule in the microalga *C. reinhardtii* is starch, one of the most abundant polysaccharide in nature. This glucose polymer is made of amorphous and crystalline domains which can be mainly found into two different structures: A and B-types. These structures have specific physicochemical properties and are valued differently in drug formulation, for example. Using high-resolution solid-state NMR Magic-Angle spinning (MAS) methods on  $^{13}\text{C}$ -labelled starches, we can assign starch and its constituents (amylopectin and amylose) in the two crystalline forms and in the amorphous state. 2D-INADEQUATE experiments enable the assignment of hereto unreported non-reducing end groups, and the assessment of starch chain length, crystallinity and hydration. Solid-state NMR can also inform us on dynamics and conformational disorder, in particular in the amorphous state.

*C. reinhardtii* also has an extracellular cell-wall mainly made of hydroxyproline-rich glycoproteins, with characteristics shared by both higher plants and mammalian extracellular matrix. Using additional 2D solid-state NMR methods on  $^{13}\text{C}$  labelled cell-wall extracts, we assign several amino acids, saccharides and links between them, revealing both molecular and dynamic complexity in the cell wall. Moreover, highly crystalline, amorphous and hydrated regions could be differentiated.

This work illustrates how high-resolution solid-state NMR can be used to enable the detection and identification of starch and cell-wall *in situ* in intact cells, therefore eliminating time consuming and potentially altering purification steps. Furthermore, we show how these NMR methods can be used to get detailed information on organic disordered solids, and even in living microorganisms, making *in situ* solid-state NMR a powerful tool to study molecules directly in the cell.

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Glycans in action: Towards disentangling the factors that modulate carbohydrates interactions

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<sup>1</sup>CIC bioGUNE, <sup>2</sup>UCM, <sup>3</sup>CIB CSIC, <sup>4</sup>CIB-CSIC

The importance of glycans in life and disease has been demonstrated during the past few years. Indeed, it is today a hot topic with diverse branches and perspectives. The field is therefore attracting increased attention and it is expected that will provide spectacular developments. Herein we describe the application of NMR methods to unravel fine structural details of molecular recognition events that involve the participation of glycans (Ardá A. and Jimenez-Barbero J., Chem. Commun. 2018).

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## P376

**Simulating Nonlinear Chemical and Physical (CAP)  
Dynamics of Signal Amplification By Reversible Exchange (SABRE)**

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The hyperpolarization of nuclear spins using para-hydrogen (pH<sub>2</sub>) is a fascinating technique that allows increasing spin polarization and, as a result, the magnetic resonance signal by several orders of magnitude. Entirely new applications become available. Signal Amplification By Reversible Exchange (SABRE) is a relatively new method that is based on the reversible exchange of a substrate, catalyst and para-hydrogen. SABRE is particularly interesting for in vivo medical or industrial applications e.g., fast and low-cost trace analysis or continuous signal enhancement. Ever since its discovery, many attempts were undertaken to model and understand SABRE, with various degrees of simplifications.

In this work, we reduce the simplifications further, taking into account nonlinear chemical and physical (CAP) dynamics. These simulations were realized using an open source software (MOIN spin library). The CAP master equation presented here takes several effects into account, that were hitherto neglected. Explicitly included are now (i) chemical processes (substrate exchange, hydrogen exchange, hydrogen entering and leaving the system), (ii) coherent spin evolution and (iii) relaxation. The model is quite flexible; it is not restricted to a specific magnetic field range, number of spins or other experimental conditions.

Using CAP, we confirmed the experimental observation that an increase of pH<sub>2</sub> pressure and flow tremendously increases SABRE polarization. Furthermore, it turned out that the NMR-invisible, transient complex plays a crucial role in the process of hyperpolarization; the NMR properties of the transient complex (J-couplings) drastically change the efficiency of SABRE. Today, little is known about the transient complex, and several parameters had to be guessed (kinetic rates, coupling constants, relaxation rates). This may change in the future if more experimental data become available.

To conclude: the CAP model promises to be very useful for understanding and optimizing SABRE, just as well as to elucidate yet unknown parameters of the SABRE system.

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P378

EPR and luminescence of the Er3+ ion doped into YGa3(BO3)4 single crystals

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The present reports the results of the experimental study of static and dynamical characteristics of the Er3+ ions in the YGa3(BO3)4 crystals. g-factors and the constants of hyperfine interaction are found. The ratio  $g_z^*A_x/g_x^{**}A_z$  is close to unity that is an evidence of insignificant admixing of the excited multiplets. The widening of the absorption lines related to the temperature increase is associated with a strong spin-phonon interaction in both pure and Er doped crystals. The spin-lattice relaxation is well interpreted appropriately by means of the Orbach-Aminov processes. The reduction of g-factor was observed at the temperature rise and it was described as the result of spin-phonon interaction, proportional to  $-1/T$ .

The crystallographic structure of the Er doped YGa3(BO3)4 at ambient conditions and at over 300 - 1073 K temperature range, as well as its stability were studied by in-situ X-ray diffraction. The temperature variation of volume thermal expansion coefficient was determined, and its average value in the studied temperature range was found to be 19.58 MK<sup>-1</sup>.

The RL spectra of the samples are qualitatively similar, featuring several emission bands due to intrinsic origin and unintentional impurities. An assumption about existence of the characteristic Er3+-related emission band located in near-infrared was made. The TSL glow curves of both samples revealed strong TSL peaks at low temperatures due to shallow traps, as well as much less intense bands located above room temperature.

Measurements of heat capacity in a wide temperature range allowed us to determine the Debye temperature. This value seems to be particularly valuable, because the Debye temperature is the important parameter, entering expressions describing various physical properties.

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P379

Structure and Dynamics of Small Proteins by NMR  
Dennis Pyper, Nina Kubatova, Krishna Saxena, Henry Jonker,  
Christian Richter, Harald Schwalbe

Small proteins encoded by small open reading frames have been largely ignored in the past, because detection and identification of the coding DNA posed significant problems[1]. Modern transcriptomics technologies have made it possible to identify more and more sORFs making the research on small proteins increasingly important. They have been found in all three domains of life and it has been shown that they play a role in a large variety of purposes e.g. cell division, morphogenesis and stress response[2,3]. We apply NMR to obtain the structures and dynamics of small proteins in their apo state as well as with ligands such as metal ions or as a part of larger protein complexes. Furthermore, NMR enables us to gain knowledge of the folding state of small proteins which include folded, partially folded, molten globule or an unstructured state.

As part of an academic collaboration with several independent research groups we investigated 29 small proteins containing between 14 and 78 amino acids. The majority of the proteins are (partially) unfolded and good targets for the investigation as complexes with other biomolecules. Two proteins have been identified to adopt structured conformations and were structurally characterized by 3D NMR giving insight into the function and dynamics of these proteins[4].

**References:** [1] G. Storz et al., "Small proteins can no longer be ignored," *Annu. Rev. Biochem.*, vol. 83, pp. 753–77, 2014. [2] S. Gottesman, "The small RNA regulators of *Escherichia coli*: roles and mechanisms," *Annu.Rev.Microbiol.*, vol. 58, no. 0066–4227, pp. 303–328, 2004. [3] G. Storz et al., "Regulation by Small RNAs in Bacteria: Expanding Frontiers," *Mol. Cell*, vol. 43, no. 6, pp. 880–891, 2011. [4] N. Kubatova et al., "Solution structure and dynamics of the small protein HVO\_2922 from *Haloferax volcanii*," *ChemBioChem* 10.1002/cbic.201900085, 2019.

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## P380

## Calculation of hyperfine couplings in paramagnetic NMR – A DFT and ab initio study

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The current theoretical model for predicting the NMR chemical shifts of paramagnetic systems is based on a modern version of the Kurland-McGarvey theory, and relies on the computation of EPR parameters (hyperfine couplings, zero-field splitting and g-tensor) with quantum-chemical methods. While the zero-field splitting and g-tensor can be calculated ab initio with sufficient accuracy using complete active space SCF (CASSCF) and n-electron valence state perturbation theory (NEVPT2), the application of density functional theory (DFT) to hyperfine coupling tensors, as done within the current computational methodology, can be problematic. In the present work, the experimental pNMR signal shifts of a series of transition metal acetylacetonate complexes have been measured and theoretically predicted with different admixtures of exact Hartree-Fock exchange energy used in the hybrid DFT functional applied to calculate the hyperfine couplings. The results vary in a wide range as the proportion of exact exchange is changed, which illustrates the shortcomings of DFT in this context. To address this issue, efforts are made to calculate also the hyperfine couplings using ab initio methods, specifically coupled cluster (CCSD+quasirelativistic pseudopotential) and, in particular, fully relativistic configuration interaction (CI). The non-trivial choice of generalized active space within the CI method is made to encapsulate both electron correlation and spin polarization effects.

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Dynamics reveals new aspects of oncogenic KRas

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<sup>1</sup>Laboratory of Structural Chemistry and Biology, Eötvös L. University, <sup>2</sup>Medicinal Chemistry Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences

Oncogenic variants of kras gene are among the most important mutations causing cancer. KRas protein is a membrane-bound small GTPase which acts as a molecular switch and plays a key role in many signal transduction pathways regulating cell proliferation, differentiation, and survival. It alternates between its GTP-bound active and the GDP-bound inactive conformers. The most frequent oncogenic mutants are G12C, G12D, and G12V, all “frozen” in the active form and thus, induces malignant tumors. Both Switch-I and -II regions playing a crucial role in autohydrolysis are known to have intrinsic dynamic properties. While the backbone dynamics of HRas was described at multiple time scales of motion by NMR, neither KRas nor the above mutants were investigated. We developed a new approach enabling us to work with the native KRas-GTP-complex without the artifacts caused by GTP analogues. Here we are providing the comprehensive backbone dynamics analysis of GDP- and GTP-bound forms of KRas and its oncogenic mutants both at the ps-ns (fast) and  $\mu$ s-ms (slow) timescales of motion.

Data were analyzed by using the Lipari-Szabó model-free analysis and results were compiled with CEST and CPMG measurements to get a more complete picture on dynamics. We found that Switch-I and -II regions are highly dynamic showing Rex-exchange in Switch-I region, while a mutation in the P-loop alters its mobility. According to HetNOE measurements, the behavior of Y32 of Switch-I turned out to be opposite namely, this region is more rigid in its GTP-bound form. This new finding reveals the key importance of Y32 in hydrolyzing GTP. Based on these experimental results in conjunction with MD and QM-MM data we do propose new and important aspects of the GTP hydrolysis mechanism. Detailed examination of these oncogenic proteins’ dynamic properties let us to better understand the oncogenic effects caused by G12 site mutations.

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**P382**

**Conformational ensemble study of the intrinsically disordered WASp Interacting Protein by Nuclear Magnetic Resonance and Fluorescence**

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Intrinsically disordered proteins (IDPs) are polypeptides lacking a well-defined structure under biologically native conditions. As a result, they are best described as an ensemble of fast-exchanging conformations. The flexible nature of IDPs allows them to adopt distinct ensembles upon binding to different partners and to carry out multiple biological functions. One such protein is WASP-interacting protein (WIP), a multi-domain polypeptide that participates in the regulation of actin cytoskeleton dynamics. Here we focus on changes in WIP conformation and dynamics in the actin-binding N-terminal region of WIP (residues 2-65), using a double-mutant actin (actin-AP) to avoid unwanted polymerization. Upon sub-stoichiometric titration of actin into the WIP(2-65) sample, the HSQC intensity profile shows the largest line broadening for residues 33-45, coinciding with the actin-binding motif-WH2. Measurement of <sup>15</sup>N rotating frame relaxation rates for WIP(2-65) in the presence of actin showed a significant increase in relaxation attributable to an exchange contribution between free and bound conformations, specifically for residues 29-44, reflecting their presence at the binding interface. These results reflect an actin-mediated change in the conformational ensemble of WIP(2-65), and validate the affinity of double-mutant actin to WIP. Based on these results we are justified in our intentions to continue studying the conformational ensemble of bound WIP2-65 and binding-folding trajectory by uncovering the binding-induced effect of actin-AP using PRE and fluorescence methods.

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## P383

### Development in the synthesis of molecular rulers and trityl spin labels for pulsed dipolar EPR spectroscopy

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The pulsed dipolar EPR spectroscopy (PDS) techniques such as double electron-electron resonance (DEER) or relaxation-induced dipolar modulation enhancement (RIDME) detect the magnetic dipolar interaction within pairs of paramagnetic species from which the spin-spin distances and distance distributions can be extracted. By combination with site-directed spin labeling (SDSL) PDS became a valuable tool in structural biology for the study of structures and conformational changes of biomacromolecules.

For the development and evaluation of PDS techniques molecular rulers consisting of a shape persistent spacer and two spin labels at both termini are of great help. We developed a construction kit approach which allows an easy and fast assembly of molecular rulers in different lengths with two identical or different spin labels and modifiable side chains. Here we demonstrate this approach with the synthesis of water-soluble nitroxide-metal rulers.[1]

Additionally, we developed a synthesis of trityl radicals of the Finland-type with two different functional groups avoiding a statistical reaction. We chose ethyne and carboxyl as the functional groups. They are chemically orthogonal and allow us to modify the trityl radicals, for example attaching water-solubilizing and bioconjugatable groups to prepare water-soluble trityl-based spin labels for SDSL.[2]

**References:** [1] I. Ritsch, H. Hintz, G. Jeschke, A. Godt, M. Yulikov, *Phys. Chem. Chem. Phys.* **2019**, *21*, 9810–9830. [2] H. Hintz, A. Vanas, D. Klose, G. Jeschke, A. Godt, *J. Org. Chem.* **2019**, *84*, 3304–3320.

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## Structural study by NMR and modeling

Al-Sc alloy formation in NaF-ScF<sub>3</sub> melts and solidified mixtures

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Rinat Bakirov<sup>6</sup>, Konstantin Maksimtsev<sup>7</sup>, Catherine Bessada<sup>1</sup>

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One of the main fields of application of scandium is the development of lightweight, high-strength aluminum alloys. Very recently, a new original technological process based on the recovery of scandium from uranium ores was developed and put into practice. The final product was scandium concentrate based on NaScF<sub>4</sub>. The main advantage of this method is the ability to use directly as obtained scandium concentrate without additional purification in order to obtain an Al-Sc alloy by adding aluminum metal into molten concentrate. Therefore, the investigation of the reaction of scandium fluoride with metallic aluminium directly in the molten bath is a relevant task from the fundamental and industrial point of view to understanding the mechanism of the formation of Al<sub>3</sub>Sc.

In situ high temperature NMR spectroscopy was used to characterize the NaF-ScF<sub>3</sub> melts with and without aluminium addition over a wide range of compositions. The interpretation of all experimental results obtained in situ in the melt is significantly enhanced by the contribution of Molecular Dynamics (MD) calculations. A new interatomic potential for the molten NaF-ScF<sub>3</sub> system was developed by using a Polarizable Ion Model (PIM). MD simulations were combined with further DFT calculations to determine NMR chemical shifts for <sup>19</sup>F, <sup>23</sup>Na, and <sup>45</sup>Sc. The agreement between the experimental NMR data and the corresponding calculated data from our applied computational protocol indicated the polymerization and network formation in the melt.

In order to identify a feasibility of the NaF-ScF<sub>3</sub> melts as solvents in production of aluminium alloys, the solubility of aluminium in molten NaF-ScF<sub>3</sub> was also determined by thermal analysis methods. The solidified mixtures have been also characterized at room temperature using high resolution solid state NMR and X-ray diffraction.

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### Nanoparticle-Assisted NMR Spectroscopy: Enhanced Detection of Analytes by Water-Mediated Saturation Transfer

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Fabrizio Mancin, Federico Rastrelli\*

*Università degli Studi di Padova*

The self-assembly of organic molecules onto the surface of gold nanoparticles (AuNPs) provides a simple way to generate protecting monolayers that can incorporate molecular receptors. By exploiting different kinds of non-covalent interactions (namely hydrophobic, ion pairing, and metal–ligand coordination) such receptors can in turn provide tailored host sites for virtually any class of guest molecules. Interestingly, the reduced translational and rotational diffusion rates resulting from the bulkiness of NPs offer a convenient route to manipulate the magnetization of monolayer spins via relaxation- and diffusion-based NMR techniques. Eventually, proper nanoparticle-assisted “NMR chemosensing” experiments can lead to the detection and identification of small molecules in complex mixtures.

However successful, the first reported NMR chemosensing protocols – based on NOE magnetization transfer – suffered from an intrinsic low sensitivity. We have recently found that, in aqueous systems, water spins in long-lived association at the nanoparticle monolayer constitute an alternative source of magnetization that can deliver a remarkable boost of sensitivity, especially when combined with high-power saturation transfer experiments. The approach is general and can be applied to analyte–nanoreceptor systems of different compositions. In this communication we provide an account of the new method, and we propose a generalized procedure based on a joint water–nanoparticle saturation to further upgrade the sensitivity, which ultimately endows selective analyte detection down to the micromolar range on standard instrumentation.

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Conformational exchange of aromatic side chains by 1H CPMG relaxation dispersion

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Aromatic side chains are attractive probes of protein dynamics on the millisecond timescale, because they are often key residues in enzyme active sites and protein binding sites. Furthermore, they allow to study specific processes, like histidine tautomerization and ring flips. Here we investigate the possibility of aromatic <sup>1</sup>H CPMG relaxation dispersion experiments as a complementary method. Artifact-free dispersions are possible on uniformly <sup>1</sup>H and <sup>13</sup>C labeled samples for histidine δ2 and ε1, as well as for tryptophan δ1 [1]. In contrast, the <sup>1</sup>H relaxation dispersion profiles in phenylalanine, tyrosine and the six-ring moiety of tryptophan display anomalous behavior caused by <sup>3</sup>J <sup>1</sup>H-<sup>1</sup>H couplings and, if present, strong <sup>13</sup>C-<sup>13</sup>C couplings. The latter one can be inhibited through <sup>13</sup>C/<sup>12</sup>C labeling schemes. <sup>1</sup>H/<sup>2</sup>H labeling prevents artifacts from <sup>1</sup>H-<sup>1</sup>H couplings and enables measuring of flat relaxation dispersion profiles in the absence of exchange or relaxation dispersion if chemical exchange is present. In summary, <sup>1</sup>H CPMG relaxation dispersion experiments with uniformly labeled <sup>1</sup>H and <sup>13</sup>C labeled or selective labeled samples extend the amount of probes in aromatic protein side chains for measuring protein dynamics on the millisecond timescale.

Reference: [1] Raum HN, Dreydoppel M, Weininger U. (2018) J Biomol NMR 72, 105-114.

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## P387

**The electronic structure of the H-cluster in [FeFe] hydrogenase as analyzed through Q-band <sup>13</sup>C ENDOR spectroscopy. Implications for the catalytic mechanism.**

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<sup>1</sup>Max Planck Institute for Chemical Energy Conversion, <sup>2</sup>Institut für Chemie, Technische Universität Berlin, <sup>3</sup>School of Chemical Sciences, University of Illinois, <sup>4</sup>Technische Universität Berlin, <sup>5</sup>Department of Chemistry, University of California, Davis

[FeFe] hydrogenases are the most active hydrogen converting enzymes in nature. Their active site contains a classical [4Fe-4S] cluster connected through one of the coordinating cysteines to a unique binuclear iron cluster [2Fe]H coordinated by CN- and CO ligands as well as a bridging aza-propane-dithiolate (ADT) ligand. The CN- and CO ligands keep the iron core in a low oxidation state (Fe(I) or Fe(II)) while the amine in the ADT ligand provides proton exchange functionality near the open coordination site at the distal iron Fe<sub>d</sub> where hydrogen splitting/formation occurs. Using EPR and NMR spectroscopy we obtained a detailed picture of the spin distribution over the H-cluster in its various redox states.[1-3] It turns out that the magnetic exchange interaction between the two sub-clusters leads to strong spin delocalization over the whole H-cluster as reflected in the observed 57Fe hyperfine interactions for the active oxidized state Hox and the CO inhibited state Hox-CO. <sup>13</sup>C triple ENDOR experiments on an H-cluster in which the ADT ligand was isotope labeled revealed spin polarization in the ADT ligand coordination sphere. DFT calculations reproduce the magnetic properties of the ligand sphere such as the ADT moiety. The origin of the observed asymmetric spin distribution over the ADT ligand is related to the specific CO/CN sphere of the H-cluster. Furthermore, <sup>13</sup>C triple ENDOR experiments on the <sup>13</sup>CN labeled H-cluster in the CO inhibited state help to resolve a controversy concerning the ligand arrangement in this state. This study demonstrates that advanced pulsed EPR spectroscopy can provide both structural and functional information on the active site of [FeFe] hydrogenase thus helping to understand its catalytic mechanism.

**References:** [1] Silakov et al. JACS. 2007, 129, 11447. [2] Rumpel et al. JACS 2017, 140, 131. [3] Rumpel, et al. JACS 2018, 140, 3863.

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### An integrative approach to study multiscale dynamics in intrinsically disordered proteins

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Intrinsically disordered proteins (IDPs) are abundant in eukaryotic proteomes and act at the heart of fundamental cellular processes such as cell cycle control and signal transduction. The aberrant function of IDPs is therefore associated with severe diseases such as cancers and neurodegenerative diseases. Protein functions are mediated via motions, therefore a detailed characterization of protein dynamics is a key step towards a mechanistic understanding of biological events. Despite remarkable progress in the structural characterization of IDPs in the past decade, an accurate high-resolution picture of dynamics in IDPs is still missing. The main obstacle is that motions in IDPs occur in multiple length- and timescales that are not accessible to a single experimental technique. Here, we introduce an integrative approach which combines high-field NMR spin relaxation with low-field proton relaxometry, nanosecond fluorescence correlation spectroscopy (nsFCS) and microseconds-long MD simulation in order to access dynamics in IDPs on a broad range of timescales. The low-field proton relaxometry allows accessing reorientational dynamics on the timescale of several nanoseconds, while the nsFCS quantifies distance dynamics on the timescale of tens of nanoseconds. After re-scaling of the time axis of the MD trajectory on the basis of these experimental data, the high-field NMR relaxation rates are used to optimize the residue-specific order parameters. Using this approach we studied dynamics in two neurodegeneration-related IDPs, alpha-synuclein and amyloid-beta. Our results indicate that reorientational dynamics in these two IDPs contain a slow component with correlation times of several nanoseconds and propose a scenario in which the timescale modulation of these motions with respect to intra- and intermolecular diffusion plays an important role in disease-related aggregation of these two proteins.

German Research Foundation (DFG) is acknowledged for a research grant to N.R-G (Grant No. RE 3655/2-1).

Reference: [1] Rezaei-Ghaleh N et al. Angew Chem Int Ed (2018) 57: 15262-6.

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## Singlet order destruction in NMR experiments

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Singlet state NMR spectroscopy is an emerging field, dealing with techniques for efficient storage of spin hyperpolarization and investigation of various slow processes. To increase the singlet-order (SO) lifetimes, special molecules have been designed, some having a SO lifetime of 1.5 hours in room temperature solutions. At the same time, to repeat the experiment with extended SO lifetimes ones should wait until it will fully relax. This often makes experiments with SO quite time-consuming.

Here we propose two approaches to Singlet Order Destruction (SOD). The idea of the first method is to coherently couple the slow-relaxing and fast-relaxing modes. This can be done by applying a continuous SLIC pulse that couples the  $T_+$  and S states in the “tilted” frame, allowing the SO to relax on the time scale of  $T_+$ . The second method makes use of repetitive blocks, with each block comprising an M2S conversion element followed by a gradient, which equalizes the triplet population in the tilted frame. The latter method has proven to be more efficient than the former one. In the case of  $T_S=200$  s and  $T_1=7$  s, it enables reducing the waiting time between successive experiments from 1000 s to 40 s.

To show the utility of this sequence, we carried out two experiments with long-lived SO: measurement of SO decay and of nutation patterns for the “forbidden” transitions in a strongly-coupled system. In both cases, the SOD scheme followed by a  $5^*T_1$  waiting time yields the same result as the standard scheme with a  $5^*T_S$  waiting time, whereas using a  $5^*T_1$  waiting time alone gives rise to strongly distorted decay curves and nutation patterns.

We thus strongly recommend using the SOD scheme in any of its versions, to make experiments with SO fast and reliable.

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**C-terminal unstructured region of UFM1-activating enzyme 5 (UBA5) forms a platform for the UBA5 interactions with other proteins of ufmylation cascade and predetermines UBA5 functionality.**

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UFM1 is an ubiquitin like protein (UBL), which shares high structural but little sequence similarities to ubiquitin. Like ubiquitin it can be covalently attached to target proteins by specific enzymatic cascade called ufmylation. Ufmylation of target proteins plays a role in central cellular events, such as gene transcription, response to cell stress and differentiation. The process of ufmylation is initiated by the E1 enzyme UBA5, which activates and transfers UFM1 to other components of ufmylation cascade. We have shown that C-terminal region of UBA5, being intrinsically disordered itself, participates in plethora of protein-protein interactions and forms a platform to attract the proteins and enzymes of ufmylation pathway to this key UFM1 ligase. Besides the components of ufmylation cascade, C-terminal region of UBA5 also interacts with the autophagy modifiers (LC3 and GABARAP proteins, with higher specificity to GABARAP subfamily proteins). However, these interactions do not associate UBA5 or/and other proteins of ufmylation pathway to the autophagy-dependent degradation, but rather link highly soluble UBA5 to the cellular membranes, preferentially to the membrane of endoplasmic reticulum.

In order to understand molecular mechanisms and requirements of the multiple interactions associated with the UBA5 C-terminal region, we used NMR spectroscopy and performed a set of investigations involving the whole C-terminal unstructured UBA5 region as well as its specific evolutionary conserved parts, and a number of UBA5-interacting proteins – UFM1, UFC1 and GABARAP/LC3. For some interactions we solved the final complex structure and revealed key intermolecular interactions stabilizing formation of the complex. The structural data were complimented and extended with functional studies, supporting our model – the C-terminal unstructured UBA5 region forms evolutionary conserved platform for functionally important protein-protein interactions, which control efficient and selective ufmylation of target proteins in cells.

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### Studying the phase transition of UCST polymers in ionic liquids with CW EPR spectroscopy

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Thermoresponsive polymers have gained a great research interest over the last decades. [1,2] The phase transition of thermoresponsive upper critical solution temperature (UCST) polymers can be characterized with electron paramagnetic resonance (EPR) spectroscopy.[2,3] Starting from standard measurements with spin probes like Fremy's salt (FS) and TEMPO in water we exchange this solvent to ionic liquid (IL)/water mixtures. In dependence of these solvent mixtures we are recording temperature series to analyze their phase transition and, if it is possible, a "pre"-transition behavior. Additionally, we vary the ratio between the mixtures of IL and water to study the IL and salt effect for the UCST-polymers.

**Reference:** [1] Hildebrand V.; Laschewsky A.; Päch M.; Müller-Buschbaum P.; Papadakis C. M.; Effect of the zwitterion structure on the thermo-responsive behaviour of poly(sulfobetaine methacrylates). *Poly. Chem.* **2017**, 8, 310-322. [2] Kurzbach D.; Junk M. J. N.; Hinderberger D. Nanoscale Inhomogeneities in Thermoresponsive Polymers. *Macromol. Rapid Commun.* **2013**, 34, 119-134. [3] Hunold J.; Wolf T.; Wurm F. R.; Hinderberger D. Nanoscopic hydrophilic/hydrophilic phase-separation well below the LCST of Polyphosphoesters. *Chem. Commun.* **2019**, 55, 3414-3417.

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### Lithium Transference Numbers in Polyester-based Electrolytes Determined by Electrophoretic NMR

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Polymer electrolytes based on polyethylene oxide (PEO) were extensively studied in the past. However, they are still suffering from low ionic conductivity and low lithium transference number. Therefore, polyester-based electrolytes like poly( $\epsilon$ -caprolactone) (PCL) have been investigated and were found to exhibit promising conductive properties, especially in a co-polymer with poly(trimethylene carbonate) (PTMC). [1]

Recently, electrophoretic NMR (eNMR) was applied to salt-in-PEO electrolytes in order to directly determine the electrophoretic mobilities of all ionic species. [2] For eNMR measurements a self-built two electrode insert for 5 mm NMR tubes is used. The electrophoretic mobility is determined from the phase shift as a function of gradient strength in an experiment based on the double stimulated echo (DSTE) pulse sequence. [3] Here, we apply eNMR to LiTfSA in PCL and PCL-co-PTMC (80:20;  $M_n = 2$  and 4 kg/mol) at 90 °C. The influence of salt concentration and molecular mass of the polymer is investigated and the results are compared to data obtained by impedance spectroscopy and pulsed field gradient NMR.

It is found that the lithium transference number is much higher than in PEO electrolytes (~0.5 compared to ~0.2). Since the total ionic conductivity is lower than in PEO, the partial lithium conductivity was calculated and found to be almost equal in PEO- and PCL-based electrolytes. Moreover, the copolymerization of PCL with 20% PTMC yields only slightly slower dynamics due to more rigid polymer domains.

To conclude, for the first time, the promising class of polyester-based polymer electrolytes was investigated by eNMR showing excellent lithium conduction properties.

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ROLE OF CONFORMATIONAL FLEXIBILITY IN THE  
FITNESS OF  $\beta$ -LACTAMASES ENZYMES

Alejandro Jose Vila, Maria Agustina Rossi\*  
*IBR-CONICET*

Alternate structural conformers are the foundation of “protein evolvability”, which refers to the aptitude of proteins to rapidly adopt new functions within existing folds or even adopt entirely new folds. The understanding of protein evolution depends on the ability to relate the impact of mutations on molecular traits to organismal fitness. Antibiotic resistance mediated by  $\beta$ -lactamases represents an evolutionary paradigm in which the organismal fitness depends on the catalytic efficiency of a single enzyme. Here, we analyze the evolution of CTX-M-14, a serine-  $\beta$ -lactamase into the clinical variant CTX-M-16 (D240G, V231A) with higher activity against ceftazidime, one of the bulkier cephalosporins, and cefotaxime, the natural substrate. CTX-M-27 (D240G) displayed a reduced catalytic efficiency against cefotaxime but it improves against ceftazidime and CTX-M-9 (V231A) showed no activity difference. These expanded hydrolytic activities are generally related to an enlargement of the active site, but this is not the case for CTX-M. We hypothesized that this could be due to an increased flexibility.

To test this hypothesis, we studied the dynamics of CTX-M-14 and its natural variants by NMR. The V231A mutation affects the CSP of the residues located on the  $\alpha\beta$  domain and the D240G does it on the  $\Omega$ -loop, an essential catalytic structure of SBLs. The ps-ns dynamics did not show any important difference between the enzymes. However, in H/D exchange experiments the double mutant displayed a much faster exchange, particularly than the wild-type enzyme, of the residues located on or near the active. These findings may suggest that, in the clinical environments, CTX-M-14 evolved to generate a variant with an increased flexibility that is able to bind and hydrolyze larger substrates.

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Exploring the folding pathway of the I27 module from the giant protein TITIN with High-Pressure NMR.

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<sup>1</sup>CBS - UM, <sup>2</sup>UM - CBS, <sup>3</sup>CBS - INSERM, <sup>4</sup>CBS - CNRS

A complete description of the pathways and mechanisms of protein folding requires a detailed structural and energetic characterization of the folding energy landscape. Simulations, when corroborated by experimental data yielding global information on the folding process, can provide this level of insight. Molecular Dynamics (MD) has often been combined with force spectroscopy experiments to decipher the unfolding mechanism of titin, the giant multi-modular protein from sarcomeres, Ig-like single- or multi-domain yielding information on the sequential events during titin unfolding under stretching. Here, we used high-pressure NMR to monitor the unfolding of titin I27 Ig-like single-domain and tandem-repeat. Since this method brings residue-specific information on the folding process, it can provide quasi-atomic details on this process, without the help of MD simulations. Globally, the results of our high-pressure analysis support the existence of an intermediate folding state caused by the early detachment of the AB  $\beta$ -sheet, often reported in previous works based on MD or force spectroscopy but never detected with chemical denaturation assays. Similarly, the A'G parallel  $\beta$ -sheet of the  $\beta$ -sandwich has been confirmed as the Achilles heel of the 3D scaffold: its disruption yields complete unfolding, with very similar characteristics (free energy, unfolding volume, kinetics rate constants) for the two constructs, suggesting the absence of folding cooperativity between the two modules in the tandem-repeat.

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NMR Studies of Phospholipid Membranes using Lanthanide Ions  
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*Imperial College London*

Biological membranes are made up of phospholipids, which form a bilayer structure along with proteins and carbohydrates. In order to study the behaviour of these membranes, phospholipid vesicles have long been used as models. Whilst they may be a far cry from the complexity of a cellular membrane, their simplicity allows researchers to study individual biophysical effects in isolation. However, NMR of phospholipid vesicles presents many challenges, one of the most notable is the difficulty of distinguishing between the inside and outside leaflets of the bilayer. To address this, we utilised ytterbium ions to induce a pseudo-contact shift on the lipids. This results in two separate signals for the head group of the lipid. These correspond to the inside and outside of the vesicle, and we have used this to study a variety of membrane compositions using NMR. It was found that the ratio of the lanthanide to lipid was shown to influence the splitting, and the magnitude of the splitting was also dependent on the lipid and composition. By introducing lyso lipids, the membrane was perturbed and increasing the temperature past the transition temperature showed merging of signal from the head group. This work demonstrates the utility of the lanthanide shift reagents. It has been shown that they are sensitive to differences in lipid formulations and capable of elucidating phenomena previously unobserved by NMR.

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### Accelerated and sensitivity improved temperature-swept pure-shift NMR Małgorzata Rytel<sup>\*,1</sup>, Paweł Kasprzak<sup>2</sup>, Piotr Setny<sup>3</sup>, Krzysztof Kazimierczuk<sup>3</sup>

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There exists a number of experimental methods which provide homodecoupled (pure-shift) <sup>1</sup>H NMR spectra. Standard techniques (like PSYCHE [2] and TSE-PSYCHE [3]) require to record spectra in a pseudo-2D mode where chunks of data are acquired as separate FIDs. However, this often leads to very time-consuming experiments [1]. The problem with time rises, when a series of pure-shift proton spectra needs to be measured under different conditions, such as temperature, pH, pressure, concentration of compound in a sample, etc.

We present two different improvements using Radon transformation in post-processing [4, 5]. The first one may be used to boost sensitivity and resolutionn in series of pure-shift spectra. In a second technique we obtain a series of pure-shift spectra in shorter time. The idea is to measure only one chunk of data per experiment and process the data with modified version of Radon transformation. Additionally, our method allows to decode rates of linear changes of peaks positions caused by variations of conditions mentioned earlier.

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### Antioxidant Effects of Some Citrus Peels for Repetitive Deep-Fat-Frying: An EPR Spin Trapping Study

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Oxidative stability of oils is an important quality issue especially during deep-fat frying process. It is well known that lipid oxidation can lead to changes in functional, sensory, and nutritive values and even the safety of fried foods. Extracts of many plants have been reported to have varying degrees of antioxidant activities, which increase the oxidative stability of fats and oils. Electron Paramagnetic Resonance (EPR) spin trapping is a powerful technique with the fast and easy applying advantage that can directly detect short-lived free radicals occurred during lipid oxidation and be used for the assessment of antioxidant activity of food based extracts.

In this study, the antioxidant effects of both some citrus peels (orange, lemon, mandarin) and BHT (Butylated hydroxytoluene; a synthetic antioxidant) on the oxidative stability of sunflower oil during deep frying process were investigated by using EPR spin trapping method. The generated radical by lipid oxidation was characterized and a relative comparison of radical intensity was done for each repetition of frying using PBN (N-tert-butyl- $\alpha$ -phenylnitron) spin trap. Using this data, the inhibitory efficiency of both citrus peel extracts and BHT on lipid free radicals in sunflower oil was determined by calculating of Antioxidant Activity (AA) values for each frying step. Since the orange extract had better AA values than BHT and the other extracts in all frying steps, it may be recommended to use it as an antioxidant for repetitive deep fat frying. Moreover, the use of citrus peels, which are released as waste in the fruit juice industry, as an antioxidant source will also contribute to recycling and thus the economy.

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Points to be Considered in EPR Analysis of Irradiated Cellulosic Foods in the Presence and Absence of Satellite Signal

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Most food products are irradiated by ionizing radiation for sterilization purposes. Electron Paramagnetic Resonance (EPR) is the only technique that allows the direct determination of the paramagnetic centers that ionized radiation can form in the structure without damaging the sample. Using the EPR technique, it is possible to determine the effects of radiation on food, whether it is irradiated within the dose range permitted by the WHO/FDA, and the minimum irradiation dose that inactivates microorganisms. Therefore, EPR technique is widely used in irradiated food detection studies.

In irradiation detection of cellulose containing foods using EPR technique, according to the CEN Protocol 1787; it is recommended to use the satellite signals that reported to belong to the “cellulose-like radical” as an indicator of irradiation. However, in many cellulose-containing foods, it was shown that satellite signals could not be measured or absent due to either the structural properties of the sample and post-irradiation storage conditions.

In this study, the EPR analysis procedure of cellulose containing foods having satellite signals was summarized and in cases where satellite signal was not observed it was shown that singlet central signal can be used for the purpose of determining the effects of radiation on the sample. It has been emphasized that microwave power and kinetic studies are important and required in order to characterize the radiation-induced paramagnetic center(s) and to use it for irradiated food detection.

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## P400

## Fractionation Strategies to Expand the Capabilities of Bench Scale NMR

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Corporate R&D, Dow Benelux B.V.

NMR spectroscopy provides detailed insight into molecular structures and is capable of quantitation without standards. An area of interest for chemical industry is the possibility to use NMR to rapidly analyze wastewater streams with anomalously high TOC (Total Organic Carbon). A high TOC excursion can lead to regulatory violations and the consequent danger of shutting down an entire manufacturing site, with a loss of millions of Euros. High-field NMR has been proven to be valuable for identifying the source of high TOC, but it is not suitable for direct on-site measurements. In addition, for complex wastewater streams, a major limitation of NMR is the analysis of mixtures and the resulting overlap of resonances and limited sensitivity.

Solvent exchange, pre-concentration and fractionation with an uncomplicated solid phase extraction (SPE) technology, has the potential to extend this capability to smaller, more cost-effective, cryogen free benchtop units that could be placed at manufacturing/support sites where a more expensive high-field instrument is not justifiable.

The SPE pre-concentration approach would allow the characterization of low-concentration components in wastewater simplifying the NMR signal patterns and overcoming solvent restrictions of NMR measurements.

In this work, we have used real-world waste streams samples for demonstrating the power of fractionation for enhancing benchtop NMR capabilities. We have developed and verified a SPE procedure, which allowed elimination of water and salt from sample matrix and complete recovery of organic components at 30-fold enrichment. In addition separate pre-fractionation into hydrophobic and hydrophilic components was achieved. 400 MHz and Bench Scale NMR spectra of SPE sample fractions were evaluated. The data demonstrated that sample preparation by SPE excellently enhanced sensitivity to allow the use of BT-NMR spectrometers for gaining insight to sample composition of wastewater streams.

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## P401

**Relative configuration determination of a  
reserpine derivative using Residual Dipolar Couplings (RDCs)**

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Natural products play a critical role in drug discovery as they provide access to new chemotypes and structural diversity. In order to take advantage of natural products as potential drug candidates, their exact chemical structure and configuration need to be known. Structure and configuration determination can be achieved by X-ray crystallography if sufficient material is available and diffracting crystals are obtained. When no crystals are available, NMR spectroscopy is a prevalent technique for determining chemical structure. Investigating the configuration of molecules containing many stereocenters is often not possible based on traditional NOE data and coupling patterns. In such cases, complementary NMR data needs to be recorded and interpreted. Recently residual dipolar couplings have become an attractive source of such additional data [1] and are considered as the key to enable the comprehensive three-dimensional structure determination of many unsolved molecules.

In this study, RDC-based analysis has been used in order to assign the relative stereochemistry of a reserpine derivative containing seven stereocenters including a tertiary amine treated as a chiral centre. The aim of the study is the reliable determination of the relative stereochemistry and the determination of the interconversion of the tertiary amine using RDCs. Experimental RDCs are used to calculate RDCs with the programs MSpin [2] and COSMOS [3]. The obtained theoretical RDCs are compared to the experimentally determined RDCs, allowing the validation of the fitting. The NMR-derived configuration is verified by X-Ray analysis.

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A High-Field ESR Study using Hexaaqua Complex Salt  
Containing High-Spin Metal Ion: The Evaluation about a Potential of  
Tutton's Salt as a Pressure Standard for High-Pressure ESR Measurement  
Yu Saito\*, Takahiro Sakurai, Shigeo Hara, Susumu Okubo, Hitoshi Ohta  
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Recently, the function of proteins is much more attract attention and measurement of high-pressure NMR about proteins showed many remarkable results in this field. We reported zero-field splitting (ZFS) parameter D term was increased depending on load pressure raise that used the hemin as a model compound of metalloprotein using ESR employing terahertz light and high-pressure (HP-THz ESR). On the other hand,  $g_z$  value was shown significant decreases in especially almost 0 to 1 GPa region, inversely. However, calibration of pressure in this system is not enough and estimated pressure value from the outer load is conceiving with containing some volume of errors. Because due to the pressure standard marker like as DPPH as magnet field marker was not reported. Hence, we considered that the ability and usability of Tutton's salt (TS) as a pressure standard marker. TS could understand from simple crystal field theory and precise ZFS parameters of various TS could be got from recent reports of Prof. Rudowicz group. A TS with  $Fe^{2+}$  ion (also called Mohr's salt,  $S=2$ ) was measured in the region of up to 1.8 GPa and 0.4 THz. The result showed significant pressure dependence of E term (H/y) as increase of  $E = 3.7782$  (0 GPa) to  $E = 4.8345$  (1.8 GPa). This increase of E term had assigned from the decrease of symmetry of orthorhombic crystal structure depending pressure rise. Especially, referencing similar study without pressure, increase of E term conclude as decrease of symmetry along x- and y-axis direction. Similarly, we also evaluated TS salt containing with  $Ni^{2+}$  ion ( $S=1$ ). In the presentation of the day, we will show the result in detail.

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## P403

## Permanent Magnet Assemblies For In Operando NMR Studies.

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KU Leuven

High-field magnetic resonance is an extremely powerful characterization technique, which cannot however be trivially adapted to perform in in operando studies in technological materials like porous solids. Limitations with space, radio-frequency compatibility, transportability and maintenance are some of the difficulties one has to fight against. On the other hand, low-field magnetic resonance makes use of permanent magnets which are more versatile and solve some of the abovementioned issues.

In this contribution we present a variety of permanent magnet assemblies which offer new possibilities for material characterization in operando conditions. Small and large single sided systems having unique field profiles simulating NMR logging tools is presented. These systems are used alone for the study of pore size distributions in rock plugs, or as single-point clinical imaging systems. We also present new enclosed pseudo-Halbach magnets producing highly homogeneous sweet-spots and offering excellent portability. All these NMR systems can be used alone as desktop analyzers, but they here we have used them simultaneously with other characterization modalities (physisorption devices, neutron scattering and imaging devices) to provide hyphenated low-field NMR approached for in operando physico-chemical characterization of materials.

Applications on mesoporous materials (MOFs, zeolites) are presented where sorption of vapors of small molecules in natural abundance is monitored in operando and relaxometric information is recorded as a function of loading pressure. This information shows that NMR offers complementary information about the sorption dynamics in a direct manner which until today was missing.

Last we show some initial results in heteronuclear magnetic resonance of metals at low magnetic field using our home-made NMR sensors. This effort can potentially offer great benefits especially in NMR of metals in materials such as batteries and catalysts.

This low-field hyphenated NMR instrumentation demonstrates sufficient generality and practicality, and seems a promising direction for dedicated industrial applications of magnetic resonance.

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Dynamics of the isolated Growth factor receptor bound protein 2 (Grb2) SH2 domain and in the context of the full-length protein.

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The cells are subjected to a precise control of survival, growth, differentiation, and apoptosis. During the process of cell proliferation it is also subject to errors which may lead to loss of cell growth control, initiating the growth above the necessity of the tissue which can cause tumors. One of the pathways that undergoes changes is the Mitogen Activated Protein Kinases (MAPK), which is mediated by some proteins such as the Growth factor receptor bound protein 2 (Grb2). The Grb2 is composed of three domains, two SH3 (C and N-terminal) and one SH2. The Grb2, besides being an adapter, it also regulates the MAPK pathway through its monomeric/dimeric forms. To understand the structural dynamics of Grb2 and its Grb2-SH2 domain, the domain resonances were assigned by triple resonance experiments and the structure was calculated by CS-Rosetta. The relaxation parameters of Grb2 showed that this protein presents a complex interdomain dynamics. Moreover, the CPMG relaxation dispersion of Grb2-SH2 showed that the two sites responsible for the phosphopeptide recognition have dynamics in different time scales, where the site I is in fast exchange regime and the site II in intermediated exchange regime. The perturbations in Grb2-SH2 chemical shifts with the addition of the hydrophobic ligand coumarin and fluorescence quenching showed an interaction at site II of SH2, suggesting that the conformational exchange observed at site II is important for coumarin access at the site. Coumarin interaction is entropically driven, with 1:1 proportion and association constant of  $K_b \sim 10^3$  M<sup>-1</sup>.

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Dynamics of *Klebsiella pneumoniae* Outer Membrane Protein A in micelles and bilayers probed at atomic resolution

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The activities of membrane proteins strongly depend on their dynamics; herein we made use of proton-detected MAS at very high-field and of relaxation violated experiments in liquid state NMR to describe the dynamics of the membrane domain of the Outer Membrane Protein A of *Klebsiella pneumoniae* (KpOmpA) in liposomes and in micelles.

From <sup>15</sup>N relaxation rates and <sup>1</sup>H-<sup>15</sup>N dipolar-coupling, we described in residue specific manner the motion of the  $\beta$ -barrel of kpOmpA reconstituted in liposome; it behaves as a rigid body element with a low amplitude collective rocking motion (i.e. about 10°). However, the loops escaped to the detection by <sup>1</sup>H-detected MAS. In line with MAS NMR data, proteolysis experiments revealed the existence of a unique Trypsin cleavage site located at the top of the extracellular loop L3 (out of 16 potential K and R sites), suggesting restricted motion and accessibility of the loops, except for the long L3. Finally, we addressed the order parameters of methyl groups from alanine residues (22 alanine spread over a selectively protonated in perdeuterated background KpOmpA solubilized in DHPC-d<sub>22</sub> micelles), we confirmed the singularity of loop 3, suggesting that it might be highly accessible to protein-protein interactions and could play a role in cellular recognition and immune response mechanisms

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Solid-state NMR investigation of the involvement of the proline-rich region in tau amyloid fibrils

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The aggregation of hyperphosphorylated tau into amyloid fibrils is closely linked to the progression of Alzheimer’s disease. To gain insight into the link between amyloid structure and disease, the three-dimensional structure of tau fibrils has been studied using solid-state NMR (ssNMR) and cryogenic electron microscopy (cryo-EM). In addition, cryo-EM structures of tau fibrils purified from patient brains showed the ability of tau to adopt different structures in amyloid fibrils. Little is known, however, about the proline-rich region of tau in the context of tau amyloid structures, despite the location of several phosphorylation sites, which have been associated with Alzheimer’s disease, in the proline-rich region of tau. In order to gain insight into the contribution of the proline-rich region of tau to amyloid fibrils, we studied in vitro aggregated amyloid fibrils of tau that contain both the proline-rich region and the pseudo-repeats. Using ssNMR we show that the sequence <sup>225</sup>KVAVVRT<sup>231</sup>, the most hydrophobic patch within the proline-rich region, loses its flexibility upon formation of amyloid fibrils. The data suggests a contribution of the proline-rich region to tau amyloid fibrils, which might account for some of the unaccounted electron density in cryo-EM studies of tau fibrils and could be modulated by tau phosphorylation.

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**The surface structure of cysteine – coated  
ultrasmall gold nanoparticles - an NMR and microscopy study**

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Cysteine as ligand molecule is the only amino acid that can bind covalently to a gold surface via its sulphur atom which allows to attach peptides and or even proteins. It is therefore essential to understand the arrangement of cysteine on gold nanoparticles as a model system and starting point for investigations of more complex ligands.

Ultrasmall gold nanoparticles with a diameter of 1.8 nm were synthesized by reduction of tetrachloroauric acid with sodium borohydride in the presence of L-cysteine, with natural isotope abundance as well as <sup>13</sup>C-labelled and <sup>15</sup>N-labelled, respectively. The surface structure and the coordination environment of the cysteine ligands on the ultrasmall gold nanoparticles were studied by a variety of homo- and heteronuclear NMR spectroscopic techniques including <sup>1</sup>H-<sup>13</sup>C-HSQC and <sup>13</sup>C-<sup>13</sup>C-INADEQUATE. Most remarkably, three coordination environments of L-cysteine on the gold surface were identified that were ascribed to different crystallographic sites, supported by geometric considerations of the nanoparticle ultrastructure. Further information on the binding situation (including the absence of residual or detached L-cysteine in the solution) and on the nanoparticle diameter (indicating the well-dispersed state) was obtained by diffusion ordered spectroscopy (<sup>1</sup>H-, <sup>13</sup>C-, <sup>1</sup>H-<sup>13</sup>C-DOSY). The particle size data and the NMR-spectroscopic analysis gave a particle composition of about Au<sub>174</sub>(cysteine)<sub>67</sub>.

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Conformational changes of the guanidine-II riboswitch

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Riboswitches are RNA structures that regulate the downstream gene expression upon binding of a small molecule ligand. The guanidine-II riboswitch is a guanidine-binding translational ON switch that is present in many proteobacterial species. The associated genes are usually involved in guanidine detoxification. This riboswitch class is characterized by two hairpins (P1 and P2) with an ACGR loop motif. The hairpins are separated by a 7-40 nt linker containing the anti-Shine-Dalgarno sequence.[1] It is proposed that upon binding of one ligand molecule to each loop an intramolecular kissing hairpin structure is stabilized through their CG base pairs.[2] This is supposed to release the Shine-Dalgarno sequence and allows translation.

We investigate the conformational dynamics of the guanidine-II riboswitch using NMR spectroscopy. The measurements show that we can differentiate between three states: free RNA, Mg<sup>2+</sup>-bound and Mg<sup>2+</sup>/ligand-bound state. In titration experiments the conformational states were confirmed and ligand binding was characterized by analysis of specific nucleotides. Therefore we use in addition to <sup>1</sup>H-NMR, also 2D NMR experiments such as <sup>1</sup>H,<sup>1</sup>H-NOESY, <sup>13</sup>C- and <sup>15</sup>N-<sup>1</sup>H-HSQC and for a <sup>13</sup>C,<sup>15</sup>N-labeled sample 3D NMR experiments.

Our goal is to understand the conformational dynamics that govern ligand binding and thereby gain insights into the regulation mechanism of the guanidine-II riboswitch.

**References:** [1] C. W. Reiss, S. A. Strobel, *RNA*, 2017, 23, 1338–1343. [2] M. E. Sherlock, S. N. Malkowski, R. R. Breaker, *Biochemistry*, 2017, 56, 352–358

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The oligomeric structure of pyroglutamyl-modified Aβ peptides studied by solid-state NMR

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The fibrillation of different amyloid β peptides and their deposition as senile plaques are a hallmark of Alzheimer’s disease. In the core of these plaques, also N-terminally truncated variants, where the first glutamate residue 3 or 11 is converted into cyclic pyroglutamate (pGlu), are found. Aggregates of these Aβ variants exhibit enhanced cell toxicity. We already showed that mature fibrils of pGlu<sub>3</sub>-Aβ(3-40) and pGlu<sub>11</sub>-Aβ(11-40) have a very similar structure as wildtype Aβ(1-40).

Since it is assumed that oligomeric states represent the cell toxic species on the fibrillation pathway, here we investigate the molecular structure of oligomers grown from of pGlu<sub>3</sub>-Aβ(3-40) and pGlu<sub>11</sub>-Aβ(11-40) variants on the single amino acid level using solid state NMR. Oligomeric samples were prepared by dropwise addition of oligomer solution into liquid nitrogen and subsequent lyophilization. The <sup>13</sup>C NMR spectra exhibit an increased line width compared to mature fibrils due to the existence of different oligomeric states in the samples. While the chemical shifts values in the second half of the peptides are quite similar for oligomers and mature fibrils, we found some interesting differences in the modified N-termini, pointing to important structural differences between oligomers and mature fibrils. This may indicates an important role of the N-terminus in early aggregation of Aβ. Also the well observed interresidual contact between Phe<sub>4</sub> and Leu<sub>34</sub> could be observed also in the oligomers of the pyroglutamated Aβ variants. The contact Glu<sub>22</sub>/Ile<sub>31</sub>, which was found for wt-Aβ(1-40) protofibrils, could not be observed.

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## P410

**Theoretical inspection of Laser-Induced Magnetic Dipole Spectroscopy (LaserIMD)**Andreas Scherer\*, Ulrich Steiner, Malte Drescher*Department of Chemistry and Konstanz Research School Chemical Biology,  
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LaserIMD allows the determination of distance distributions in the nanometer region between a photo label and a permanent radical.[1] The photo label is initially diamagnetic and forms a triplet upon photo excitation. In double electron-electron resonance (DEER) the dipolar information is obtained by a  $\pi$ -pulse that induces a transition of  $\Delta m = \pm 1$  in a pump spin.

For LaserIMD the situation is different because the singlet state passes to a superposition of triplet states. As this transition is initiated by spin-orbit coupling, the  $T_+$  and  $T_-$  states are equally populated. This results in a net magnetization of zero during the evolution of the dipolar oscillation.

For the calculations we introduced three spins: a permanent radical  $S$  that acts as the observer spin and two spins  $L$  and  $I$  that form the singlet and triplet state of the photo label. We derived the commutation relations according to the product operator formalism and used them to perform an analytical calculation of the LaserIMD pulse sequence. The singlet-triplet transition was introduced by taking the partial trace over the  $L$  and  $I$  states and forming the Kronecker product between the density operator of the spin  $S$  and a triplet of the  $L$  and  $I$  spins. This means that the coherences will change their position in the density matrix. The transfer allows them to undergo a dipolar evolution resulting in the LaserIMD signal.

A calculation in the spin state picture showed that the transition that is responsible for the dipolar signal is between the  $D_{+y} T_x$  and the  $D_{-y} T_y$  state. Here  $D_{(\pm)y}$  are the eigenstates of the doublet spin  $S$  in the  $y$ -direction and  $T_x$  and  $T_y$  are the zero field eigenstates of the triplet.

**Reference:** [1] Hintze, C.; Bücker, D.; Domingo Köhler, S.; Jeschke, G.; Drescher, M., J. Phys. Chem. Lett. 2016, 7 (12), 2204–2209.

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Conformations of the Full-Length Guanidin-II Riboswitch Monitored by PELDOR

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Riboswitches are RNA elements that are involved in the regulation of genes. They bind small metabolites and undergo upon metabolite binding conformational changes that switches the corresponding genes on or off. The Guanidine-II Riboswitch binds guanidine and is composed out-off two stem loops, P1 and P2, connected via a single strand. X-ray crystallography on the isolated P1 and P2 stem loops revealed that they form homodimers  $(P1)_2$  and  $(P2)_2$  with the guanidine bound between the loops. This lead to the working hypothesis, that binding of the guanidine to the full-length guanidine-II riboswitch induces intraRNA P1P2 contacts with the guanidine bound between both loops.

We set out to investigate this in solution by a combination of site directed spin labelling and Pulsed Electron-Electron Double Resonance (PELDOR or DEER). The labelling was done via click chemistry in solution and RNA strand ligation in case of the full-length riboswitch. We find that the isolate stem loops P1 and P2 occur in form of duplexes or single strands in the absence of ligand. Adding guanidine leads in both cases to the formation of the homodimers  $(P1)_2$  and  $(P2)_2$ . Mixing both isolated stem loops induces the formation of duplexes and of the heterodimeric  $(P1P2)$  complex after adding guanidine. In contrast, single and double nitroxide labelling of the full-length riboswitch reveals that predominantly P1P2 contacts are formed upon adding guanidine.

This shows that PELDOR in combination with site directed spin labelling and ligation strategies is a valuable tool to follow ligand induced conformational changes of riboswitches.

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## P412

## Ever faster magic-angle spinning – 150 kHz solid-state NMR spectroscopy

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Almost invariably, the information content of the NMR spectrum, both in terms of structure and dynamics, increases with improved spectral resolution. For this reason, most biomolecular applications have been based on carbon detection since proton signals were too broad, principally due to proton-proton dipolar coupling. Through technical developments in faster magic-angle spinning, the line width in deuterated and fully back-protonated as well as in fully protonated proteins has been reduced to a degree which makes proton-detected solid-state NMR an attractive and accessible alternative with the positive side effect that much smaller sample amounts are needed for a typical application.

The development of proton detection in solid-state NMR has come with an increase in mass sensitivity of two orders of magnitude. Consequently, one might ask if we can expect to benefit from a continued investment in faster spinning. To address this question, MAS up to 150 kHz is used to investigate a complex of archaeal RNA polymerase subunits 4 and 7, in which the Rpo4 unit with 107 amino-acid residues is at natural isotopic abundance while the 187 residues of Rpo7 are <sup>13</sup>C-<sup>15</sup>N-labeled.

Using a rotor with an outer diameter of 0.51 mm and a uniformly <sup>13</sup>C- and <sup>15</sup>N-labeled sample content of approximately 100 µg, we observe a linewidth improvement of a factor 1.3 for 150 kHz compared to 100 kHz. This compensates (to ~90 %) for a factor ~1.5 of signal reduction due to the smaller rotor size necessary for faster spinning, considering the fact that the dimension of the detection coil is decreased as well. We show linear improvement of coherence lifetimes in a range of 100 to 150 kHz MAS and demonstrate that the technical challenges of investigating proteins at this MAS frequency can be overcome, concluding that continued efforts toward faster spinning are thus meaningful and timely.

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## P413

### Insights on water interaction at the interface of nitrogen functionalized hydrothermal carbons

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Hydrothermal carbon (HTC) materials derived from biomass are of considerable current interest due to low cost, environmentally desirable properties, and a broad range of potential applications, for example as functional materials, catalysts, absorber, or electrode material. Especially nitrogen-doped carbon materials are now playing an important role in energy conversion and storage technologies like water-splitting. Therefore, studying the molecular dynamics of water interactions with N-HTC based materials is important for understanding their general properties.

N-HTC were specifically synthesized by glucose and urotropine as precursors. Three different molar precursor ratios were used to analyze the effect of (i) the type and amount of N-functional groups and (ii) the synthesis temperature on the structure of the resulting material and on the correlated water interaction properties. Structural analysis were performed by <sup>13</sup>C and <sup>15</sup>N MAS spectroscopy, revealing trends of functional groups formed in this systematic approach. Water saturated (N)-HTC were analyzed by diffusion NMR and relaxation NMR. Results show N-functional groups are more important than pore size distribution and O-functional groups in affecting water movement in N-HTC materials. Finally, the degree of water interaction can be tailored by adjusting the synthesis temperature and the ratio of precursors.

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## P414

### Impact of the Abelson tyrosine kinase N-terminus on the conformations of its regulatory core

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Abelson tyrosine kinase (Abl) is an ubiquitously expressed kinase associated with a number of cellular signaling processes. The regulatory core of Abl consists sequentially of the N-cap, the SH3 and SH2 domains, and the kinase domain (KD) N- and C-lobes. The N-Cap differs between two splice variants, 1a and 1b. Abl 1b is 19 residues longer and N-terminally myristoylated. Under physiological conditions, Abl 1b is autoinhibited in an assembled conformation where SH2 and SH3 domains dock onto the kinase C- and N-lobes, respectively. This conformation is stabilized among others by the docking of the N-terminal myristoyl into a cavity of the KD C-lobe. Disruption of the interactions results in a disassembled, active conformation.

An erroneous chromosomal translocation leads to the deregulated Bcr-Abl fusion protein lacking the myristoylation and causes Chronic Myeloid Leukemia (CML). Effective ATP site inhibitors represent the front-line therapy against CML. However, spontaneous point mutations render these inhibitors ineffective in a fraction of patients. Recently, the promising inhibitor asciminib has been developed, which targets the myristoyl binding pocket. This allosteric inhibition mechanism is not well understood.

We have shown previously that a large part of the apo Abl regulatory core comprising the SH3-SH2-KD domains adopts the assembled autoinhibited conformation in solution[1]. Surprisingly, however, binding of imatinib and all other type II ATP site inhibitors induces the disassembled, but nevertheless inhibited conformation[2]. The assembly and disassembly of the core strictly correlate with the conformation of the Abl activation loop. Here, we present solution NMR data on the effect of the N-cap on the assembly state of Abl 1b and 1a in various ligand-bound forms

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## P415

### Structure and dynamics of ionic-liquid-containing polymer gel electrolytes as seen by NMR

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Polymer gel electrolytes based on the copolymer poly(vinylidene fluoride-hexafluoropropylene) (PVdF-HFP) are being explored as materials for batteries, supercapacitors and electromagnetic shielding materials. Gels containing ionic liquids instead of organic solvents result in inflammable, nonvolatile, highly conducting, and thermally as well as chemically stable films. The materials properties can be tuned by adding various kind of fillers. Addition of in-situ prepared silica enhances ion conductivity, possibly due to a decreased degree of polymer crystallinity. BaTiO<sub>3</sub> [1] or Fe<sub>3</sub>O<sub>4</sub> [2,3] nanoparticles in combination with multi-walled carbon nanotubes (MWCNT) have been used to improve electromagnetic shielding [1-3]. We have taken a multinuclear solution and solid-state NMR approach to investigate these complex materials. <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F NMR spectra provide information on specific interactions between the polymer backbone and the ions. Relaxation time measurements, including those of <sup>7</sup>Li, are employed to detect differences in the dynamics of ions and polymers for different samples. Finally, diffusion constants of the ions, obtained by pulsed-field gradient NMR, are compared with the conductivities of the polymer gel electrolytes.

#### Acknowledgement

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## P416

**Phase Sensitive Spectral De-Convolution for the Analysis of  
Ion Transport in Concentrated Electrolytes**

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Electrophoretic NMR (eNMR) is a versatile method for the investigation of drift velocities of charged and neutral species within an electric field. The application of an electric field during a diffusion NMR experiment causes a zero order phase shift of the NMR signal depending on the parameters of the diffusion experiment and the applied electric field strength. However, the experimental parameters have to be chosen very carefully since the heating and decomposition of the sample may cause significant measurement artifacts.

Ionic liquids and solvent-in-salt electrolytes gained a lot of attention during the last decade for possible applications in next generation batteries. In order to improve their properties, the considered electrolyte mixtures became more complex incorporating additives or a combination of solvents. When investigating highly concentrated electrolyte solutions via eNMR, one usually obtains small phase shifts due to the low mobilities of the electrolyte's constituents. This makes it hard to obtain the individual mobilities from the conventional mobility ordered spectroscopy (MOSY) method using 2D fast fourier transformation due to a poor resolution in the indirect dimension and averaging of superimposed signals.

In this work, we used phase sensitive spectral de-convolution to analyze superimposed proton eNMR spectra yielding reliable phase information and valuable information on the investigated electrolytes. We investigated the influence of mono- and multidentate additives on the migration behavior of lithium in an ionic liquid and observed lithium changing its migration direction from the "wrong" to the "right" direction depending on the chelate effect. We also found different lithium coordination sites in bi-solvent-in-salt electrolytes with different mobilities probed by the migration behavior of the solvent molecules.

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## P417

**Food Profiling: Determination of the authenticity of walnuts by NMR-Spectroscopy**Caroline Schmitt\*<sup>1</sup>, Markus Fischer<sup>2</sup>, Thomas Hackl<sup>3</sup>

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NMR spectroscopy has proven a valuable tool in differentiation of the geographical origin of various food products.[1] The geographical origin is influenced by the composition of metabolites due to different exogenous factors such as soil, temperature or water availability. The concept of a metabolomics-based approach based on the determination of a molecular fingerprint which is provided by the detection of the metabolites by NMR spectroscopy. Walnuts have been chosen as a matrix, since they are economically important for the confectionery industry and often used as a part of different food products.[2]

First, there must be established an own standard operation procedure (SOP) for the extraction of metabolites, because each food product of plant origin constitutes an individual matrix and a unique metabolite composition. Various work up procedures and solvent mixtures of different polarity were compared and stability measurements of two extracts were also carried out. Since the data obtained is too complex for manual evaluation, multivariate data analysis methods such as principal component analysis (PCA) or machine learning tools can be used. Principal component analysis was applied to the results of two different extraction methods to compare them.

**References:** [1] R. Bachmann, S. Klockmann, J. Haerdter, M. Fischer, T. Hackl, J. Agric. Food. Chem. 2018, 66, 11873-11879. [2] L. Eliseeva, O. Yurina, N. Hovhannisyan, Annals of Agrarian Science 2017, 15, 71-74.

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P418

Conformational dynamics in the bacterial beta-barrel transporter  
FhaC studied by solid- and solution-state NMR

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The Two-Partner Secretion (TPS) pathway in Gram-negative bacteria is dedicated to the export of large proteins serving notably as virulence factors. TpsB transporters are transmembrane   -barrel proteins secreting their TpsA substrates across the outer membrane. They belong to the ubiquitous Omp85 superfamily mediating protein insertion into or translocation across membranes. In the whooping cough agent *Bordetella pertussis*, the TpsB transporter FhaC mediates secretion of the adhesin FHA. Its resting-state crystal structure composed of a 16-stranded   -barrel preceded by two periplasmic POTRA domains is known, but its mechanism of protein transport has so far remained elusive [1].

FhaC is characterized by considerable dynamics. EPR, mutagenesis and accessibility studies have shown that crucial elements of its structure populate a dynamic equilibrium already in the absence of FHA. Notably, helix H1 and loop L6, which block the translocation pore in the resting state, may be displaced from the pore [2,3]. Such movements are likely implicated in the transport mechanism.

Our aim is to characterize the conformational dynamics of FhaC in molecular detail and to elucidate its role in transport. We study perdeuterated, Ile-  1 methyl-labeled FhaC samples in liposomes and nanodiscs by solid- and solution-state NMR. We report on methyl <sup>13</sup>C R1   and CEST experiments probing dynamics on the   s to ms time scale, as well as paramagnetic relaxation enhancement experiments to visualize transient minor conformations.

**References:** [1] Clantin, B. et al. *Science* 317, 957–961 (2007). [2] Gu  rin, J. et al. *Mol. Microbiol.* 92, 1164–1176 (2014). [3] Gu  rin, J. et al. *Mol. Microbiol.* 98, 490–501 (2015).

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P419

Development of Heteronuclear-Detected NMR Experiments for  
Characterization of NH- and NH<sub>2</sub>-Groups in RNA

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Heteronuclear-detected NMR experiments are becoming increasingly more common due to the constant development in cryogenic probes. These experiments are particularly beneficial with respect to nucleic acids due to the inherent low proton-density and limited <sup>1</sup>H-chemical shift dispersion. Here, we present <sup>15</sup>N- and <sup>13</sup>C-detected NMR experiments that enable characterization of imino and amino groups in RNA. Both of those functional groups are central in many types of nucleobase interactions, however, different exchange processes impede their characterization via <sup>1</sup>H-detected NMR experiments.

We apply <sup>15</sup>N-detected H-N correlation experiments for imino groups, on a set of five different RNAs and investigate the effect of molecular size on resolution, sensitivity and relaxation behavior. With no improvement in sensitivity of <sup>15</sup>N- over <sup>1</sup>H-detected H-N correlation experiments we move on to characterize nucleobase interactions through amino groups. They are difficult to detect as their proton-resonances usually exhibit large line widths due to a restricted rotation around the C-NH<sub>2</sub> bond. Herein, we introduce a <sup>13</sup>C-detected C(N)H-HDQC experiment which enables the detection of sharp resonances for all amino groups independent of their bond rotation through the evolution of <sup>1</sup>H-double quantum chemical shift. In addition, we have developed a <sup>13</sup>C-detected 'amino'-NOESY experiment where inter-residual amino NOE contacts can be observed, which are not detectable in conventional <sup>1</sup>H-detected NOESY experiments. The newly obtained NOE contacts are compatible with structure calculations and improve the structure's precision especially for RNAs with scarce experimental data. Those new amino experiments facilitate characterization of dynamic RNAs, as for example apo-states of riboswitches, where imino proton resonances are often not accessible due to a fast solvent exchange. Thus, amino protons can be used as a new standard for characterization of nucleobase interactions in nucleic acids.

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P420

Characterization of the binding sites of the  
Wnt signaling inhibitor Sclerostin using solution NMR  
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The Wnt signaling pathway is a crucial regulator of bone development in vertebrates. Through its activation, bone mass and mineral density is increased. A negative regulator of this pathway is the protein sclerostin. By binding the LRP5/6 co-receptor of the canonical Wnt pathway, sclerostin suppresses the binding of the Wnt ligand and consequently the bone growth and remodeling. In the presence of glycosaminoglycans (GAGs), it is shown that sclerostin loses its inhibitory effect. Therefore, we are interested in a detailed understanding of the sclerostin-GAG interaction to understand how this suppresses the binding towards LRP5/6 co-receptors, as this is may be a promising target to improve bone healing.

The structure of sclerostin (191 amino acids) contains a rigid cysteine-knot motif as well as extremely flexible termini (with approximately 50 amino acids each). For the investigation of the sclerostin binding mechanism using solution NMR, we established a purification and refolding protocol with a yield of 30 mg per liter fermented culture. In initial experiments, different GAGs were titrated to <sup>15</sup>N-labeled sclerostin. In the <sup>1</sup>H-<sup>15</sup>N-HSQC spectrum, shifting peaks were observed that belong to the predicted GAG binding site of sclerostin.

To mimic the binding of sclerostin towards the receptor, a peptide derived from the binding region of the LRP6 co-receptor was synthesized. After titration with this peptide, some peak shifts were observed in the corresponding <sup>1</sup>H-<sup>15</sup>N-HSQC spectrum of sclerostin that belong to some so far unassigned peaks. Upon assignment of these peaks, it will be possible to identify those residues, where the chemical environment was changed during the binding towards GAGs and LRP6.

Prospectively, a successive titration of a GAG and the peptide mimicking the LRP6 co-receptor to <sup>15</sup>N-labeled sclerostin will generate a more detailed picture of the interaction of sclerostin, GAGs and LRP5/6.

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## P421

**EPR investigation of a tyrosine dyad in a  
ribonucleotide reductase-inspired model system**

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Amino acid radicals are involved in essential processes in primary metabolism such as photosynthesis, respiration, and biosynthesis of DNA building blocks. They serve as one-electron redox cofactors in biocatalysis and multistep proton-coupled electron transfer (PCET) reactions. *E. coli* class Ia ribonucleotide reductase (RNR), which catalyzes the reduction of nucleotides to their corresponding deoxynucleotides, is a paradigm for studying PCET in biology. The radical transfer mechanism in RNR involves at least five tyrosine residues. A  $\pi$ -stacked, cofacially aligned tyrosine dyad is required for the fidelity of the radical transfer during PCET, and thus of the catalytic activity.[1] The remarkable structure of the two tyrosines might affect the redox chemistry in a way that they do not provide two sequential radical transfer steps but act as a collective property. In this work, we used pulsed EPR and orientation selective <sup>1</sup>H/<sup>2</sup>H ENDOR to investigate whether the perturbed redox properties of the tyrosines is due to the shared hydrogen bond within the dyad. In order to reach our goal, we used a model system, called DPX, that mimics the tyrosine dyad in RNR. In DPX two phenols are cofacially positioned at a fixed distance on a xanthene backbone.[2] Pulsed EPR showed the phenoxy radical could be generated and trapped after photogeneration with a 266 nm lamp. Combination of orientation selective ENDOR data, their corresponding simulations and DFT calculations revealed the existence of a hydrogen bond that is perpendicular to the aromatic ring plane. Our results strongly support that the non-covalent interaction between two tyrosines perturbs the well-studied one-electron redox chemistry of tyrosine radical in RNR. Here we report the first example that suggests a collective act of a tyrosine pair in an enzyme.

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P422

Identifying Structural Changes in Map1B Light Chain upon Interaction with Microtubules

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Proteins of the MAP1 (microtubule-associated protein) family are implicated in the regulation of microtubule spatial organisation and stability. One of its members is MAP1B, a predominantly neuronal protein required for neuronal network formation and synaptic maturation in murine brain development. [1],[2] Its importance is further highlighted by its implication in several human diseases. [3],[4] Despite this involvement, the function of MAP1B still remains elusive. Binding of MAP1B to microtubules is achieved in part through a microtubule binding domain (MTBD) in the N terminus of its light chain. [5] Previous work allowed us to determine the assignment of the intrinsically disordered light chain MTBD. [6] Here we report on our progress in using paramagnetic relaxation enhancements (PREs) to study the bound form of MTBD. Updated recombinant expression protocols allowed us to introduce cysteine mutations in order to attach MTSL spin labels to the polypeptide. Interaction conditions of the MAP1B light chain MTBD and microtubules, purified from pig brain, were then optimised to obtain free MTBD in exchange with its microtubule bound form. PRE measurements are ongoing at this time. The available data confirms the interaction of the N-terminal MTBD of the light chain of MAP1B with microtubules and reveals a compaction of the protein upon interaction.

**References:** [1] Tortosa et al., *J Biol Chem* **2011**, 286, 40638-40648. [2] Meixner et al., *J Cell Biol* **2000**, 151, 1169-1178. [3] Allen et al., *Nature* **2005**, 438, 224-228. [4] Zhang et al., *Cell* **2001**, 107, 591-603. [5] Noiges et al., *J Neurosci* **2002**, 22, 2106-2114. [6] Orban-Nemeth et al., *Biomol NMR Assign* **2014**, 8, 123-127.

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## P423

## Structure and dynamics of the nanophase separated stiff comb-like polymer PPDOT

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Structural and dynamic properties of polymers with stiff main chains and alkyl side chains are generally relevant in applications of semi-conducting polymers used in solar cells or as organic LEDs. Such systems are often characterized by a surprisingly high crystallinity, which is in fact a relevant feature for the electric performance.

Here, we investigate a model system, the comb-like polymer, poly(1,4-phenylene-2,5-n-didecyloxy terephthalate) with 10 carbons in the the alkyl side chain (PPDOT). With <sup>13</sup>C MAS NMR spectroscopy we analyze the structure and molecular motions in PPDOT with respect to its two polymorphic states. Both modifications, called *A* and *B*, exhibit a morphology with well-ordered backbones in  $\pi$ - $\pi$ -stacks separated by nanodomains which are formed by the methylene sequences [1, 2].

A change from the as-synthesized PPDOT-*B* to PPDOT-*A* occurs through a solid-solid phase transition above 70°C. At ambient temperatures, the polymorph *A* slowly converts back to the thermodynamically more stable state *B*.

We use <sup>13</sup>C CP MAS spectra to observe the structural changes during the phase transition and the differences in both modifications. In <sup>13</sup>C DIPSHIFT experiments, which inform about motionally averaged <sup>1</sup>H - <sup>13</sup>C dipole-dipole couplings, we do not see any indication of an amorphous backbone phase and therefore we emphasize that the complete sample exhibits a well ordered  $\pi$ - $\pi$ -stacking for  $T < 180^\circ\text{C}$ . The polymorphs differ in the conformational statistics and dynamics of the side chains, and above 180°C we detect the formation of a liquid crystal phase.

**References:** [1] G. Gupta et. al., *J. Phys. Chem. B* **121**, 4583-4591 (2017) [2] V. Danke et. al., *Eur. Polym. J.* **103**, 116-123 (2018).

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P424

Binding characteristic of neuronal protein  
FA-transporter P2 characterized with spin-labeled fatty acids  
Florian Schöffmann<sup>\*1</sup>, Petri Kursula<sup>2</sup>, Dariush Hinderberger<sup>1</sup>

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The protein P2 is a member of the class of FABPs (Fatty Acid Binding Proteins). Its function in the organism is to transport fatty acids (FAs) out of the intercellular system and bring them to the myelin sheath around axons in the PNS (peripheral nervous system).

A reversible neuronal disease known as “Charcot-Marie-Tooth disease” leads to a reversible myelin degeneration and one theory assumes that mutations on P2 may play a decisive role in the onset/process. Our goal is to use spin-labeled fatty acids to shed light on the binding pocket of P2 and its mutants. Initial studies have already shown that the mutants absorb the fatty acids differently qualitatively as well as quantitatively. The change in binding behavior can be directly related to the point mutations in P2. For the investigation, 5-DSA and 16-DSA (doxyl-stearic acid) were used, whereby each partial area of P2 the binding pocket could be illuminated by CW EPR spectroscopic measurements in temperature and concentration series.

In future experiments, P2 will be investigated with other neuronal proteins on neuronal lipid membranes. The influence of the neuronal membrane on the binding properties of P2 will be investigated by measuring P2 with other neuronal proteins in the presence of a myelin sheath model.

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## P425

## Global and local parameters to characterize peptide-membrane mimetics interaction

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Understanding the physico-chemical basics of protein-membrane interactions are of key importance in functional characterization of the cell membrane. In order to simplify this complex system the best membrane mimetics are bicelles, which at  $q=0.5$  (long chain/short chain lipid) ratio are ideal for solution state NMR studies[1,2].

We chose biologically relevant protein fragments: the lysine-rich segments of the plant stress protein ERD14 and the C-terminal domain of a small  $\text{Ca}^{2+}$ -binding, metastasis associated protein S100A4 to follow changes in size and morphology occurring upon interaction with neutral DHPC/DMPC and negatively charged DHPC/DMPC/DMPG bicelles under physiological conditions (pH=7.4; 150 mM NaCl).

Local environmental changes for both partners (typically 1mM/2mM unlabeled peptide and 150mM/300 mM lipids) were followed by 2D  $^1\text{H}$ - $^{13}\text{C}$  spectra, peptide amide environment using  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra, lipid phosphate headgroup environment by  $^{31}\text{P}$  NMR.

Translational diffusion experiments enable determination of the diffusion coefficient  $D$  that permits calculation of an effective hydration radius  $r_{\text{H}}$ . SAXS measurements were performed on the same sample under the same experimental conditions. Fitting of a lentil core-shell model (with axis  $a, b, t_a, t_b$ ) on the scattering curve enables a shape prediction and determination of gyration radius  $r_{\text{G}}$ .

For all studied systems significant line broadening of the DMPC peak in the  $^{31}\text{P}$  spectra was detected upon peptide interaction; while peptide  $\text{H}_{\text{N}}$  resonances shifted or broadened below detection limit. Diffusion coefficients were the same for the bicelle (followed on the DMPC methyl group) and for the peptide, showing that a peptide-bicelle complex was formed. Corresponding changes in  $r_{\text{H}}$  and  $b$  axis parameters enable elucidation of morphology variation. The structural changes of peptides were also followed by CD spectroscopy.

**References:** [1] Vold et al. *J. Biomol. NMR* 9 (1997) 329–335. [2] Bodor et al. *Biochim. Biophys. Acta* 1848 (2015) 760–766.

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P426

Geopolymers and their formation, traced by 27Al- and 29Si-NMR approaches

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Geopolymers are inorganic polymers, mainly based on minerals of geological origin (see e.g. ref. [1]). Their acid resistance, as well as low CO<sub>2</sub> footprint, make it an attractive construction chemical.

Geopolymerization is a multi-step reaction, typically conducted in alkaline media. It involves dissolution of a precursor, re-organization & condensation, and hardening, on a time scale of hours to days.

In addition to rheology and differential scanning calorimetry, NMR provides valuable microscopic insight to the complex reaction mixture (see e.g. ref. [2]).

Here, we report how molecular species can be discriminated during geopolymerization by means of their quadrupolar coupling constant using a simple, time-resolved static <sup>27</sup>Al NMR spectroscopy, and an appropriate theoretical framework.

Furthermore, bulk and surface properties of precursors and reaction products are compared by means of <sup>27</sup>Al- or <sup>29</sup>Si-detected direct excitation, cross-polarization and dynamic nuclear polarization NMR experiments under magic angle spinning.

**References:** [1] Davidovits, J. Ceramic Science and Technology 8:365 (2017). [2] Favier et al., Cement and Concrete Research 75:104 (2015)

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## P427

**Multidimensional Laplace NMR study of surfactants in aqueous solutions**Anne Selent<sup>\*,1</sup>, Ilari Ainasoja<sup>1</sup>, Pär Håkansson<sup>1</sup>, Otto Mankinen<sup>1</sup>,Sanna Komulainen<sup>2</sup>, Nønne Prisle<sup>3</sup>, Ville-Veikko Telkki<sup>1</sup><sup>1</sup>NMR Research Unit, University of Oulu, <sup>2</sup>NMR Research Unit, University of Oulu,<sup>3</sup>Synchrotron-based Atmospheric Research Group, University of Oulu

Surfactants are a group of chemicals, which lower the surface tension of liquids [1]. They may be completely unnoticeable, but we come across them in multiple different ways. They are used extensively in industry and science as detergents, wetting agents, emulsifiers, foaming agents and dispersants. However, these substances can also be found directly in our day-to-day life as they are used in soaps, paints, inks, cosmetics, toothpastes, cleaning agents, medications, etc. Surfactants can also be found in atmosphere as aerosols [2]. Due to their abundance around us it is important to understand their behavior and properties.

Most of the surfactants comprise of a hydrophobic tail and a hydrophilic head. In aqueous solutions, after a critical concentration is met, surfactants tend to form aggregates such as micelles and vesicles in different shapes and forms. The shape of the aggregates depends on the features of the solution and the surfactant itself. For example, the size difference between the hydrophilic head and hydrophobic tail and the pH of the solution have an effect on the aggregation.

In this work, we investigate aqueous surfactant solutions with multidimensional relaxation and diffusion NMR methods (called Laplace NMR [3]). We demonstrate that the multidimensional Laplace NMR combined with relaxation modeling reveals detailed information about the structures of aggregates, which is not available with other methods.

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## P428

# Study of the chain dynamics in irradiated high density polyethylene (HDPE) by proton low-field double-quantum (DQ) NMR

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Industrial grade samples of HDPE with a broad molecular weight distribution, cross-linked via irradiation below the melting point, were probed by low-field <sup>1</sup>H time domain NMR to provide information about the crystallinity in the semi-crystalline state and chain motion in the molten state. As the cross-linking reactions only occur in the amorphous region, the produced samples are not uniformly cross-linked, which means in the molten state, the sample contain a mixture of linear chains with different lengths, which follow different dynamic regimes, leading to very complex dynamics. For studying the chain motion with the double-quantum (DQ) NMR experiment, two methods were applied for analyzing the measured data. The first method, used for probing rubbers and hydrogels in previous studies [1], extracts the averaged residual dipolar coupling (Dres) which mirrors the amount of anisotropic motions caused by entanglements and cross-links. The second novel method is based on a power-law model of the orientation autocorrelation function (OACF) C(t), which estimates the amplitude of the OACF and the time scaling exponent  $\kappa$  (slope in the plot of C(t)~t<sup>- $\kappa$</sup>  against time in logarithmic scale) [2]. The  $\kappa$  values, which can vary between ~0 (a perfect network) and ~0.5 (a linear entangled polymer) in this sample, can demonstrate how the cross-linking affects the chain motions in such a complex system. Complementary rheological measurements also showed that the decaying slope of storage modulus (G') vs. angular frequency ( $\omega$ ) correlates with the irradiation intensity, which is similar to what  $\kappa$  values reflected.

**References:** [1] Lange F, Schwenke K, Kurakazu M, Akagi Y, Chung U-i, Lang M et al. *Macromolecules* 2011;44:9666–74. [2] Mordvinkin A, Saalwächter K. *J. Chem. Phys.* 2017;146:94902.

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**<sup>19</sup>F NMR-based fragment screening for detection of secondary binding sites**

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Carbohydrate-protein (lectin) interactions play a crucial role in cell-cell communication and host-pathogen interaction and thus, determine the progress of health and disease. Consequently, lectins are attractive for the development of therapeutics; however, only a few inhibitors have been designed to interfere with this interaction. Here, we aim to establish a broadly applicable methodology for development of non-carbohydrate based inhibitors for lectins. For this, a set of bacterial lectins involved in formation of antibiotic-resistant biofilm by *Pseudomonas aeruginosa* (LecA, LecB) and *Burkholderia ambifaria* (BamBL) has been chosen to explore the potential of our discovery pipeline [1].

<sup>19</sup>F NMR-based fragment screening was used to assess the druggability of various lectins. <sup>1</sup>H- and <sup>19</sup>F-based libraries were screened including 950 fragments overall. For LecA, <sup>19</sup>F NMR screening resulted in hit rates of <1% and 5% for the carbohydrate- and secondary binding sites, respectively. Interestingly, <sup>19</sup>F NMR screening against BamBL showed a hit rate of 48% for carbohydrate- and secondary binding sites, respectively. Several hits were followed up for BamBL in protein-observed <sup>1</sup>H-<sup>15</sup>N HSQC-TROSY NMR and orthogonal biophysical techniques to validate the binding. This resulted in a hit rate of 45%, which is consistent with our fragment-observed <sup>19</sup>F NMR screening.

Taken together, these data suggest the existence of secondary binding sites in lectins that potentially can be used for the design of allosteric inhibitors for lectins.

**Reference:** [1] Imberty, A.; Varrot, A., Microbial recognition of human cell surface glycoconjugates. *Current opinion in structural biology* **2008**, 18 (5), 567-76.

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Use of L-PGDS protein as a theranostic agent for  
early detection of amyloid- $\beta$  aggregation  
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Lipocalin-type prostaglandin D synthase (L-PGDS) is an endogenous brain protein, shown previously as a major chaperone for amyloid  $\beta$  ( $A\beta$ ) peptide. It binds to monomeric  $A\beta$  as well as mature fibrils and is capable of inhibiting their aggregation. We have also recently found that L-PGDS can successfully breakdown mature  $A\beta$  fibrils (manuscript submitted), which opens avenues for its use as a therapeutic agent in cases of Alzheimer's disease (AD).

This work is aimed at utilizing the theranostic potential of L-PGDS early detection of  $A\beta$  in AD mice using MRI. For this purpose, we conjugated the recombinant L-PGDS protein with iron oxide-based nanoparticles with different outer coatings. The functionality of the conjugated protein and its inhibition activity towards  $A\beta$  were compared to choose the correct composition. Effect on cellular viability and tendency to form aggregates was also compared. Four different nanoparticles were compared for their T2 contrast enhancement. Taking all results together, biological probes developed by covalent conjugation of magnetic resonance- active ferritin nanocages with L-PGDS were used for in vivo studies in AD mice. Injections in diseased mice showed hypointensity in mouse brain areas correlated with the presence of amyloid-rich structures compared to age-matched healthy mice.

All MRI data were acquired using Bruker spectrometers and Paravision 6.0 software was used for processing and analysis. T2 relaxation curves were prepared for nanoparticles to compare relaxivity differences in biological buffers and 1% agarose phantoms. Observable differences in T2-star weighted image intensities were identified upon non-invasive administration of conjugated probes in control mice brain. Conjugates injected in the ventricular chambers were shown to disperse upon incubation and accumulate in amyloid-rich brain regions in AD mice. To this end, we have shown here that L-PGDS protein has tremendous potential as a diagnostic agent for early identification of AD hallmarks in disease-prone populations.

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## P431

**Accelerated pure-shift acquisition based on prior knowledge from proton spectrum**

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Pure-shift NMR [1] is a branch of techniques allowing the suppression of homonuclear J-couplings in an NMR spectrum. This gives a boost in resolution as multiplets in a spectrum collapse into singlets. The techniques rely on echo-type pulse sequences with selective pulses. In between the pulses, a short chunk of an FID is acquired. The whole FID is then constructed from these chunks.

The type of the acquisition which yields spectra of better resolution and fewer artifacts resembles a standard two-dimensional

measurement where each chunk is measured in a separate increment (pseudo-2D acquisition). The one-dimensional FID is then reconstructed from these increments. An example is widely used PSYCHE pulse sequence [2]. However, such experiments are time-consuming. This leads to an idea to accelerate them with non-uniform sampling (NUS). Normally, NUS in NMR means omitting some measurement points and reconstructing them mathematically afterwards. Here, one can only omit whole chunks, not separate points. This situation is potentially more challenging, as any regularity can increase undersampling artifacts and hamper the consequent reconstruction. However, this procedure still works, as was demonstrated in [3].

On the other hand, we have prior information on the spectrum: the conventional 1H spectrum can tell us, within some tolerance, where to anticipate peaks in a pure-shift spectrum. In this work, we show how to optimize the burst sampling scheme taking the advantage of this prior information. Our aim is to minimize the risk of high undersampling artifacts and thus assist the reconstruction. We demonstrate that the optimization procedure eliminates the otherwise present risk of the reconstruction failure. We also provide a step-by-step workflow.

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## P432

**Long-lived nuclear spin states in  $^{15}\text{N}$  labeled trans-azobenzene**

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Long-lived nuclear spin state of the  $^{15}\text{N}$  pair in doubly  $^{15}\text{N}$  labelled trans-azobenzene have been studied. The singlet state of the two  $^{15}\text{N}$  spins is accessible due to magnetic non-equivalence<sup>1</sup>. It is possible to convert  $^1\text{H}$  or  $^{15}\text{N}$  magnetization to the long-lived state and backwards<sup>2</sup>. The lifetime of the singlet order under the spin-locking fields was measured at 9.4 and 16.4T magnetic fields and appeared to be  $1060 \pm 110$ s in both cases. The signal intensity derived from the long-lived component reached up to 160% of  $^{15}\text{N}$  thermal polarization using adiabatic SLIC pulse<sup>3</sup> on the  $^1\text{H}$  channel. Adiabatic methods are favorable compare to the resonance methods (such as M2S<sup>4</sup> and SLIC<sup>5</sup>): up to 26% (260%) level of  $^{15}\text{N}$  magnetization can be obtained after MSM<sup>2</sup> pulse sequence using the initial nitrogen (proton) spin magnetization with adiabatic methods, whereas 16% (160%) are provided by the resonant methods.

The field dependence of the long-lived component was measured using a field-cycling setup revealing features at fields of 6T and 2T. At these fields the singlet lifetime reached  $400 \pm 50$ s and  $500 \pm 50$ s respectively, whereas at other fields it was  $130 \pm 20$ s in the absence of spin-locking. A similar non-monotonous dependence was observed at 16.4T for the lifetime as a function of the amplitude of the spin-locking field applied on the  $^1\text{H}$  channel. We argue that such effects are possible due to changes of the singlet-triplet leakage rates in the 12-spins system of trans-azobenzene.

**References:** [1] K. F. Sheberstov et. al, Appl. Magn. Reson., 2018, 49, 293-307. [2] Y. Feng et. al, J. Chem. Phys., 2014, 141, 134307. [3] B. A. Rodin, et. al, J. Chem. Phys., 2019, 150, 064201. [4] M. C. D. Tayler et. al, Phys. Chem. Chem. Phys., 2011, 13, 5556-5560. [5] S. J. DeVience et. al, Phys. Rev. Lett., 2013, 111, 173002.

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Allosteric Activation of GDP-Bound Ras Isoforms by Bisphenol Derivative Plasticisers

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Stefanie Löffek<sup>2</sup>, Iris Helfrich<sup>2</sup>, Hagen S. Bachmann<sup>3</sup>, Jürgen Scherkenbeck<sup>4</sup>, Raphael Stoll<sup>1</sup>  
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The protein family of small GTPases controls cellular processes by acting as a binary switch between an active and an inactive state. The most prominent family members are H Ras, N Ras, and K Ras isoforms, which are highly related and frequently mutated in cancer. Bisphenols are widespread in modern life because of their industrial application as plasticisers. Bisphenol A (BPA) is the best known member and has gained significant scientific as well as public attention as an endocrine disrupting chemical, a fact that eventually led to its replacement. However, compounds used to replace BPA still contain the molecular scaffold of bisphenols. BPA, BPAF, BPB, BPE, BPF and an amine substituted BPAF derivate all interact with all GDP-bound Ras Isoforms through binding to a common site on these proteins. NMR-, SOScat-, and GDI- assay based data revealed a new Bisphenol-induced, allosterically activated GDP bound Ras conformation that define these plasticisers as Ras allosteric agonists.

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MODULATION OF CENP-ACse4 BY ITS BINDING PARTNERS

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The centromere in eukaryotes is epigenetically marked by the presence of a specialised nucleosome wherein Histone H3 is replaced by a variant CenH3. In budding yeast, it is called Cse4. Cse4 has an unusually long N-terminal tail when compared to other CenH3s and the role of this long tail is not fully understood.

Since Cse4 is the component that distinguishes the specialized nucleosome from the canonical one, understanding its structure, its interactions with various proteins and its regulation is indispensable to understand centromere function. Cse4 interacts with H4 in the nucleosome as well as with its chaperone Scm3. However, due to lack of structural information about these interactions, it has led to many contradicting theories. There is a gap of information as only the C-terminus of Cse4 which harbors the histone fold domain has been well characterized with functions such that of centromere targeting of Cse4.

We have reported using NMR and other biophysical techniques, for the first time, that Cse4 exists in a ‘closed’ conformation wherein the N and the C-terminus are involved in interaction. Upon H4 binding, structural changes occur which ‘opens’ Cse4 as a result of which the long N-terminal tail becomes free. We also found that the tail interacts with its chaperone, Scm3 which was missing in the previous structural studies. This interaction is unique as both the proteins are intrinsically disordered and do not acquire a ‘definite’ structure even after binding, forming a ‘fuzzy like complex’, even though the interaction is strong. This type of association may be required to facilitate the interaction of multiple proteins with both the N-terminal tail and Scm3 while they interact with each other at the centromere laying the basis of kinetochore formation.

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Cleavage of DNA fragments: the role of the metal center combined to structural rearrangements for hydrolytic and oxidative reactions

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Cleavage of DNA is a vital process in all living systems. Such process is involved in replication, transcription and other cellular transactions, where the leading role belongs to enzymes and specific cofactors. In general, three different types of DNA cleavage can be distinguished, namely i) DNA hydrolysis, ii) photochemical cleavage and iii) oxidative cleavage, although the last two categories are quite closely related. DNAzymes (also known as deoxyribozymes) are DNA sequences that can replace the classical enzymatic architectures and still catalyze selectively the chemical reaction under investigation. Although several fascinating and promising aspects of those compounds are supported by several biochemical studies, information on their structures and mechanistic features are severely lacking. Interestingly, very often those systems have as cofactors metals and lanthanides, as more conventional enzymatic adducts. Thus, methods of choice to study DNAzymes are with no hesitations CW and pulsed EPR/ESR techniques. Compared to enzymatic complexes, DNAzymes may exhibit a minor selectivity/specificity but the relatively lower costs with respect to enzymes and their higher stability boosted their applicability in recent years. Loss of specificity/selectivity does not seem to affect the catalytic efficiency. In this study the local and global structural features of DNAzymes are described by advanced EPR techniques involving dipolar and hyperfine spectroscopy. Kinetics studies, distance measurements and HYSCORE experiments highlight the role of the copper ions within the hydrolytic cleavage. Selectively modified nucleobases have been used in a covalent and non-covalent scenario to point out the binding efficiency towards the metal center. Preliminary data on the oxidative cleavage catalyzed by manganese ions are also presented.

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Intrinsic Disorder of Huntingtin Exon-1 Fibrils

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Cross- $\beta$  fibrils (aka amyloid fibrils) are a hallmark of many neurodegenerative diseases. Every mechanism of potential cross- $\beta$  fibril toxicity will have to be mediated by the (abnormal) interaction of the fibril surface with cellular binding partners. We are studying the fibril surface in the context of huntingtin exon-1 (HTTex1) important in Huntington's Disease (HD). The core of HTTex1 fibrils is formed by its polyQ domain, which is expanded in HD making the protein prone to fibril formation. The Pro-rich C-terminus of HTTex1 fibrils is highly dynamic as shown by solid-state NMR and EPR. Together with electron microscopy, we proposed a bottle brush model of HTTex1 fibrils in which the C-terminus forms the surface of the fibrils in the form of dynamic bristles.

To describe the dynamics and conformational space of this dynamic C-terminus in more detail, we used a combination of NMR, EPR, and molecular dynamics simulation. With solution NMR techniques under MAS, we site specifically assigned the most dynamic residues of the C-terminus. Based on this assignment we measured site-specific  $R_1$  and  $R_2$  rates as well as residual dipolar couplings. Using DEER EPR, we measured distance distributions throughout the C-terminus. Finally, we ran molecular dynamics simulations of the HTTex1 C-terminus that are in good agreement with the experimental data. With a detailed description of the HTTex1 fibril surface in hand, we are studying the interaction of HTTex1 C-terminus with other cellular components. A first target is the HSP40 co-chaperone DNAJB1 for which we will present solid-state NMR and EPR data showing the interaction of DNAJB1 with HTTex1.

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### Intra-residue methyl-methyl correlations of leucine and valine to aid chemical shift assignments

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Using Methyl-TROSY HMQC based experiments in selectively labelled proteins is an important strategy for studying large and dynamic systems[1]. A key issue faced is the chemical shift assignment of the methyl resonances in proteins where experiments that link side-chains to the backbone are unfeasible. Recent structure-based chemical shift assignment methods obtain the methyl assignment independently of the backbone[2,3]. Linking intra-residue methyl groups in Valine and Leucine is vital in these protocols as it vastly reduces the solution space from approximately  $n!$  to  $(n/2)!$ , where  $n$  is the number of Leucine and Valine side-chains to assign.

This work presents a method utilising three-bond  $^1\text{H}$ - $^{13}\text{C}$  scalar-coupling transfers to link intra-residue methyl resonances in  $\text{U-}[^2\text{H},^{12}\text{C}] \text{ L/V-}[^{13}\text{CH}_3/^{13}\text{CH}_3]$  samples. This HMBC-HMQC strategy has significant advantages over previous NOESY based methods, as only one correlation is obtained per methyl group, which is independent of the protein's correlation time or internal dynamics. Furthermore the information obtained from the through-bond transfer can help to deconvolute crowded regions of the Methyl-HMQC spectrum.

To demonstrate the utility of this method we have applied it to two systems: Malate Synthase G (80 kDa) and the 360-kDa  $\alpha 7\alpha 7$  particle from the *T. acidophilum* proteasome. In these proteins we linked 100% and 93% of intra-residue Leucine and Valine methyl groups respectively. In cases where the methyl HMQC is severely crowded we show that the HMBC-HMQC can be used to identify the intra-residue cross peak in 4D NOESY spectra recorded with long mixing times.

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Strucutral properties of APP WT and four single point mutants

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Cleavage of the C99 fragment of amyloid precursor protein (APP) at alternative positions in the transmembrane (TM) helix by  $\gamma$ -secretase is considered as one of the triggers that lead to the development of Alzheimer’s disease. Yet, how this process takes place in the lipophilic environment and which details of the substrate sequences are essential for proteolysis are questions that are currently under debate. As no specific consensus sequence has been detected for  $\gamma$ -secretase, dynamic parts within the TM helix are discussed as potential recognition sites. Cleavage is believed to be initiated at alternative  $\epsilon$ -sites that leads to release of the most abundant A $\beta$ 40 and the minor A $\beta$ 42 and A $\beta$ 38 peptides plus minor species. Numerous mutations are known to increase the A $\beta$ 42/40 ratio often leading to early onset Alzheimer’s disease (FAD).

Here we compare the structure of the TM helix of APP to that of four single point mutations in 80% trifluoroethanol. The mutations comprised two FAD mutants (V44M, I45T). The V44M mutation leads to increased A $\beta$ 42/A $\beta$ 40 ratios due to a shifted  $\epsilon$ -cleavage preference from L49 to T48 whereas I45T does not affect the site but reduces  $\epsilon$ -cleavage efficiency dramatically. The other two mutants were designed to disrupt the double GlyGly motif (G38P, G38L). These mutants do not alter  $\epsilon$ -cleavage preferences, yet lead to reduced cleavage efficiency. Previous results have shown that the intrinsic structural properties and flexibility of the transmembrane domains are affected by these single point mutations (Götz et al, Biophys J. 2019 and Götz et al, Sci Rep. 2019). Structures calculated from NMR-data suggest that TMD helical structure is only slightly altered by the mutations. However, the relative conformation of N- and C-terminal TMD-helices and therefore the TMD flexibility differs between wild type and mutants, suggesting that helix flexibility might affect the formation of the enzyme substrate complex.

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Modulation of protein (de)stabilisation by Ionic Liquids: an NMR view

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Over the last few years, the use of ionic liquids as co-solutes to modulate protein activity/stability or amyloidogenesis has attracted considerable attention. This is due to the large amount of possible cation/anion combinations that allow to develop tailor-made ILs with properties matched for specific applications - the control of protein stability or folding landscapes (Weingartner *et al.* PCCP 2012). In order to gain a structural insight into ILs effects on proteins we have been systematically studying different protein models and ILs using mainly NMR spectroscopy. Our previous studies with aqueous imidazolium-based ILs (Figueiredo *et al.* PCCP 2013, Silva *et al.* PCCP 2014) showed that protein destabilisation is a consequence of direct preferential binding of anions/cations to the protein surface in combination with ion hydration.

Small charged molecules are abundantly found in the cytoplasmic milieu, and some of these ions can naturally form ILs, leading to questions about the biological relevance of IL-protein interactions. Based on this, we designed a biocompatible IL, choline glutamate ([Ch][Glu]), that has so far shown striking properties in terms of effects in protein stability. We have studied IL effects on a highly-stable and well-structured protein GB1; and a metastable protein drkN SH3 domain ( $\Delta G_u \sim 0$ ). In this communication, mainly comparing the effect of different ILs, [1-buty-3-methyl-imidazolium][dicyanamide] destabiliser vs [Ch][Glu] stabiliser through different NMR-based methodologies (chemical shift mapping, <sup>15</sup>N relaxation and ZZ-exchange combined with biophysical studies) we present a mechanism for protein (de)stabilisation promoted by ILs and their differences with “common” salts/chemical denaturants. Our work will contribute to a deeper understanding of the implications of the modulation of protein stability/folding landscapes by small charged metabolites with biological relevance.

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Towards highly sensitive EPR of various paramagnetic systems at mK temperature

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Leonid Abdurakhimov, Christoph Zollitsch, John J. L. Morton  
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Major advances are currently being made in the area of cavity quantum electrodynamics and the development of superconducting quantum circuits which can offer a significant improvement in the EPR sensitivity [1]. These include recent developments of the ultra-high-Q superconducting microresonators, quantum-limited cryogenic microwave amplifiers and the use of millikelvin temperatures accessible in dilution refrigerators [2,3]. The combination of such developments has already led to the record-breaking EPR sensitivity of 65 spins/ $\sqrt{\text{Hz}}$  [4]. However, these demonstrations have typically studied paramagnetic impurities within the substrates on which the superconducting resonators are patterned [1-4]. Here, we discuss the potential of extending the use of highly sensitive EPR at millikelvin temperatures, making use of both 3D cavities and patterned microresonators to study a broader and more general set of systems including spin labels and various intrinsic paramagnetic centers in solid state and biological compounds.

**References:** [1] A. Bienfait, J.J. Pla, Y. Kubo, M. Stern, X. Zhou, C.C. Lo, C.D. Weis, T. Schenkel, M.L.W. Thewalt, D. Vion, D. Esteve, B. Julsgaard, K. Molmer, J.J.L. Morton, P. Bertet, Nat. Nanotechnol. 2015, 11, 253. [2] J.J.L. Morton, P. Berter, J. Magn. Reson. 2018, 287, 128. [3] G. Dold, C.W. Zollitsch, J. O’Sullivan, S. Welinski, A. Ferrier, P. Goldner, S.E. de Graaf, T. Lindström, J.J.L. Morton, Phys. Rev. Appl. 2019, 11, 054082. [4] S. Probst, A. Bienfait, P. Campagne-Ibarcq, J.J. Pla, B. Albanese, J.F. Da Silva Barbosa, T. Schenkel, D. Vion, D. Esteve, K. Molmer, J.J.L. Morton, R. Heeres, P. Bertet, Appl. Phys. Lett. 2017, 111, 202604.

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## P441

## Investigation of the ligand configuration in Th(IV) N-donor ligand complexes

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For the separation of trivalent actinides and lanthanides highly selective ligands are required. Soft N-donor ligands like bis(triazinyl)pyridines (BTPs) are known for their high separation factors. Though several investigations on the interaction of actinides with these ligands are available, the molecular origin of the selectivity remains largely unclear and is a topic of fundamental scientific interest. NMR spectroscopy is a valuable tool to determine interactions between metal ions and donor ligands. For actinides and lanthanides, the interactions are dominated by electrostatic interaction. However, due to the overlap of frontier orbitals, the interaction is also partially covalent, resulting in a change of electron density distribution in the ligand. It was found that the fraction of covalency in the metal nitrogen bond in Am(III) complexes is significantly higher than in similar weak paramagnetic lanthanide complexes [1].

In the present work, we performed NMR investigations on diamagnetic Th(IV) complexes with nPr-BTP and iPr-BTP. For the first time, we observed different ligand configurations for  $[\text{Th(IV)(BTP)}_4(\text{OTf})_4]$  depending on the solvent that was used. Compared to Ln(III)/Am(III) the higher charge results in a stronger complex-solvent interaction which leads to a cis/trans isomerisation of the 1,2,4-triazinyl rings depending on the solvent polarity. In polar aprotic solvents, the nitrogen atoms in 2-position of both 1,2,4-triazinyl rings interact with the metal ion (cis), resulting in exactly the same number of signals in the NMR spectra as observed for Ln(III)/Am(III) complexes. In contrast, NMR spectra taken in polar protic solvents show twice as many signals indicating that one 1,2,4-triazinyl ring flips and binds via the nitrogen in 4-position (trans).

These results provide valuable information on the bonding mode and bonding differences in lanthanide/actinide N-donor complexes, which are expected to be the driving force of the ligands' selectivity.

Reference: [1] Adam et al. Dalton Trans. 2013, 42, 14068.

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SPIN-LABELING EPR STUDY OF HYBRID NANO MATERIALS

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North Carolina State University

Design of new bio-nano hybrid systems incorporating cellular membranes calls for understanding and accounting for the influence of nanosupport and nanoconfinement on the structure and properties of lipid bilayer, lipid-solid and lipid-protein interfaces. Further progress in this field requires development of spectroscopic methods capable of characterizing nanoparticle interfaces and, particularly, interfacial electrostatic properties. Here we report on spin-labeling EPR studies of surface electrostatics of silica nanoparticle by two pH-sensitive labels. We then employed pH-sensitive lipids and specifically labeled protein side chains to assess effects of silica on the surface electrostatic potential of solid-supported lipid bilayers and effective pKa of the membrane-buried peptide ionisable sidechains. Specific covalent attachment of EPR probes ensures that the spectroscopic signal originates from well-defined location at the interfaces. EPR titration data allow us to characterize local dielectric constant at the silica nanoparticle surface and show that the surface potential associated with silanol groups develops over wide range of pH starting at pH≈4 and increasing up to pH≈9 where we reach the probe sensitivity limit. We have shown that bilayers formed from zwitterionic or mixed lipids on silica nanoparticle surfaces possess a higher negative electrostatic potential than the unsupported bilayers with the potential of mixed bilayers containing negatively charged lipids being significantly more sensitive to the silica support. Effect the silica nanoparticle size on the lipid bilayer surface electrostatic potential was also observed for particles smaller than 100 nm. pH-sensitive EPR probes were then employed to label model WALP peptide known to form an α-helix when integrated into a lipid bilayer. The silica support exerted pronounced effects on WALP dynamics and the effective pKa of the ionizable probe. It was demonstrated that the silica nanoparticles shift the effective pKa of the ionizable nitroxide probe in a membrane depth-dependent manner. Supported by NSF 1508607 to TIS.

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## P444

**Magnetic Field Influence on Asphaltene Aggregation Observed by DOSY NMR**

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Asphaltenes, the heaviest, the most polar and the least reactive molecules in crude oil can aggregate and cause many problems during oil recovery and refining process. Asphaltenes isolated from the Middle East crude oil, atmospheric and vacuum residues were studied in this work.

It will be demonstrated that asphaltene aggregates form different molecular weight species with different composition and number of monomer units. Furthermore, aggregation process has been found to be affected by the magnetic field [1].

A significant increase of diffusion coefficients of aggregates upon the increase of the magnetic field strength will be shown and discussed in terms of higher molecular mobility of present species at higher magnetic fields that could lead to a degradation of aggregates.

**Reference:** [1] J. Parlov Vuković, P. Novak, T. Jednačak, M. Kveštak, D. Kovačević, V. Smrečki, I. Mikulandra, S. Glanzer, K. Zangger, J. Dispers. Sci. Technol. 40 (2019) DOI: 10.1080/01932691.2018.1561302

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## P445

## Lanthanide binding tags – placing fragments in fast exchange using PCS

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<sup>1</sup>Helmholtz Zentrum Muenchen, Technische Universitaet Muenchen

Paramagnetic NMR using lanthanide binding tags has proven to be very useful for structural characterization of proteins and strongly bound ligands in slow exchange. This is based on the distance and orientation dependence of pseudocontact shifts (PCS) induced by the paramagnetic center. A combination of NMR titrations and binding kinetics from other techniques such as AlphaScreenTM can be used to tackle the challenge of using this approach with molecules binding in fast exchange, for example fragment molecules in drug discovery programs, to characterise the binding pose. This could aid in fragment placement for key drug development projects where crystallisation or soaking fragments is difficult, as is often the case in early stage drug discovery. We use DOTA-M8(a) and vinyl-dipicolinic acid(b) to determine the binding site of a small fluorinated fragment that binds in fast exchange to *Trypanosoma cruzi* PEX14 N-terminal domain using Lutetium, Ytterbium and Thulium. This is a medically relevant target in the treatment of Chagas Disease, prevalent in South America: interruption of the Protein-Protein Interface (PPI) between this and PEX5 has been shown to lead to death of the parasite(c) but soaking and co-crystallisation of the T. cruzi variant with fragments has proved to be challenging.

- (a) DOTA-M8: An Extremely Rigid, High-Affinity Lanthanide Chelating Tag for PCS NMR Spectroscopy (Häussinger et al, JACS 2009)
- (b) A Dipicolinic Acid Tag for Rigid Lanthanide Tagging of Proteins and Paramagnetic NMR Spectroscopy (Su et al, JACS 2008)
- (c) Inhibitors of PEX14 disrupt protein import into glycosomes and kill Trypanosoma parasites (Dawidowski et al, Science 2017)

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### NMR Crystallographic Investigations of a Series of Organic Cocrystals Exhibiting Tetrel Bonds

Scott A. Southern\*, Michael West, Maressa Bradshaw, David L. Bryce  
University of Ottawa

In recent years, the concept of noncovalent bonding has expanded to include a specific and interesting class of interactions analogous to hydrogen bonding, called  $\sigma$ -hole interactions.[1] These interactions result from the depletion of electrostatic density on the opposite end of a covalent bond between an electron-withdrawing substituent and a 'donor' atom from groups 14-17 in the periodic table.

The tetrel bond (TB) is an example of a  $\sigma$ -hole interaction where the bond donor is one of the group 14 elements (T=C, Si, Ge, Sn, Pb).[2] While the tetrel elements, particularly carbon, are well known to form TBs, their solid state NMR (SSNMR) responses can often be overwhelmed by other effects including those of crystal packing. Furthermore, tetrel atoms in organic cocrystals often bond with four substituents, giving rise to steric problems which may impact the strength of these interactions.

This work expands on a previous SSNMR study of organic cocrystals exhibiting carbon TBs.[3] Both SSNMR and computational approaches are used to examine a series of cocrystals formed from either caffeine or theophylline and a number of other small acceptor molecules. The formation of TBs with oxygen atoms on the acceptor molecules gives rise to  $^{13}\text{C}$  chemical shift changes of approximately 3-5 ppm for the methyl carbon TB donor atoms. These NMR responses are not necessarily straightforward, and can be conflated with other crystallographic effects. This study attempts to rationalize  $^{13}\text{C}$  NMR responses by investigating the effects of inter- and intramolecular features such as ring-currents and hydrogen bonds using complementary plane wave pseudopotential DFT calculations, thereby providing further insights into TBs from an NMR crystallographic perspective.

**References:** [1] Clark, T. *et al.*, *J. Mol. Model.*, **2007**, 13, 291. [2] Bauzá, A., *et al.* *Angew. Chem. Int. Ed.*, **2013**, 52, 12317. [3] Southern, S.A., Bryce, D.L., *J. Phys. Chem. B.*, **2015**, 119, 11891.

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 **$\beta$ -BPSE: fast DOSY acquisition of small molecules and macromolecules**Sebastian Spann<sup>\*,1</sup>, Burkhard Luy<sup>2</sup><sup>1</sup>Institute of Organic Chemistry, KIT, <sup>2</sup>Institute for Biological Interfaces 4, KIT

The application of Diffusion Ordered NMR Spectroscopy has vastly increased in the last years, which illustrates the benefits of monitoring diffusion in solution NMR. Consequently much effort has been ventured to improve the application of diffusion measurements in NMR [1]. In this work we introduce a pulse sequence that enables the spectroscopist to perform DOSY experiments in a matter of tens of seconds, providing the opportunity to perform reaction monitoring via diffusion. The newly derived sequence utilises  $\beta$ -angle excitation, a well established method for decreasing the overall experimental time in NMR spectroscopy [2]. Additionally, the following spin echo is realised with bipolar gradients, therefore the sequence is called  $\beta$ -Bipolar Spin Echo ( $\beta$ -BPSE). To suppress the signal loss due to J-modulation during the diffusion time, the mixing sequence DIPSI2 is implemented during  $\Delta$ . The isotropic mixing during the  $\beta$ -BPSE sequence increases the signal by the factor of 2 compared to the conventional stimulated echo sequence. The  $\beta$ -BPSE sequence was applied to small molecules with measurement times of 9 seconds. Moreover, extending the  $\beta$ -BPSE on larger molecules such as a sequence-defined decamer [3], illustrates the sequence's applicability on macromolecules.

**References:** [1] Cohen Y., Avram L., Frish L., *Angew. Chem. Int. Ed.* 44, 520-554, 2005. [2] Ross A., Salzmann M., Senn H., *J-Bio NMR* 10, 389-396, 1997. [3] Solleder S. C., Zengel D., Wetzel K. S., Meier M.A.R., *Angew. Chem. Int. Ed.* 55, 1204-1207, 2016.

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## P448

### Structural, Functional and Toxicological investigation of novel surface- acting agents from marine bacterial strains

Styliani Chasapi<sup>1</sup>, Aikaterini Zompra<sup>1</sup>, Karina Salek<sup>2</sup>, Theodora Mantso<sup>3</sup>, Georgia Voulgaridou<sup>4</sup>, Ioannis Anastopoulos<sup>5</sup>, Tony Gutierrez<sup>6</sup>, Alex Galanis<sup>4</sup>, Aglaia Pappa<sup>4</sup>, Mihalis Panagiotidis<sup>3</sup>, Stephen R. Euston<sup>6</sup>, Georgios A. Spyroulias<sup>\*,1</sup>

<sup>1</sup>Department of Pharmacy, University of Patras, <sup>2</sup>School of Engineering & Physical Sciences, <sup>3</sup>Department of Applied Sciences, Northumbria University, <sup>4</sup>Department of Molecular Biology & Genetics, Democritus University of Thrace, <sup>5</sup>Department of Molecular Biology & Genetics, Democritus University of Thrace, <sup>6</sup>School of Engineering & Physical Sciences, Heriot Watt University

Biosurfactants are active surface molecules produced by microorganisms and characterized by low toxicity and high biodegradability. The aim of this study is the functional and chemical characterization of biomolecules produced by bacteria associated with a marine phytoplankton bloom of MARISURF EU Horizon 2020 project -an international consortium that aims to produce natural and environmentally-friendly surface-active agents (SAs) with unique characteristics for various commercial applications. Two biopolymers-SAs (from *Ruegeria* sp. and *Halomonas* sp.), will be discussed in detail, including structural characterisation, functional properties and toxicological evaluation. For these most promising high-molecular biopolymers (based on functional and toxicological profile) chromatographic analysis and isolation of the components was accomplished using size exclusion chromatography. Furthermore, in order to gain structural information about the selected biopolymers, they were further investigated through the acquisition of 1D and 2D NMR experiments. Functional and structural characterization suggest one of the biopolymers to have peptide or peptide-like nature with saccharide characteristics and also containing fatty acid with long aliphatic chain(s), with very good foaming, gelling and emulsifying properties. The other one is high molecular weight biopolymer with exopolysaccharide and lipid characteristics according to the biophysical characterization. For both biopolymers, a safety profile as well as functional and structural characterizations was determined justifying their exploration in various industrial applications.

Acknowledgments: "MARISURF" project ([www.marisurf.eu](http://www.marisurf.eu)) funded by the European Union Framework Programme for research and innovation, Horizon 2020 (Grant No. 635340).

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P449

**NMR study of the Heme-Nitric Oxide/Oxygen Binding (H-NOX) domain from *Nostoc* sp, a homologue of soluble Guanylate Cyclase (sGC)**

Aikaterini Zompra<sup>1</sup>, Aikaterini I. Argyriou<sup>1</sup>, Garyfallia I. Makrynitsa<sup>1</sup>,  
Styliani Chasapi<sup>1</sup>, Minos-Timotheos Matsoukas<sup>1</sup>, Stavros Topouzis<sup>1</sup>,  
Andreas Papapetropoulos<sup>2</sup>, Georgios A. Spyroulias<sup>\*,1</sup>

<sup>1</sup>University of Patras, Department of Pharmacy,

<sup>2</sup>University of Athens, School of Health Sciences, Faculty of Pharmacy

Soluble Guanylate Cyclase (sGC) is the primary receptor for nitric oxide (NO) and a potential therapeutic target. sGC has a key role to essential and diverse physiological processes such as blood pressure regulation, memory formation, platelet aggregation and muscle relaxation. sGC is a heterodimeric hemoprotein which consists of an H-NOX domain, a Per/ARNT/Sim (PAS) domain, a coiled-coil (CC) domain and a catalytic domain. The heme domain of sGC belongs to a recently discovered family of heme-based gas sensor proteins called Heme-Nitric Oxide/Oxygen (H-NOX) binding domains that are conserved across eukaryotes and bacteria. Also, due to the implication of sGC in cardiovascular diseases, sGC stimulatory compounds are vigorously sought.

Overall, our research includes the expression, purification, isolation and the Nuclear Magnetic Resonance (NMR) study of the heme-bound H-NOX domain from *Nostoc* sp, which shares 35% sequence identity with the H-NOX domain of the  $\beta 1$  subunit of human sGC. In the present study we investigate the conformational and functional properties of H-NOX domain (Ns H-NOX) from *Nostoc* sp., with sGC activators using molecular biology methods and NMR spectroscopy.

Ns H-NOX domain was expressed in bacterial *E.coli* strains, in isotopically labelled M9 minimal growth media and isolated by anion exchange and size exclusion chromatography. The 1H-15N TROSY NMR spectra exhibits significant signal dispersion and narrow line widths indicating a well-folded domain in solution with stable tertiary structure. NMR driven interaction studies were also performed with sGC activators to identify the residues which play key role to interaction with these compounds.

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## P450

**Evidence for the role of intracellular water lifetime as a  
tumour biomarker obtained by in vivo field-cycling relaxometry**

Rebecca Steele<sup>\*,1</sup>, Maria Ruggiero<sup>2</sup>, Simona Baroni<sup>2</sup>, Silvio Aime<sup>2</sup>, Gianni Ferrante<sup>1</sup>

<sup>1</sup>Stelar s.r.l., <sup>2</sup>University of Torino, Molecular Biotechnology and Health Sciences

Conventional diagnostic magnetic resonance imaging (MRI) techniques have focused on the improvement of the spatial resolution by using high magnetic fields (1-7T). High field allows the visualization of small tumour mass but lacks to give a precise evaluation of tumour grading and metastatic potential. Recently,[1,2] we showed that the intracellular water lifetime represents a hallmark of tumour tissue cells status that can be easily monitored by measuring T1 at different and relatively low magnetic field strengths, ranging from 0.2 to 200 mT. A fast exchange through cell membranes indicates a high metabolic rate and thus a high activity of the tumor cells. Thus it is possible to measure the high metabolic pressure by an enhance water exchange with the exterior of the cell. Therefore, intracellular water lifetime can be considered an important tumour biomarker directly depending on the rate of cell proliferation, cell migration and in responding to external stimuli as hypoxia or extracellular acidosis. Moreover, currently tumour responses to therapy are monitored primarily by imaging evaluating essentially the decrease of tumor size. This approach, however, lacks sensitivity and can only give a delayed indication of a positive response to treatment. In our study, we propose the use of FFC-NMR to provide relevant information about response to treatment by monitoring changes of water exchange rates through cell membranes that are directly dependent on the metabolism alterations caused by the chemo- or radio-therapy.

**References:** [1] Ruggiero et al Angew Chem Int Ed Engl 57, 7468-7472 (2018). [2] Ruggiero et al Molecular physics 117, 968-974 (2019).

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**In Vivo FFC-NMR of Tumour-Associated Macrophages (TAMs) in Murine Melanoma with Assessment of Extra- and Intra-Cellular Localization of Iron Oxide Particles**  
Rebecca Steele<sup>\*.1</sup>, Simona Baroni<sup>2</sup>, Maria Rosaria Ruggiero<sup>2</sup>, Silvio Aime<sup>2</sup>, Gianni Ferrante<sup>1</sup>  
<sup>1</sup>*Stelar s.r.l.*, <sup>2</sup>*Department of Molecular Biotechnology and Health Sciences, University of Torino*

Tumour associated macrophages (TAMs) are forced by the cancer cells to adopt an anti-inflammatory phenotype and secrete factors to promote angiogenesis and tumor invasion [1]. For these reasons, sensitive and non invasive methods to detect TAMs are needed for tumour classification and individual patient stratification to stronger or targeted therapies.

Herein we propose a new, alternative diagnostic protocol to assess the localization of an USPIO-NP (ferumoxytol) in TAM in melanoma tumours. The method is based on the acquisition of *in vivo* NMRD profiles on a FFC relaxometer endowed with a wide bore magnet and a dedicated transmitter/receiver solenoid detection coil placed around the mouse's leg [2]. The slopes of the obtained  $R_1^{POST}-R_1^{PRE}$  profiles acquired 3 and 24h after ferumoxytol injection appear the most significant parameter that can act as an unequivocal reporter of nanoparticle intra- or extracellular localization thus allowing an unambiguous TAM quantification. In fact, 24h after the injection the remaining ferumoxytol is taken up by macrophages as confirmed by histological analysis (Pearls assay). This finding open new horizons for the field of cell tracking applications [3].

**References:** [1] R. Noy1 and J. W. Pollard *Immunity* 41, 49–61 (2014). [2] M. R. Ruggiero, S. Baroni, S. Pezzana, G. Ferrante, S. Geninatti Crich and S. Aime *Angew. Chem.* 57, 7468-7472 (2018). [3] K. J. Pine, G. R. Davies and D. J. Lurie *Magn. Reson. Med.* 63, 1698-1702 (2010).

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## P452

## Anion Binding Studies of a Triazole Containing Catalyst

Pascal Steinforth\*, Julia Bamberger, Olga Garcia Mancheño, Monika Schönhoff  
*University of Muenster*

Selective anion binding to a catalytic host is a promising topic on the route to develop systems triggering catalytic activity. In this regard, cooperative hydrogen bonding by multiple 1,2,3-triazoles establishes a suitable environment for different anions. On this basis, the asymmetric dearomatization of quinolines was successfully introduced by anion binding catalysis [1]. However, the active catalytic properties and the binding modus is still unknown.

In this work,  $^1\text{H}$  NMR spectroscopy is used under variation of the host-to-guest ratio and evaluation of the chemical shifts employing an analysis via Job plots and application of different binding models (BindFit [2]) to gain insight into the stoichiometry, binding constant and binding partners. In addition, spin relaxation studies provide insights into the immobilization of the relevant protons involved in the binding motif. Anion-specific differences of the binding are found when comparing tetrabutylammonium chloride (TBACl) and tetrabutylammonium benzoate (TBAB). Furthermore, an interesting influence of the type of cation on anion binding is observed using ammonium benzoate ( $\text{NH}_4\text{B}$ ), which is interpreted by competing interactions of the anion with the cation and the host, respectively.

Concerning anion binding, a 1:1 stoichiometry was determined for TBAB, while TBACl showed a 1:2 stoichiometry. For both anions binding constants and binding partners were successfully identified. No binding was observed in the case of  $\text{NH}_4\text{B}$ . We suggest ion pairing in solution as a possible reason.

**References:** [1] M. Zurro, S. Asmus, S. Beckendorf, C. Mück Lichtenfeld, O. Garcia Mancheno, *J. Am. Chem. Soc.* **2014**, 136, 13999-14002. [2] D. B. Hibbert, P. Thordarson, *Chem. Commun.*, **2016**, 52, 12792-12805, <http://supramolecular.org>

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Rational design in Gd(III) and bis-nitroxide polarizing agents for high-field MAS-DNP

Gabriele Stevanato<sup>\*1</sup>, Dominik Kubicki<sup>1</sup>, Gilles Casano<sup>2</sup>, Karthikeyan Ganesan<sup>2</sup>, Anne-Sophie Chauvin<sup>1</sup>, Katharina Keller<sup>3</sup>, Laura Esteban Hofer<sup>3</sup>, Sébastien Abel<sup>2</sup>, Claire Sauvée<sup>2</sup>, Georges Menzildjian<sup>1</sup>, Maxim Yulikov<sup>3</sup>, Gunnar Jeschke<sup>3</sup>, Anne Lesage<sup>4</sup>, Olivier Ouari<sup>2</sup>, Marinella Mazzanti<sup>1</sup>, Lyndon Emsley<sup>1</sup>  
<sup>1</sup>EPFL, <sup>2</sup>Aix Marseille Universite, <sup>3</sup>ETH, <sup>4</sup>CRMN Lyon

Since the last 15 years MAS-DNP has developed into an important research tool in the structural investigation of materials and surface science. The sensitivity gain is provided by highly polarized electron spins transferring polarization to nuclear spins upon microwave irradiation. The polarizing agent (PA) is represented typically by exogenous radicals with the gold-standard bis-nitroxides AMUPol [1] and TEKPol [2] leading to 1H enhancements of around 250 at 9.4 T and 100 K.

Here we show that the, so far overlooked, local conformation around the N-O● region can dramatically impact the MAS-DNP performance, with the new synthesised HydrOPol returning a 1H enhancement of 330 at 9.4 T and 100 K: about 30% better enhancement than the current standard in the field.

However, a major limitation is that nitroxides are not always compatible with sample formulations, loosing for example the capability to yield any enhancement in reducing environments as encountered in cells or in many catalysts. We show as an example that AMUPol fails to yield any enhancement on a solution of ascorbic acid. A possible way to overcome the problem is using alternative Gd(III)-based PAs[3].

By selecting the chelating ligand in [Gd(tpatcn)] we can polarize ascorbic acid and achieve a maximum 1H enhancement of 37 at 9.4 T and 100 K (a factor 2 better than [Gd(dota)(H2O)]-.[4] Moreover, by using a simplified theoretical model tested on different Gd(III) complexes we show that the reduction of zero-field splitting impacts quadratically the expected MAS-DNP signal enhancement, therefore establishing an important design parameter.

Overall, by rational design of either the local geometry around the N-O● region in bis-nitroxides or of the chelating ligand in Gd(III)-systems, we show that HydrOPol and [Gd(tpatcn)] currently yield the best reported enhancements at 9.4 T and 100 K in their own class of PAs.

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3D structural determination of unstable enzymatic  
intermediate complex via paramagnetic tagging  
Jia-Liang Chen, Xun-Cheng Su

Short-lived and low-abundance enzyme intermediate complexes represent a mainstream of transient protein complexes in enzyme catalysis and signal transductions. Many transient and unstable protein intermediate complexes are generated in real-time reactions or non-equilibrium conditions, however, resonance assignments of these unstable complexes are a challenge and structural determination of these intermediates are even harder. Herein, we show that resonance assignments of these low-abundance cross-peaks can be made by selectively isotope labeling and paramagnetic tagging approach. As a consequence, 3D structure of the unstable intermediate complex can be determined using pseudocontact shift (PCS) as structural restraints.

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## P455

**Direct  $^{13}\text{C}$  NMR to unveil chemistry of graphite oxide frameworks**

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With a staggering energy density of  $142 \text{ MJ kg}^{-1}$ , three times larger than that of petroleum, hydrogen became one of the most valuable and environmentally-friendly fuels [1]. Hydrogen is an energy carrier that does not suffer from energy dissipation (electricity as a direct energy source does), since the energy is chemically stored; however, it lacks of adequate means of confinement and transportation. Hence the bottleneck to the mass production of hydrogen fuels and fuel cells, is located in development of systems with proper storage capacity and parameter-controllable adsorption/desorption processes [1, 2]. The ability of carbon to alter its hybridization is a key characteristic that proves the versatility of carbonaceous materials in hydrogen storage applications, and thus graphite oxide frameworks are prompted in this work for such an application.

Graphite was electrochemically oxidized (EGO) and later functionalized with different amines (e.g. 1,8-diaminooctane, p-phenylenediamine, melamine, etc.) in order to obtain rigid porous frameworks (EGOF). EGOF will be simultaneously reduced with transition metal precursors to obtain composite frameworks utilized for hydrogen sorption. The oxidation and functionalization were conducted following mild conditions as to preserve intrinsic graphite precursor morphological characteristics. In this work, direct  $^{13}\text{C}$  NMR measurements were carried out as means to investigate the chemistry of the obtained frameworks. The deduced analyses were further supported by XRD, HRTEM, EELS and SAED results. The obtained NMR spectra had subtle differences due to the mild functionalization conditions that preserved precursor EGO characteristics. However, fine deconvolution of spectra unveiled distinct carbon environments that matched with similar ones reported in the literature.

**References:** [1] Tozzini, V. and V. Pellegrini, *Prospects for hydrogen storage in graphene*. Phys Chem Chem Phys, 2013. 15(1): p. 80-9. [2] Han, S.S., et al., *Stability of hydrogenation states of graphene and conditions for hydrogen spillover*. Physical Review B, 2012. 85(15): p. 155408.

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## P456

**Chemo-enzymatic position-specific labeling of RNAs with  
modified nucleotides for NMR spectroscopic studies**

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RNA is a versatile biomolecule that plays an important role in many cellular processes e.g. translating the genetic information, gene regulation, and reaction catalysis in organisms. NMR spectroscopy can be used to obtain conclusive informations about the structure and dynamics of a system, as well as to investigate interactions of RNA with proteins, ions or ligands. However, long RNAs with more than 100 nucleotides arise signal overlays in the NMR spectrum, which often complicate a detailed characterization. Here we develop a method for position-specific labeling of RNAs with NMR active modified nucleotides to overcome the size limitation of the NMR spectroscopy. In this project flourine or methoxy modified nucleotides are incorporated into RNA by using a chemo-enzymatic method.[1] <sup>19</sup>F has a high gyromagnetic ratio. Thus, fluorine is a very sensitive nucleus in NMR spectroscopy. In addition, fluorine does not occur in native RNA, allowing unambiguous assignment of signals in the <sup>19</sup>F spectrum. Therefore currently, we are working on the synthesis of the aptamer domain of the 2'-dG sensing riboswitch with one fluorine modified cytidine that enables us the NMR spectroscopic investigations of the RNA folding, ligand binding and kinetics.

**Reference:** [1] S. Keyhani, T. Goldau, A. Blümmler, A. Heckel, H. Schwalbe, *Angew. Chemie - Int. Ed.* 2018, 57, 12017–12021.

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## Photomagnetic Effects of Nitroxide Radical Liquid Crystals

Yuki Sugiyama, Takuya Akita, Daichi Kiyohara, Yushiaki Uchida, Norikazu Nishiyama

Rod-shaped compounds with a five-membered ring nitroxide radical (NR) moiety in the mesogen core show unique intermolecular magnetic interactions in their liquid crystalline (LC) and isotropic liquid (Iso) phases above room temperature; their paramagnetic susceptibilities often rise steeply at phase transitions from crystalline (Cr) to LC phases [Y. Uchida *et al.*, *J. Mater. Chem.*, **2008**, 18, 2872.]. We found that this effect originates from inhomogeneous intermolecular magnetic interactions by analyzing electron paramagnetic resonance (EPR) spectra with Voigt function [Y. Uchida *et al.*, *J. Phys. Chem. B*, **2012**, 116, 9791.]. The phase transitions from Cr to LC phases are usually irreversible and slow. In contrast, the phase transitions from LC to Iso phases can immediately occur in response to external stimuli like light. In fact, we found that a new NR compound showing a chiral smectic C (SmC\*) phase shows relatively large increase of paramagnetic susceptibility at the SmC\*-to-Iso phase transition. Furthermore, since the SmC\* compound doped with a LC compound with an azobenzene moiety, BMAB, shows photo-fluidization owing to the photoisomerization of the azobenzene moiety, the mixture exhibits light-induced reversible switching of magnetic properties, which was named “photomagnetic effects” [T. Akita *et al.*, *Commun. Chem.*, in press.]. Here, we report the design of a new NR compound with an azobenzene moiety (Azo-NR) and the photo-induced reversible change of the magnetic properties due to the changes of the shapes owing to the photoisomerization of the azobenzene moiety. We will present the comparison of the photomagnetic effects of the azobenzene-based NR compound with those of the above-mentioned mixture of the NR compound and BMAB.

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P458

Probing Insulin/hIAPP Interactions and Insulin Fibrillation by NMR Spectroscopy  
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Amyloid fibrils are  $\beta$ -sheet-rich protein aggregates commonly found in the organs and tissues of patients with various amyloid-associated diseases.[1] An aggregated form of the human islet amyloid polypeptide (hIAPP) is believed to be associated with  $\beta$ -cell death in type-II diabetes (T2D). Environmental elements of  $\beta$ -cell granules including high concentrations of insulin are known to inhibit IAPP aggregation in healthy individuals.[2] However, the molecular details that underlie the insulin-induced inhibition of hIAPP aggregation have not been determined. Here we investigated interaction of IAPP with insulin using solution-state NMR under various pH conditions, backbone amide NH chemical shift perturbations were used to identify the interaction interface between the two peptides. Our results suggest that IAPP binding with insulin may compete with homodimers formation, by shielding available monomer-monomer interaction surface of each protein and preventing the formation of the higher-molecular-weight species. The strong dependence of this interaction on the pH underlines the importance of electrostatics in this binding. In the second part of the work, we explore the molecular conformation of insulin peptide in its amyloid fibril form. Towards this end, we prepared isotopically  $^{13}\text{C}/^{15}\text{N}$  labeled insulin fibrils in vitro and examined their MAS (Magic Angle Spinning) NMR spectra. 2D  $^{13}\text{C}$ - $^{13}\text{C}$  DARR spectra displays high resolution and yields a single set of chemical shifts, suggesting the presence of a single fibril morphology. The sequential assignment of NMR resonances was achieved using 3D NCACX and NCOCX experiments, resulting in the identification of the insulin fibril core. Measurements of  $^{13}\text{C}$ - $^{13}\text{C}$  and  $^{13}\text{C}$ - $^{15}\text{N}$  long-range distance restraints are currently in progress. In summary, preliminary solid-state NMR results and an insulin fibril model will be presented.

**References:** [1] Selkoe, D. J. (2003) Nature, 426, 900–904. [2] Larson, J. L. & Miranker, A. D. (2004) J. Mol. Biol., 335, 221–231.

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### Structural and Kinetics Study of Serum Amyloid A – Principle Component of AA Amyloidosis

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AA amyloidosis is characterized by fibrillar aggregates of protein that accumulate in extra-cellular region of tissues and organs and consequently compromise their function. The main component of AA amyloidosis was found to be Serum Amyloid A (SAA) 1.1 protein. SAA is a protein secreted during the acute phase of inflammation. It is present in the blood serum and is associated with HDL. SAA is synthesized in the liver by stimulation of cytokines like IL-1, IL-6 and TNF. It has been observed that in state of infection and inflammation, SAA expression level increases up to 1000 times. Therefore, it has been proposed that SAA dissociates from HDL at high concentrations and induces the formation of fibrils.

We aim to solve the structure of murine SAA fibrils and understand the mechanism of fibril formation. The kinetics of the aggregation process are studied by a Thioflavin T (ThT) binding assay. Ex vivo seeds were used to prepare isotopically labeled fibrils and to mimic in vivo structure. It was observed that seeding shortens the lag phase of amyloid formation. Fibrils formations was confirmed by TEM. 3D MAS solid-state NMR measurements were performed to assign the resonances of the individual atoms in the fibril and to obtain distance restraints.

In addition, we characterized SAA fibril formation by means of Circular Dichroism Spectroscopy and Dynamic Light Scattering in presence of lipids, nanodiscs and polysaccharides to better understand the role of the cellular environment on aggregation. Our study will provide an insight into the structure of SAA fibrils, its kinetics and role of cellular component for fibril formation.

**References:** [1] Lu, J., et al. Structural mechanism of Serum amyloid A-mediated inflammatory amyloidosis, PNAS, 2014, 111(14):5189-519. [2] Stephanie Claus, et al., Cellular mechanism of fibril formation from serum amylois A1 protein, EMBO Rep., 2017, 18(8):1352-1366.

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## P460

## Molecular Orientation Distributions in Fibers

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Molecular orientation distributions of polymeric fibers and films are known to be responsible for mechanical and electro-chemical properties. A quantitative interpretation of the molecular orientation distribution can be made by analyzing rotor phase separated CSA sideband intensities during slow rotation rates. The 2D rotor synchronized spectrum is matched with a theoretical model of the same experiment with a Legendre polynomial orientation distribution, where each polynomial contribution is weighted by a scalar named as the “order parameter”. Challenges typically occur in the validation and interpretation of such measurements, therefore our NMR experiments are cross-correlated with equivalent interpretations using polarized Raman spectroscopy and X-ray scattering. We demonstrate a direct application to regenerated cellulose fibers, an important environmental benign cotton alternative. Unlike many other techniques, solid-state NMR provides a higher degree of chemical selectivity, which enables quantitative molecular anisotropy analysis of composites. The applied method has so far not been used with DNP enhancement, but should be applicable if slow rotation probes are available.

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## P461

### 15N MRI of biologically relevant hyperpolarized molecules via SABRE hyperpolarization

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Signal Amplification By Reversible Exchange (SABRE) is a method based on the transfer of parahydrogen ( $p\text{-H}_2$ ) polarization to the substrate nuclei due to the constant exchange of  $p\text{-H}_2$  and ligands over metallic Ir center of metal-organic complex. Generated levels of polarization provide the higher signal of the ligand. In the experiments, when SABRE process occurs in high magnetic field polarization from hydrogen atoms of  $p\text{-H}_2$  can be transferred to heteronuclei of the substrate using SLIC-SABRE pulse sequence, which generates the conditions of level anti-crossings.

We combined SLIC-SABRE method for polarization with FLASH (fast low angle shot) or SPI (single point imaging) pulse sequence for imaging. As a result,  $^{15}\text{N}$  MRI of  $^{15}\text{N}$ -labelled pyridine ( $^{15}\text{N}$ -Py) and nicotinamide ( $^{15}\text{N}$ -NA) was obtained. Imaging time using FLASH pulse sequence ( $< 1$  second) in two orders smaller comparing with SPI ( $\approx 6$  minutes for  $^{15}\text{N}$ -Py and 27 minutes for  $^{15}\text{N}$ -NA). Therefore, despite lower spatial resolution ( $0.15 \times 2.4 \text{ mm}^2/\text{pixel}$  for  $^{15}\text{N}$ -Py and  $0.3 \times 4.8 \text{ mm}^2/\text{pixel}$  for  $^{15}\text{N}$ -NA using FLASH;  $0.6 \times 0.6 \text{ mm}^2/\text{pixel}$  using SPI) FLASH pulse sequence is more suitable for  $^{15}\text{N}$  MRI in biomedicine because of the necessity of shorter imaging time.

Consequently, FLASH was used for imaging of 4-aminopyridine (fampridine) and dimethylaminopyridine (DMAP) with natural abundance of  $^{15}\text{N}$  nuclei. Fampridine is a drug used for treatment of multiple sclerosis.  $^{15}\text{N}$  NMR of fampridine and DMAP showed  $^{15}\text{N}$  polarization level  $\approx 8\%$  that allowed to do  $^{15}\text{N}$  MRI. The resulted images had spatial resolution  $0.3 \times 2.4 \text{ mm}^2/\text{pixel}$  which was enough to observe 1.6 mm capillary through which  $p\text{-H}_2$  was supplied. Signal-to-noise ratio was equal 49 and 109 for DMAP and fampridine correspondingly. This work is the first demonstration of  $^{15}\text{N}$  MRI of biomolecules with natural abundance of  $^{15}\text{N}$  nuclei using SLIC-SABRE for polarization.

This work was supported by grant from RSF #17-73-20030.

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## P462

**H $\alpha$  detected NMR approach for the assignment of intrinsically disordered proteins**Csenge Lilla Szabó<sup>\*1</sup>, Fanni Sebák<sup>1</sup>, Beáta Szabó<sup>2</sup>, Ágnes Tantos<sup>2</sup>, Andrea Bodor<sup>1</sup><sup>1</sup>*Institute of Chemistry, Eötvös Loránd University,*<sup>2</sup>*Institute of Enzymology, Hungarian Academy of Sciences*

Studying intrinsically disordered proteins (IDPs) under physiological conditions may present a great challenge using methods based on H<sub>N</sub> detection. The main drawback is the signal loss caused by chemical exchange of amide protons with water. Thus, conventional triple-resonance methods such as HNCA, HNCACB and HNCO fail to provide satisfactory information. The alternative way of assignment can be <sup>13</sup>C-detected experiments[1] or H $\alpha$  detection.

Though the signal dispersion in the H $\alpha$  and H<sub>N</sub> region is similarly low (about 1 ppm), the H $\alpha$  detected approach has the advantage that the alteration of measurement conditions does not have a significant effect on signal intensities. Moreover, these experiments reveal proline environments as well. Drawbacks may represent the proximity of the water signal and the multiple couplings, that has to be overcome.

The H $\alpha$  detected triple resonance experiments such as HCAN[2], the complementary HCA(CO)N and HCA(N)CO enable the detection of sequential connectivities even with PP motifs. These measurements provide backbone H $\alpha$ , C $\alpha$ , N and C' chemical shifts which are characteristic for the secondary structure. The acquisition time required for H $\alpha$  detection are comparable with those for H<sub>N</sub> detection, moreover, sensitivity and resolution can be further enhanced by applying real-time homodecoupling (BASEREX)[3].

We present this approach for backbone assignment of the 106 residues long loop region of EZH2 protein. Using chemical shift data collected under physiological conditions, we proved that the protein is highly disordered. Our results demonstrate the applicability and usefulness of the H $\alpha$  detected approach for assignment of intrinsically disordered proteins.

**References:** [1] Bermel, W et al., Angew. Chem. Int. Ed. 44, 3089-3092 (2005). [2] Kanelis, V et al., J. Biomol. NMR 16, 253-259 (2000). [3] Haller JD et al., J. Magn. Reson. 302:64-71 (2019).

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## P463

## Self-induced recognition of enantiomers: from NMR characterization of aggregation to applications in chiral analysis

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The determination of the enantiomeric purity of chiral drug molecules is an important task involving rigorous regulatory demands in the pharmaceutical industry. Besides chiral HPLC and CE, NMR spectroscopy is a widely applied method for this purpose. It is commonly held that in order to separate the NMR signals of enantiomers so that they can be individually integrated, diastereomeric complexes must be formed by using “chiral solvating agents”. In reality, enantiomers with functional groups for non-covalent interactions may undergo intermolecular self-association in sufficiently concentrated solutions, as a result of which transient diastereomeric homo- and heterochiral associates are formed. In a nonracemic mixture of the enantiomers, the ensuing equilibria involving the various monomers and associates becomes asymmetric for the two enantiomers, which will thereby exhibit distinct NMR signals. This phenomenon, called Self-Induced Recognition of Enantiomers (SIRE), is less well known, although it potentially offers a simple method for enantiomeric ratio measurements [1].

Our research aimed to assess the general applicability of SIRE for molecules with H-bond donor and acceptor sites. We managed to achieve the self-induced enantiomeric separation of <sup>13</sup>C NMR signals for selected model compounds in CDCl<sub>3</sub>: ibuprofen, diethyl tartrate, dimethyl malate, dibenzyl tartrate and N-Fmoc-valine [2]. Aggregation constants were derived by the mathematical evaluation of the <sup>1</sup>H and <sup>13</sup>C chemical shift changes upon the serial dilution, and <sup>1</sup>H NMR-based diffusion coefficients were also measured to assess the size of the self-associates. Certain analytical aspects of SIRE, such as its accuracy or its limit of detection, were also studied.

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## P464

**Self-diffusion and microstructure of ammonium  
bis(trifluoromethanesulfonyl)imide-based ionic liquids**

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Applications of ionic liquids (ILs) are continuously expanding. Current applications include ILs as electrolyte material in lithium batteries, ultracapacitors, media for chemical reactions, drug delivery systems. The potential of ILs lies in the flexibility and customisation due to chemical variability of their components. That being said, the properties of ILs are tailored via the selection of the cation and anion, which modulates the nature of the interactions. This way, we can provide a medium where some of the chemical reaction rates are likely to be more favourable than others if compared to commonly applied molecular solvents. Furthermore, the relationship between microstructure and molecular dynamics is a fundamental issue for the design and performance of ILs. Numerous methods can be used to study molecular mobility in fluids, with Nuclear Magnetic Resonance (NMR) being an exceptionally informative technique for studying translational motion. Diffusion NMR is a ubiquitous and fundamental process characterised by the random motion of elementary constituents of matter, most notably of atoms and molecules, due to their thermal energy, and provides insight into physicochemical properties.[1][2] The accessibility of multinuclear pulsed field gradient spin-echo (PGSE) techniques for accurate measurements of the ionic self-diffusion coefficients has enabled many fruitful studies in the area of ILs. In these systems, diffusion is the process of charge transport via cations or anions activated by internal kinetic energy. Self-diffusion combined with other bulk properties, such as viscosity, density, and electrical conductivity, provides a thorough understanding of molecular transport in ILs.[3] The aim of this work is to provide a better understanding of bis(trifluoromethane)sulfonimide ionic liquid with varied length of the alkyl chain. We focus mainly on the self-diffusion process, measured via NMR. Measurements were carried out using Agilent 14T spectrometer.

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## P465

## Insights into p-Doping of P3HT by Electron Paramagnetic Resonance

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Efficient and controlled molecular doping of organic semiconductors promises to significantly advance the field of organic electronic devices. However, an in-depth understanding of the doping process is required to guide the design of improved semiconductor materials and molecular dopants.[1] Electron Paramagnetic Resonance (EPR) Spectroscopy is uniquely suited to investigate the nature and dynamics of the paramagnetic species generated by introduction of dopant molecules into solutions or films of organic semiconductors.[2,3] In this study, a multi-frequency continuous-wave and pulse EPR approach was used to investigate p-doping of P3HT with two molecular dopants: F4TCNQ and tris(pentafluorophenyl) borane.

Continuous-wave EPR was used to quantify the concentration of paramagnetic species as a function of dopant concentration and revealed significant differences in the spectral signatures depending on the dopant molecule and the doping concentration. Measurements at different microwave frequencies provided further insights into the nature of the detected paramagnetic species and allowed the separation of signals arising from the polaron on P3HT and the paramagnetic state of the dopant. Hyperfine pulse EPR techniques for the characterisation of the interaction of the unpaired electron with its surroundings further aided the assignment of different EPR spectra and characterisation of the P3HT radical cation. The extent of delocalisation was determined using ENDOR (Electron Nuclear Double Resonance) to measure the hyperfine couplings between the hole and the protons on the polymer chain. A comparison of doped regioregular and regiorandom P3HT shows clearly reduced hole delocalisation in regiorandom P3HT, which, combined with a significantly reduced spin concentration, supports the hypothesis that increased hole delocalisation improves doping efficiency by promoting the separation of charge-transfer states.

**References:** [1] Salzmann et al., Acc. Chem. Res. 49, 370-378 (2016). [2] Aguirre et al., Phys. Chem. Chem. Phys. 10, 7129-7138 (2008). [3] Steyrlleuthner et al., Phys. Chem. Chem. Phys. 19, 3627-3639 (2017).

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## P466

## New Progress toward High-Dimensional Biomolecular SSNMR

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Solid-state NMR has played an important role in structural analysis of various insoluble and heterogeneous biomolecules such as amyloid fibrils. The lack of spectral resolution, however, still poses a serious problem. Although multi-dimensional experiment can potentially enhance spectral resolution, higher dimensional SSNMR experiments beyond 4D are challenging due to exponential increase of the machine time as a function of the dimension. In this study, we examined the feasibility of data collection of 5D protein SSNMR as well as fast data collection of 4D protein SSNMR. As a benchmark, we performed 5D HACACONH and 4D HACANH experiments for uniformly <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N-labeled the  $\beta$ 1 immunoglobulin binding domain of protein G (GB1) in order to examine the usefulness of these high-dimensional SSNMR experiments for structural study of protein in terms of spectral resolution and sequential back bone assignment. To perform 4D in a range of a few hours, three methods were combined to accelerate the experiment. First, we reduced the repetition time by paramagnetic-assisted condensed data-collection (PACC) method for sensitivity improvement with a GB1 microcrystal sample doped with 20 mM Cu-EDTA. Second, we utilized <sup>1</sup>H detection under ultra-fast magic angle spinning (MAS) at 60 kHz to improve sensitivity. Finally, nonuniform sampling (NUS) was adopted at indirect dimensions to reduce acquired data points substantially. Under these conditions, a 4D HACANH spectrum was obtained for the GB1 sample within an experimental time of ~1 hour with sufficient signal-to-noise ratios except for signals from high mobility residues. Next, we collected a 5D HACACONH spectrum for the GB1 sample using the same strategy within 19 hours. While the 4D data yielded information on intra-reside resonance connectivity, the 5D data offered that on inter-reside connectivity for sequential resonance assignment. Further improvements of these 4D-5D experiments with selectively deuterated GB1 sample will be also discussed.

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Ultralow-field NMR of liquids confined in ferromagnetic and paramagnetic materials

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<sup>2</sup>University of Cambridge - Magnetic Resonance Research Center

Nuclear magnetic resonance (NMR) techniques provide unique insight into the physical and chemical behavior of fluids, including composition, dynamics and reactivity. Such insight is permitted even when the fluid is completely enclosed in a structure that is opaque to visible radiation. Historically, however, NMR in metallic or electrically conductive enclosures is a technical challenge, since (i) induced gradients obstruct the spatial and spectroscopic resolution and (ii) the AC signals may be strongly attenuated due to the skin depth effects. These problems can be mitigated by detecting NMR at a sufficiently low nuclear precession frequencies, using spin-exchange relaxation-free (SERF) atomic vapor magnetometers[1,2].

Here we report on ultralow-field <sup>1</sup>H NMR of liquids inside sample vessels or nanoscale confinement that are weakly ferromagnetic (i.e. have a magnetic moment even at zero applied field) and/or paramagnetic (i.e. have a magnetic moment proportional to the applied field) detected with a SERF <sup>87</sup>Rb magnetometer. Both types of magnetism may contribute to the net field experienced by the nuclear spins and may consequently broaden or shift the center frequency of the NMR spectral line. Ultralow-field NMR is an advantageous method to distinguish each contribution[3]. We demonstrate results for closed containers of an aluminum alloy (showing a remanent magnetic field of around 50 nT) and for nano-disperse cobalt oxide on porous silica.

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Proton detected magic-angle spinning NMR and DFT to understand a conformational change upon drug binding in a proton channel

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The M2 protein from influenza is a membrane protein that forms a tetramer and acts as a proton channel. M2 inhibitors cause large conformational changes upon binding.<sup>1</sup> A histidine cluster (His 37) localized in the middle of the pore has been identified as a main component of the proton shuttle mechanism. However, the histidine cluster has been reported as structured in either a box or a parallel conformation.<sup>2,3</sup> In this work, proton detected ssNMR at 55 and 105 kHz magic-angle spinning of fully protonated M2 reconstituted in lipids have been used in combination with DFT calculations to characterize ligand protein interaction as well as conformational changes.

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**Nonlinear sampling in ultrafast Laplace NMR**  
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Laplace NMR (LNMR), comprising of relaxation and diffusion experiments, provides detailed information about molecular rotational and translational motion. The resolution and information content of LNMR can be increased substantially by a multidimensional approach. However, the approach leads to very long experiment times due to need for repetitions with an incremented evolution time or gradient strength. Furthermore, the need for repetitions significantly hinders the use of modern hyperpolarization methods.

Recently, we have shown that the experiment time of multidimensional LNMR can be shortened by one to three orders of magnitude by encoding the incremented variables of an indirect dimension into the layers of the sample.[1,2] The method is called ultrafast LNMR, and it has found diverse applications in the investigation of porous media, chemical analysis, identification of intra- and extracellular metabolites as well as mobile NMR.[1-6] The single-scan approach also facilitates significantly the use of hyperpolarization to boost sensitivity by many orders of magnitude. Originally, the spatial encoding was exploited in ultrafast NMR spectroscopy.[7]

In this contribution, the principles and recent progress of ultrafast LNMR is described. Furthermore, a method for non-linear sampling of the indirect dimension in UF-LNMR measurements is introduced.[8] This leads to an optimized detection of exponential experimental data and significantly improved resolution of LNMR parameters.

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Improved signal fidelity in orthogonal 4-pulse DEER: avoiding crosstalk signals between nitroxide and gadolinium spin labels

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DEER on orthogonally spin labeled proteins [1] is a versatile technique that allows to retrieve a much greater information content from a single sample than DEER on proteins spin labeled with just single type of label. The reason behind is that orthogonal spin labels can be addressed independently in DEER experiments due to spectroscopically non-overlapping central transitions and/or distinct relaxation times and transitions moments. Accordingly, samples orthogonally labeled with NO and Gd give access to three DEER channels, namely NO-NO, NO-Gd and Gd-Gd. However, it has been reported in the literature before [2] that crosstalk signals between individual DEER channels can occur.

These crosstalk signals can be a severe problem when studying complex systems (e.g. hetero-oligomeric protein complexes). To address this issue, we performed orthogonal DEER experiments using mixtures of NO-NO, NO-Gd and Gd-Gd biradicals characterized by clearly distinct distances to systematically study under which conditions crosstalk signals occur and how they can be distinguished or suppressed in order to improve signal fidelity [3].

**References:** [1] Yulikov, Maxim "Spectroscopically orthogonal spin labels and distance measurements in biomolecules." *Electron Paramagnetic Resonance* 24 (2015): 1-31. [2] Gmeiner, Christoph, et al. "Spin labelling for integrative structure modelling: A case study of the polypyrimidine-tract binding protein 1 domains in complexes with short RNAs." *PCCP* 19.41 (2017): 28360-28380. [3] Teucher, Markus, et al. "Improved signal fidelity in orthogonal 4-pulse DEER: avoiding crosstalk signals between nitroxide and gadolinium spin labels" *Manuscript in preparation* (2019).

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Exploring the structural properties of brevinin-1BYa and its bioactive analogues  
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Brevinin-1BYa (FLPILASLAAKFGPKLFCLVTKKC) is a 24-amino acid residue host-defence peptide, first isolated from the skin secretions of the foothill yellow-legged frog *Rana boylii*. The peptide is of interest as it shows broad-spectrum antimicrobial activity, and is particularly effective against opportunistic yeast pathogens. Its potential for clinical use, however, is hindered by its latent haemolytic activity. The peptide's structure, as well as the structures of two analogues, the less haemolytic [C18S,C24S]brevinin-1BYa and the more potent *cis*-dicarba-brevinin-1BYa, were investigated in a 33% 2,2,2-trifluoroethanol (TFE-d<sub>3</sub>)-H<sub>2</sub>O solvent mixture and in membrane-mimicking sodium dodecyl sulphate and dodecylphosphocholine micelles by <sup>1</sup>H-NMR spectroscopy and molecular modelling techniques.

While the peptides do not possess a secondary structure in aqueous solution, the peptides' structures in membrane-mimetic media are characterised by two α-helices, which extend from Pro<sup>3</sup> to Phe<sup>12</sup> and from Pro<sup>14</sup> to Thr<sup>21</sup>, and are connected by a flexible hinge located at the Gly<sup>13</sup>/Pro<sup>14</sup> residues.

With the aid of molecular dynamics simulations and 5-doxyl-labelled stearic acid and manganese chloride paramagnetic probes, it was determined that the peptides' helical segments lie parallel to the micellar surface, with their hydrophobic residues facing towards the micelle core and the hydrophilic residues pointing outwards, suggesting that both peptides exert their biological activity

by a non-pore-forming mechanism. While the native and dicarba peptides are fully associated with the micellar surface, the acyclic analogue's C-terminus is only weakly associated with the micellar surface and is in direct contact with the surrounding aqueous solvent. The loss of the cyclic conformation is therefore seen to affect the peptide's ability to establish hydrophobic interactions with the lipid bilayer and induce disordering events by which it exerts its biological activity. These novel findings create a new avenue of research to explore the impact of peptide-membrane interactions on the peptide's therapeutic index.

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**Triplet fullerene for nanoscale distance measurements by EPR**

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Nanoscale distance measurements by pulsed dipolar (PD) Electron Paramagnetic Resonance (EPR) play crucial role in structural studies of biomolecules and their complexes. The properties of spin labels used in this approach are of paramount importance, since they determine the sensitivity limits, attainable distances and proximity to biological conditions, including physiological temperatures. The typical distance measurement employing nitroxide labels requires low temperatures as 80 K and 10-100  $\mu$ M biomolecules concentration.

Herewith, we propose and validate the use of photoexcited fullerenes as spin labels for PD EPR distance measurements. Stronger electron spin polarization and narrower spectrum of fullerenes compared to other triplets (e.g., porphyrins) boost the sensitivity, and superior relaxation properties allow PD EPR measurements up to a near-room temperature. The capabilities of new approach are demonstrated using covalently-bound fullerene-nitroxide and fullerene-triarylmethyl pairs, as well as supramolecular complex of fullerene with nitroxide-labeled protein.

Therefore, photoexcited triplet fullerenes can be considered as new paradigm-shifting spin labels with the cutting-edge spectroscopic properties for future structural studies of biomolecules.

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**<sup>77</sup>Se-edited CPMG-HSQC NMR Methods to Monitor Protein-Glycan Interactions**

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László Szilágyi, Hans-Joachim Gabius, Katalin E. Kövér

The fundamental importance of protein-glycan recognition calls for specific and sensitive high-resolution methods for detailed analysis. Binding of small molecules to proteins is often monitored by observing <sup>1</sup>H NMR resonances of the ligands due to the inherent high sensitivity of <sup>1</sup>H detection. Ligand-observed schemes may, however, fail, because of spectral overlap, if potential ligands are present as a mixture, which is typically the case when screening of compound libraries. <sup>19</sup>F NMR via using fluorinated ligands has been proposed to resolve the overlap issue. Here we present a new <sup>77</sup>Se NMR methodology for complementary studies of selenoglycans with superb resolution and optimised sensitivity, where direct NMR detection on <sup>77</sup>Se is replaced by indirect observation in a 2D <sup>1</sup>H,<sup>77</sup>Se heteronuclear correlation experiment. The proposed 2D <sup>1</sup>H,<sup>77</sup>Se HSQC pulse sequence is based on <sup>1</sup>H,<sup>77</sup>Se CPMG-INEPT out-and-back transfer via <sup>2,3</sup>J<sub><sup>1</sup>H,<sup>77</sup>Se</sub> long-range couplings.[1] CPMG-INEPT long-range transfer ensures maximum detection sensitivity, clean signal phases, and reliable ligand ranking. As an example, signal attenuation due to binding induced line-broadening in the 2D <sup>1</sup>H,<sup>77</sup>Se HSQC spectrum of a mixture of selenosugars efficiently monitors selenodigalactoside recognition by three human galectins and a plant toxin. The range of applications is large, furthermore selenium introduction via O/Se exchange has far less potential influence on molecular interactions and it can target more sites than OH/F exchange. By monitoring competitive displacement of a selenated spy ligand, the selective <sup>77</sup>Se NMR approach may be used to screen non-selenated compounds as well. Moreover, <sup>1</sup>H,<sup>77</sup>Se CPMG-INEPT transfer allows to combine further NMR sensors of molecular interaction with the specificity and resolution of <sup>77</sup>Se NMR spectroscopy.

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**Reference:** [1] M. Raics, et al, ChemBioChem 2019, doi:10.1002/cbic.201900088.

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Including protons in solid-state NMR resonance assignment and secondary structure analysis for RNA polymerase II subunits Rpo4/7

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<sup>1</sup>H-detected solid-state NMR experiments with fast magic-angle-spinning (MAS) frequencies deliver <sup>1</sup>H chemical shifts of proteins in solids, which enables their interpretation in terms of secondary structure. We present <sup>1</sup>H and <sup>13</sup>C-detected NMR spectra of the RNA polymerase[1] subunit Rpo7 in complex with unlabeled Rpo4 and use the <sup>13</sup>C, <sup>15</sup>N and <sup>1</sup>H chemical-shift values deduced from them to study the secondary structure of the protein in comparison to a known[2] crystal structure. We applied the automated resonance assignment approach FLYA, including <sup>1</sup>H solid-state chemical shifts, and show its performance in comparison to manual spectral assignment. Further assessing the secondary structure in comparison to the known crystal structure, our results confirm that <sup>13</sup>C secondary chemical shifts (SCS) are a bona fide predictor of secondary structure elements. In cases where <sup>13</sup>C chemical shifts are not available, secondary structure elements can be identified using the sum of <sup>1</sup>H $\alpha$  and <sup>1</sup>H<sup>N</sup>N SCS, while using instead either only 1H $\alpha$  or 1HN SCS or TALOS showed an increased uncertainty in the boundaries of observed secondary structure elements compared to the crystal structure<sup>2</sup>. We discuss the potential as well as pitfalls of secondary structure determination based on solid-state NMR including <sup>1</sup>H chemical shifts.

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## SCREAM-DNP on biologically relevant small molecules

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Dynamic Nuclear Polarization (DNP) is a powerful method for sensitivity enhancement during solid-state NMR experiments. One recently discovered mechanism in DNP is Specific Cross-Relaxation Enhancement by Active Motions (SCREAM-DNP). SCREAM-DNP can be observed when molecular motions support the specific condition for heteronuclear cross-relaxation between <sup>1</sup>H and <sup>13</sup>C, resulting in a negative <sup>13</sup>C enhancement during a direct DNP experiment.[1] SCREAM-DNP was first observed for molecules bearing methyl groups due to reorientation dynamics active at typical DNP temperatures around 100 K. The efficiency of cross-relaxation can be controlled through variation of the sample temperature.[2]

We investigated SCREAM-DNP of methyl-bearing amino acids and of methyl bearing molecules of interest for biological research (lactate, pyruvate, caffeine and nicotine). Investigation of amino acids with SCREAM-DNP is the first step toward the utilization of this effect as a site specific probing technique using methyl groups in protein systems. Lactate and pyruvate are biologically interesting molecules that are utilized as biomarkers for cancer cell detection.[3] Another approach for using SCREAM for site-specific probing is the use of methyl-carrying ligands such as caffeine and nicotine as the source of magnetization. Those molecules could be used for binding dynamics studies between the ligand and the receptor.

In this poster we discuss the changes in methyl-dynamics and SCREAM-DNP efficiencies regarding sterical hindrance and different temperatures in the above-mentioned molecules that are of interest in biological research. The different methyl-dynamics of the amino acids could be distinguished through SCREAM-DNP. Operating temperatures for efficient SCREAM-transfer for both nicotine and caffeine could be found. Different methyl-dynamics for Lactate and Pyruvate could be shown.

**References:** [1] Daube et al., J. Am. Chem. Soc., 2016, 138, 16572. [2] Aladin et al., Angewandte Chemie, 2019, 58, 4863. [3] Albers et al., Cancer Re., 2008, 68, 8607-8615.

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4D-CHAINS/autoNOE-Rosetta, an automated pipeline for rapid NMR structure determination

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CEITEC – Central European Institute of Technology, Masaryk University

The automation of NMR structure determination remains a significant bottleneck towards increasing the throughput and accessibility of NMR as a structural biology tool to study proteins. The chief barrier currently is that obtaining NMR assignments at sufficient levels of completeness to accurately define the structures by conventional methods requires a significant amount of spectrometer time (several weeks), and effort by a trained expert (up to several months). We have recently addressed both bottlenecks by presenting a complete pipeline that meets the key objectives of NMR structure determination; minimal data collection, least human intervention, and applicability to large proteins [1]. Key to our approach was the development of 4D-CHAINS algorithm that provides highly accurate and near-complete NMR resonance assignments from only two 4D spectra. In combination with autoNOE-Rosetta, 4D-CHAINS provides a robust approach leveraging a highly automated process to obtain reliable structures in a matter of days [2, 3]. Besides illustrating the merits of our pipeline for timely NMR structural studies, novel concepts in automation will be discussed aiming to harness the powerful advantages of the next-generation NMR spectrometers with magnetic strengths of 1.2 GHz.

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**Thermal properties, proton conductivity, and  
proton dynamics in imidazole-doped nanocrystalline cellulose**

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A continuous increase in the energy consumption and care of our planet forces the search for clean energy sources such as, for example, the proton exchange fuel cell. In our laboratory, we are looking for materials that could replace Nafion as a proton exchange membrane but show proton conductivity above 100 °C and under anhydrous conditions, be easily accessible, environmentally friendly, easy and cheap to produce. One of the possibilities is to use cellulose doped with imidazole (Im). Cellulose serves as supporting material and imidazole acts as a proton donor and proton acceptor at the same time. Encouraged by the good value of the conductivity ( $2.0 \times 10^{-4}$  S/m at 160 °C) obtained for imidazole-doped microcrystalline cellulose we continue our search to obtain an even better cellulose-based proton conductor. In new composites, we replaced microcrystalline cellulose with nanocrystalline cellulose (CNC). This work presents the thermal, electrical and dynamic properties of CNC-Im nanocomposites. The nanocomposite with the highest concentration of imidazole shows a conductivity of  $10^{-1}$  S/m at 150 °C. The thermal properties of the CNC, Im, and CNC-Im are supported by the kinetic analysis of the processes observed. The combined interpretation of experimental results of impedance and <sup>1</sup>H solid-state NMR spectroscopy gives us some insight into the macroscopic and microscopic processes involved in proton transport in CNC-Im. Local processes such as reorientation of imidazole rings and breaking of hydrogen bonds are identified and their activation energies are calculated and compared to the energy obtained from impedance spectroscopy.

The studies fully verified the hypothesis that by replacing microcrystalline cellulose with nanocrystalline cellulose we are able to obtain a composite with a higher concentration of imidazole, and thus a higher electrical conductivity.

**Acknowledgments**

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**P478**

**Investigating Metabolic Changes in a Cell Model of an  
Autoimmune Skin Disease by NMR-Spectroscopy**

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Although metabolomics has been widely applied to study metabolic changes in various diseases including autoimmune diseases, it is still very limited used? in autoimmune skin diseases. Our study is focused on pemphigus vulgaris (PV) which is a life-threatening autoimmune skin disease resulting from intercellular dissociation in the epidermis. By using a keratinocyte cell model of PV which resembles the intercellular dissociation and applying NMR spectroscopy we aim to measure temporal changes of intra- and extra-cellular metabolites. This will help to reveal the sequence of pathogenic cellular and molecular events. Since many of the disease-related signaling pathways also play important role in energy metabolism, we initially compared metabolic changes under high glucose and low glucose conditions to investigate in future if there is a secondary effect on the pathological processes.

In addition, since the dissociation only takes place between the basal layer and the suprabasal layer of epidermis while the upper differentiated layers remain intact, we speculate that the basal cells and differentiated cells will show different biochemical responses under PV stimulation. Thus, cells are cultured under low and high calcium contents to acquire basal and differentiated status respectively and compare their metabolic profiles. Since calcium may also have an effect on energy metabolism, we first determine the metabolic profiles of our cells under different calcium and glucose levels. We also determined the NMR compatibility of several culture media for extracellular metabolite analysis. The findings demonstrated that media containing HEPES are not suitable and media with high calcium content yielded extra peaks of unknown origins. A series of NMR measurement was performed to identify the metabolites. The temporal metabolic differences across various culture conditions will be presented.

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### 13C Longitudinal Relaxation Times for Advanced Applications of Nanodiamonds in Medicine

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Nanodiamonds (NDs) are a subject of increased application interest based on the possibilities of their use in medical imaging [1] and as drug delivery systems [2, 3]. Fluorescent NDs are addressed as they emit single-photon luminescence due to a number of optically sensitive paramagnetic defects, particularly nitrogen-vacancy centres. The knowledge on how the defect types and distribution in the structure affects the relaxation is of crucial importance as they affect the physical properties of the NDs and play an important role in the choice of NDs types.

The pure diamonds have long <sup>13</sup>C spin lattice relaxation times, measured with NMR, due to the low <sup>13</sup>C natural abundance. On the other hand, the presence of defects in the diamonds, either inherent or intentionally induced allows additional relaxation pathways and thus accelerates nuclear relaxation. The most common natural paramagnetic defects in diamonds include sp<sup>3</sup>-type dangling bonds on carbons, and single nitrogen substitutions known as P1 centres.

We investigated the dependence of the <sup>13</sup>C longitudinal relaxation times of a series of non-fluorescent and fluorescent NDs in solid state and in solution. Severe change in the linewidths for the non-fluorescent to the fluorescent NDs as powder samples and upon their solution was observed. The <sup>13</sup>C longitudinal relaxation times of the NDs were measured with saturation recovery experiment and the obtained values from the exponential data fitting were compared.

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NMR IN ELLAGITANNIN CHARACTERIZATION

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Ellagitannins, a sub-class of hydrolysable tannins are widely distributed polyphenolic plant secondary metabolites that have shown various antiherbivore and antiparasitic as well as human health-promoting activities [1–3]. In many cases, their relatively complex chemical structures combined with difficulties in purifying reasonable amounts of pure compounds set challenges to their structure elucidation. Furthermore, ellagitannins consist of more carbon than hydrogen and many of the hydrogens are exchangeable due to numerous hydroxyl groups in their structures, and these reasons often make the structure elucidation of some parts of the molecules difficult even with modern NMR correlation techniques.

Recently, the correct structures of hippophaenins B and C [2], and salicarinin D [3] were confirmed with the aid of combined use of MS, NMR and ECD spectrometric methods. Especially, selective HMBC, 1D-ROESY and 1D-TOCSY experiments proved to be essentially important in the structure determinations. The ECD spectra were utilized to determine the correct axial chiralities of the hexahydroxydiphenoyl (HHDP), nonahydroxytriphenoyl (NHTP) and valoneoyl groups in the structures, and thus, to finally reveal the absolute configurations of the studied compounds.

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Interdomain contacts between the J-domain and the substrate binding domain of the co-chaperone ERdj3 regulate its function

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Biozentrum, University of Basel, Switzerland

J-proteins are a large and very diverse class of indispensable chaperones that are mainly known as co-chaperones for the ATP-dependent chaperone Hsp70. Only together with J-proteins, Hsp70 can carry out such different functions as protein folding, prevention of protein aggregation and the facilitation of protein degradation (1). The task of J-proteins is to deliver client proteins to Hsp70 and stimulate its ATP-hydrolysis. Besides their highly conserved J-domain needed for the stimulation of ATP hydrolysis of Hsp70, J-proteins comprise a substrate binding domain (SBD) and, in many cases, diverse additional domains. However, little information about J-proteins in general and their interaction with client proteins in particular is available (2).

Here, we present structural and functional data of the human tetrameric J-protein ERdj3 (154kDa), cellularly localized inside the endoplasmatic reticulum (ER) (3). 2D[15N,1H]-TROSY-HSQC spectra of the full-length protein and the three-dimensional structure of its J-domain demonstrates that the GF-linker, connecting the J-domain to the SBD, dynamically interacts with the J-domain. In addition, the J-domain interacts with the SBD as evidenced by 2D[15N,1H]-TROSY-HSQC spectra and mass spectrometry-crosslinking experiments. Addition of a client protein peptide leads to the disruption of the interaction and subsequently to the release of the J-domain from the SBD. To conclude, the sterical interference of the SBD by the J-domain and its dynamic rearrangement represents a new mechanism for the chaperone function of J-proteins. This regulation mechanism might also coordinate the interaction with Hsp70 since the client protein induced release of the J-domain increases its accessibility by Hsp70.

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**Comprehensive NMR Analysis of Pore Structure of Superabsorbing Cellulose Nanofibril Aerogels**

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The vast volume of sea oil transport and rise in the number of underwater drilling platforms all around a world creates a great risk for environment. To minimize the ecological impact of possible water spills there is a need for cheap, oil selective materials used for cleaning oil spills from the sea surface.

In the recent years a new branch of aerogel material made up of cellulose nanofibrils (CNFs) has been developed [1]. These materials can act as superabsorbents for oil and chemical cleanup from water. Their properties arise from the capillary action which is depended upon the pore size distribution and type of material.

In this work, we used a set of NMR techniques to characterize the porous structures of CNF aerogels. The CNF materials were manufactured from waste cellulose fibers [2]. The fibers underwent nanofibrillation and hydrophobic modification (silylation) resulting cellulose nanofibril sponges, which had ultralow density (0.0029 g/cm<sup>3</sup>) and high porosity (up to 99.81). The four samples of hydrophobic cellulose aerogel of consistencies: 0.3%, 0.5%, 0.75% and 1% were analyzed using NMR cryoporometry, diffusometry, MRI, FESEM and laser microscopy for thorough characterization of their porous structures.

The NMR cryoporometry allowed us to determine the size distribution of nanopores inside the solid framework of the aerogels. The diffusion measurements revealed the size distribution of the dominant, micrometer scale pores as well as the tortuosity of the materials. The MRI analysis provided an access to the size distribution of the largest pores (hundreds of micrometer in scale) in the sample. The results of the NMR analysis were in good agreement with the microscopic analysis.

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Optically detected spin transfer from Gd<sup>3+</sup> and Tb<sup>3+</sup> ions to  
Ce<sup>3+</sup> emitters in garnet crystals

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The method of optically detected magnetic resonance (ODMR) was used to observe spin transfer processes from the paramagnetic Gd<sup>3+</sup> and Tb<sup>3+</sup> ions to Ce<sup>3+</sup> emitters in garnet crystals. The intensity of photoluminescence excited by circularly polarized light into Ce<sup>3+</sup> absorption bands can be used for selective monitoring the population of the Ce<sup>3+</sup> ground-state spin sublevels. It is shown that two mechanisms of spin transfer occur in these systems, and in both cases these processes are due to cross relaxation. In the first system, Gd<sup>3+</sup> to Ce<sup>3+</sup>, cross-relaxation occurs between Zeeman levels in a magnetic field, while in the second case, Tb<sup>3+</sup> ions to Ce<sup>3+</sup>, cross-relaxation takes place inside the optical transitions of the two systems intersystem crossing.

Direct evidence of the cross-relaxation effects in garnet crystals containing two electron spin systems, i.e., the simplest one of Ce<sup>3+</sup> ions with the effective spin  $S = 1/2$  and the system of Gd<sup>3+</sup> ions with the maximum spin  $S = 7/2$ , has been demonstrated. Magnetic resonance of Gd<sup>3+</sup> has been found by monitoring Ce<sup>3+</sup> emission in cerium and gadolinium co-doped garnet crystals, which implies the impact of the Gd<sup>3+</sup> spin polarization on the optical properties of Ce<sup>3+</sup>.

ODMR of Tb<sup>3+</sup> with resolved hyperfine structure has been found by monitoring the intensity of Ce<sup>3+</sup> luminescence excited by circularly polarized light in cerium and terbium co-doped garnet crystals, which unambiguously indicates the transfer of energy with conservation of spin from Tb<sup>3+</sup> ions to Ce<sup>3+</sup> ions. The processes of Tb<sup>3+</sup>, <sup>U</sup>i Ce<sup>3+</sup> radiative and nonradiative energy resonant transfer is known to occur simultaneously in Ce<sup>3+</sup> and Tb<sup>3+</sup> co-doped garnets. There is overlap of the Tb<sup>3+</sup> emissions series with the absorption band of Ce<sup>3+</sup>. This overlap can cause radiative energy transfer by reabsorption or nonradiative energy transfer due to multipolar or exchange interactions.

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Human and Yeast PCNA dynamics:

A comparative study using NMR Methyl Relaxation Dispersion

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The Proliferating cell nuclear antigen (PCNA) is a ring-shaped DNA sliding clamp (~90 kDa homo-trimer) that acts as a processivity factor of replicative DNA polymerases. PCNA is a very promiscuous protein and recognized to interact with many proteins via what is known as PCNA Interaction Protein sequence (PIP-Box). The human (h) PCNA and its yeast (sc) variant hold 35% sequence identity and the same three-dimensional structure, however variable dynamics in the seconds to hour time scale in the apo form. This had been confirmed independently using H/D exchange studies by NMR[1] and MS[2] and the dynamics were attributed to the stability of the protein via hydrogen bonding networks.

Here we report an attempt to quantify the  $\mu$ s-ms conformational dynamics of hPCNA and scPCNA studied in their apo and holo forms (bound to PIP-Box motifs of p21 and cdc9 respectively) via methyl (ILV) relaxation dispersion NMR (RD NMR) spectroscopy. Primarily the methyl group assignments of ILV residues were carried out using protein prepared using the precursors with deuterated and linearized carbons. While the proteins were labeled using precursors that have isolated methyl groups in ILV position for the RD NMR measurements. The preliminary methyl RD NMR results show that hPCNA is more dynamic in the unbound form whereas the scPCNA is dynamic in the bound form. The complete analysis of RD NMR results will further reveal about the intermediate conformations that participate in the binding events of PCNAs.

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Mussel-glue inspired adhesives: A study on the relevance of L-Dopa and the function of the sequence at nanomaterial-peptide interfaces by using NMR spectroscopy and modeling

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Mussel glue-proteins undergo distinct structural responses during adhesion processes at the material interface by optimizing binding kinetics and multivalent surface contacts (Waite et al., *Science* 2015). Aspects of this intriguing behavior are mimicked by a mussel-glue mimetic peptide (HSYSGWSPYRSG (Y\*=L-Dopa)) that has been previously selected by phage-display to adhere to Al<sub>2</sub>O<sub>3</sub> after enzymatic-activation. However, the underlying structural secrets facilitating the interaction of these synthetic mussel-inspired peptides with Al<sub>2</sub>O<sub>3</sub> surfaces was not understood, so far. Using solution NMR methods combined with molecular dynamics simulations were able to establish sequence-structure and function relationships at the interface. By taking advantage of favorable peptide exchange kinetics and correlation time, the synthetic mussel-inspired oligomer peptide sequence is subdivided into two sub-motifs, is subdivided into two sub-motifs, each containing one Dopa “anchor” (either Motif-1 or Motif-2). 1H-NMR titration experiments, where Al<sub>2</sub>O<sub>3</sub> nanoparticles (NPs) are steadily added to peptide solutions shine a light on interactions at the interface. The sign of the NOE cross peaks in 2D-NOESY spectra of each of the sub-motifs with NPs distinguishes the free and bound peptide; STD enables extracting conformation and orientation of the bound sub-motifs. While Motif-1 at Al<sub>2</sub>O<sub>3</sub> adopts a wobble in a cone structure (“M” shape), Motif-2 forms a compact hook structure (“C” shape). Notably, Motif-1 contacts the Al<sub>2</sub>O<sub>3</sub> surface dynamically, whereas Motif-2 exhibits stronger binding capability optimizes the surface contacts of the full-length peptide. The structural features of the oligomer bound-peptide are elucidated by molecular modeling, taking the NMR data of the sub-motifs as constraints. An in-depth analysis reveals not only the conformational aspects of the oligomer peptide representing an elongated “Hook” shaped structure with both Dopa residues anchored at the inorganic surface. This valuable knowledge can transfer into bio-inspiration for the development of synthetic adhesives in wet environments, such as in dental, orthopedic, and other biomedical applications.

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Solvation and ionic pairing effects of choline acetate  
ionic liquid electrolyte in protic and aprotic solvents

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Ionic liquids (ILs) are nowadays well known as alternative media for organic, catalytic and electrochemical applications[1]. Nonetheless, traditional ILs tend to incorporate imidazolium or pyridinium cations which have high toxicity, low biodegradability and high cost[2]. For that reason, ILs synthesized from bio-organic molecules have gained considerable interest and their mixtures with organic solvents have been shown for example to increase their dissolution, stabilization as well as electrochemical characteristics[3]. In most cases what has been found to control these characteristics is the degree of intermolecular cation, anion, solvent electrostatic and/or hydrogen bonding interactions, which in turn promote pair or aggregate formation[4].

In this study solvation and ion pairing effects have been exploited for the biodegradable choline-based quaternary ammonium (N,N,N-trimethylethanolammonium) salt with acetate as anion ( $[\text{Ch}]^+[\text{OAc}]^-$ ). NMR titration experiments have been performed in water ( $\text{D}_2\text{O}$ ) and dimethylsulfoxide ( $\text{DMSO-d}_6$ ) by stepwise addition of the salt and the changes in  $^1\text{H}$  chemical shift ( $\delta$ ) and diffusion coefficient ( $D$ ) have been followed. It was found that in  $\text{DMSO}$  both  $\delta$  and  $D$  follow a characteristic 1:1 binding isotherm which was attributed to  $[\text{Ch}]^+ \cdots [\text{OAc}]^-$  pair formation, whereas in  $\text{D}_2\text{O}$  the opposite effect has been observed upon addition of the salt. From the corresponding equilibrium association constants the Gibbs free energy of formation for the dimeric species has been obtained. Furthermore, the diffusion coefficients found to follow the Stokes-Einstein (or SEGWE) equation[5] over the concentration range and hence the hydrodynamic radii of the monomeric and dimeric species were extracted.

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### Stability of Diphosphates and Diphosphonates in Si-O-P Compounds - A Solid-State NMR Study

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In general, Si-O-P compounds are structurally interesting materials because they possess many different structural motives including higher coordinated silicon atoms. Well-known examples are crystalline silicophosphates like  $\text{SiP}_2\text{O}_7$ . [1] These and other Si-O-P compounds can be synthesized by different reaction routes at ambient pressure. We established a so-called “water-free” sol-gel route, [2,3] which can be adapted as a general synthetic procedure to obtain various Si-O-P compounds by using diphosphoric or diphosphonic acids instead of crystalline phosphoric acid.

Here, the reaction of tetraethoxysilane with either diphosphoric acid (SiOP1) or 1-hydroxyethane 1,1-diphosphonic acid (SiOP2) yielded amorphous products. The local structure of these Si-O-P materials could be elucidated primarily by solid-state NMR spectroscopy. The evidence for higher coordinated silicon atoms was given by  $^{29}\text{Si}$  MAS NMR spectroscopy due to chemical shift data. [4] Additionally,  $^{13}\text{C}$  and  $^{31}\text{P}$  MAS NMR spectra could verify the existence of diphosphate and diphosphonate structures. Furthermore, FSLG-HETCOR and  $^{31}\text{P}$ - $^{29}\text{Si}$  CP-REDOR experiments were able to deliver, inter alia, detailed information about  $^1\text{H}$ - $^{29}\text{Si}$  and  $^1\text{H}$ - $^{31}\text{P}$  heteronuclear correlations and the first coordination sphere of the phosphate and phosphonate groups. In the case of SiOP1, the phosphorous atoms of the main structure are connected via oxygen to three silicon atoms. [5]

**References:** [1] H. Rabaâ, F. Bkiri, *Can. J. Chem.* **2006**, *84*, 1024–1030. [2] S. Jähnigen, E. Brendler, U. Böhme, G. Heide, E. Kroke, *New J. Chem.* **2014**, *38*, 744–751. [3] S. Jähnigen, E. Brendler, U. Böhme, E. Kroke, *Chem. Commun.* **2012**, *48*, 7675–7677. [4] T. R. Krawietz, P. Lin, K. E. Lotterhos, P. D. Torres, D. H. Braich, A. Clearfield, J. F. Haw, *J. Am. Ceram. Soc.* **1998**, *120*, 8502–8511. [5] J. Kowalke, C. Arnold, I. Ponomarev, C. Jäger, P. Kroll, E. Brendler, E. Kroke, *Eur. J. Inorg. Chem.* **2019**, *2019*, 828–836.

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Phosphorylation modulates dynamics and conformation of the nuclear factor of activated T-cells

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The nuclear factor of activated T-cells (NFAT1) is arguably the most important transcription factor in the immune system. NFAT initiates immune response by up-regulating the production of several cytokines (IL-4, TNF-alpha, IFN-gamma, etc.). Besides its prominent role in infectious disease response as well as e.g. organ transplant rejection, NFAT has been linked to a number of mechanisms related to cancer. For instance, NFAT1 has been shown to be involved in tumor suppression in model cells whereas it participates in invasion and drug resistance in lung and breast cancer. Moreover, activation of NFAT has been demonstrated to improve immunotherapies for cancer patients. However, its mechanistic role has to be elucidated.

NFAT is comprised of a well-known Rel-like DNA binding domain flanked by two intrinsically disordered regions (IDRs). The N-terminal IDR contains a transactivation domain (TAD) as well as a regulatory domain and a nuclear localization sequence. At least 18 phosphorylation sites have been reported, targeted by about 6 different kinases. It is well established that dephosphorylation of those sites by calcineurin drives nuclear translocation of NFAT, although the mechanism remains elusive.

Here, we investigate the effect of Ser23 phosphorylation by the kinase p38 on a subdomain of the N-terminal IDR containing the transactivation domain (1-130). We show that NFAT TAD has significantly different ps-ns dynamics compared to the rest of the IDR. CPMG relaxation-dispersion allows to detect a sub-populated state exchanging with the main state on a timescale of 0.8 ms. We show that Ser23 phosphorylation modulates this process, which translates into increased binding affinity to e.g. to co-activator CBP/p300. Moreover, Ser23 phosphorylation also modulates long range effects that could be determinant for the translocation mechanism of NFAT to the nucleus.

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## P489

**Topology of Delignified Wood Fibers using MAS DNP at 9.4 T**

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Efficient delignification processes of lignocellulosic biomass are required in the biorefinery, pulp and paper industries in order to easily integrate biomass utilization into value-added products. Recently, it has been shown that integrating formaldehyde in the process allows high yield delignification which does not affect the cellulose digestibility. However, its exact role and structure at the surface of the residual biomass is still not yet understood. (L. Shuai, et al. Science 2016, 354, 329.)

Here, we use magic angle spinning dynamic nuclear polarization (MAS DNP) NMR spectroscopy methods to elucidate the delignification process, reveal the topochemistry of the solid residue, and characterize the exact nature of the components that remain inside the residual cell wall after the fractionation process of poplar wood.

In particular, we exploit the potential of MAS DNP to study the topology of poplar wood materials after pretreatment and fractionation processes and show that this approach clearly distinguishes between different sections of the wood cell wall. The regioselectivity feature of relayed DNP C-13 CPMAS experiments allows to distinguish between the external cell wall and the inner middle lamellae, as polarization transfer is effective only in a range of 40 to 200 nm. We first observe that lignin and hemicelluloses in poplar wood were almost completely extracted after a two-hour pretreatment with dioxane/H<sub>2</sub>O/formaldehyde/HCl, while pretreatment process times of 30 min do not completely delignify poplar wood.

We then show that the condensed lignins which were not extracted in the absence of dioxane and formaldehyde accumulate in a 40 nm region at the surface of the cell wall. Using carbon-13 enriched formaldehyde to follow the depolymerization mechanism we also show that 1 % of the total amount of carbon in the material could be assigned to self-polymerization.

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Correlation of Serum and Tissue Biomarkers with Hyperpolarized 13C Lactate Production in the Monitoring of Neuroendocrine Prostate Cancer

Seth Vigneron, Jinny Sun, Shubhangi Agarwal, Robert Bok, John Kurhanewicz, Renuka Sriram

Introduction

Neuroendocrine prostate cancer (NEPC) is a subtype that arises as the tumor develops resistance to androgen deprivation therapy (ADT). Preliminary data has displayed the ability of hyperpolarized (HP) 13C pyruvate MRI to detect high-grade PC and provide early evidence of metabolic response to therapy<sup>1,2</sup>. The goal of this study was to develop a serum marker to measure the development of NEPC during ADT in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model and use immunohistochemical staining (IHC) to quantify the NEPC % of the tumor to correlate with HP 13C MRI findings.

Methods

TRAMP mice that developed 0.5-1cc MRI detectable tumors at ~12 weeks received ADT. Mice with a ≥20% increase in tumor volume two weeks post-treatment were labeled non-responders, containing varying amounts of androgen-insensitive adenocarcinoma (CRPC) and NEPC. Single time-point, frequency-specific 13C 3D imaging was performed 35s after an injection of polarized [1-13C] pyruvate using a gradient spin-echo (GRASE) sequence<sup>2</sup>. ~100uL of blood was drawn and an enolase activity assay was performed to determine serum neuron specific enolase (sNSE) activity. Tumor sections were stained for NEPC using NSE and synaptophysin stains and was quantified by pixel HSV values.

Results

sNSE activity increased significantly in ADT resistant mice as compared to mice with androgen-sensitive adenocarcinoma and mice without tumors. sNSE activity was linearly correlated to the amount of NEPC. HP lactate/pyruvate (Lac/Pyr) ratio significantly increased with increasing amounts of neuroendocrine dedifferentiation.

Conclusion

This study demonstrated that sNSE activity correlates to NEPC dedifferentiation as measured by quantitative IHC of the resected tumor, thereby providing a means for selecting tumors for correlation with HP 13C MRI findings and further treatment. Preliminary HP 13C pyruvate studies suggest that the HP Lac/Pyr ratio can differentiate between CRPC and NEPC based on the degree of increased HP 13C lactate production.

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**Intrinsic paramagnetic susceptibility tensors for a complete lanthanide series**

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Pseudocontact shift (PCS) NMR is a powerful technique to study interactions, dynamics and structures of proteins, protein – protein and protein – ligand complexes in solution. Trivalent lanthanide ions are the most useful class of paramagnetic centres for PCS NMR and allow to determine the position of nuclei over distances larger than 80 Å from the metal. Lanthanide chelating tags (LCTs) have been developed in order to attach lanthanides in a stereo-chemically pure and rigid manner to proteins. The size of the observed PCSs is proportional to the anisotropic part of the magnetic susceptibility. So far the anisotropy parameters  $\Delta\chi_{ax}$  and  $\Delta\chi_{rh}$ , describing the anisotropic part of the magnetic susceptibility, have been determined only on proteins where they are reduced by motional averaging. The intrinsic anisotropy a lanthanide exhibits within a LCT is unknown. Studying the anisotropy directly on a free tag is not possible by using conventional 2D-NMR assignment strategies, as they are rendered useless by the extremely short T2 times. To obtain information about the observed shifts, we applied a combination of different selective <sup>2</sup>H and <sup>13</sup>C labelling schemes, as well as double resonance techniques. Fitting of the anisotropy parameters revealed the presence of significant contact shifts. We present here the intrinsic anisotropy parameters  $\Delta\chi_{ax}$  and  $\Delta\chi_{rh}$  for the complete series of paramagnetic lanthanides on the DOTA-M7FPy tag.

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Exact distance measurement and spatial sampling in RNA

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RNA does not only translate the genetic code into proteins, but also carries out important cellular functions. Understanding such functions requires knowledge of the structure and dynamics at atomic resolution. Almost half of the published RNA structures have been solved by NMR, but as a result of severe resonance overlap and low proton density, high-resolution RNA structures are rarely obtained from NOE data alone. Instead, additional semi-empirical restraints and labor-intensive techniques are required for structural averages, while there are only a few experimentally derived ensembles representing dynamics.

We have replaced the standard NOE-based procedure for protein structure determination by an approach that employs tight averaged distance restraints derived from exact NOEs (eNOEs). We have recently shown that our protocol is also able to define a 14-mer RNA tetraloop structure at high resolution without other restraints. Here, we redefine the limits of the eNOE methodology in terms of retrieving spatial sampling and the RNA size amenable to such studies:

We use eNOEs to calculate a two-state structure of the tetraloop. Then, we demonstrate that a molecular dynamics trajectory generated without experimental restraints features a distinct and previously unknown low-populated state that improves the agreement with the eNOEs.

Since fully sampled NOESY buildup series can take 10 days or more to acquire, eNOE studies are both expensive and problematic in the case of unstable samples. We investigate the fidelity of eNOE restraints recorded with non-uniform sampling (NUS) of the indirect dimensions. NUS imparts negligible errors on the eNOE distances down to 10% sampling, but there is a noticeable decrease in the eNOE yield below 40%.

Our innovations to the eNOE protocol may open an avenue to obtain high-resolution structural ensembles of small RNA with moderate experimental efforts and average structures of larger RNA.

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The effect of charge transfer in graphite intercalation materials as probed by <sup>13</sup>C NMR

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Graphite exhibits a quasi-two-dimensional structure and highly anisotropic properties. Despite the abundant literature in the past on the physical properties and applications of graphite, there is still a number of open questions with respect to magnetic and transport behavior of graphite intercalation compounds. Sensitive and metastable graphite intercalation compounds can be measured in a short time by high-degree enrichment with <sup>13</sup>C isotope. Using solid state NMR we characterized the structural changes occurring in <sup>13</sup>C-enriched fine-grained graphitic materials upon chemical and electrochemical modification. The <sup>13</sup>C MAS NMR spectra of pristine graphitic materials and those subjected to a thermal pressure-assisted modification and a room-temperature treatment by gaseous Br<sub>2</sub> feature a single very broad asymmetric line. Upon the electrochemical insertion of alkali metals in graphitic material, we observe a dramatic line narrowing, shift and an intensity increase with a factor of five for Li and of two for Na intercalation. We associated these effects to anisotropic diamagnetic susceptibility and charge transfer. A physical scenario thus implies that some <sup>13</sup>C atoms in the graphitic regions subjected to the demagnetizing fields become unobservable by <sup>13</sup>C NMR. Complete (in the case of lithiation) or partial (in the case of sodiation) destroying of the demagnetizing fields, however, enables the accessibility of these <sup>13</sup>C spins.

In addition, our results clearly demonstrate charge transfer in the direction perpendicular to the graphitic layers, which exceeds the thickness of a single graphite layer, as lithium ions are intercalated. This knowledge can be applied for advanced high-power-density electrode materials for safe and fast-charging metal-ion batteries or for novel spintronic concepts with controlled spin-polarized charge carrier injection and transport, combined with the possibility to manipulate magnetic anisotropy.

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## Molecular fluid dynamics description of Rheo-NMR

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Abnormal protein aggregation is a hallmark of Alzheimer's disease and numerous other neurodegenerative disorders. Despite tremendous interest in pathogenesis of such diseases, no existing method can capture initial protein aggregate nucleation and subsequent growth at atomic resolution in real time.

To fill this gap, we have recently established high-sensitivity rheological NMR ("Rheo-NMR") spectroscopy that enables us to detect atomic-level structural changes of a protein during amyloid formation in real time (Anal. Chem., 2017, 89). By using the newly developed apparatus, we are able to detect site-specific structural information on amyloidogenic proteins during their amyloid formation in situ, thereby gaining insight into the mechanism underlying amyloid nucleation at atomic resolution.

However, NMR experiments do not provide a truly visual **picture** of protein motion under shear. Nevertheless, we are keen to understand what is actually going on inside the NMR tube during a Rheo-NMR experiment.

Therefore, we are using molecular dynamics simulations to study the motion of protein particles in a simple shearing flow. We are also starting to study the fluid itself numerically by computational fluid mechanics.

Connecting fluid mechanics, biomolecular simulations, and experiments applying shear flow in situ appears to be a promising strategy to understand protein motion, dynamics, and deformation under shear.

**References:** [1] Morimoto D. et al., Anal. Chem., 2017, 89(14), 7286-7290. [2] Walinda E. et al., Biochim. Biophys. Acta Gen. Subj., 2019, in press.

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**The Companion of Cellulose Synthase 1 confers salt tolerance through a Tau-like mechanism in plants.**

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Abiotic stress, such as salinity, drought, and cold, causes detrimental yield losses for all major plant crop species. Understanding mechanisms that improve plants' ability to produce biomass is therefore of great importance for agricultural activities. The companion of cellulose synthase (CC) proteins sustain cellulose synthesis during salt stress by regulating the cortical microtubule (MT) network and cellulose synthase localization in *Arabidopsis thaliana*.

In our work, we show that the disordered cytosolic domain of CC1 promotes polymerization and bundling of MTs and can diffuse bidirectionally along the MT lattice.

Using NMR-spectroscopy, we probed the interaction of CC1 with MTs. Upon complexation, the protein does not fold into a rigidly bound structure, but rather binds locally with conserved, short hydrophobic binding motifs that are interconnected by flexible linker regions. Mutation of key residues in the N-terminal binding motif impaired binding in vitro and abolished MT-guided cellulose synthesis resulting in a salt sensitive phenotype in vivo.

The biophysical properties and MT-binding behavior of CC1 are reminiscent of those of the prominent mammalian Tau/MAP2/MAP4 family. Hence, we propose that CC1 sustains cellulose synthesis during salt stress via a Tau-like mechanism.

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**Micro-fluidic NMR for in-situ monitoring of the kinetic process of molecular assembly**Xinchang Wang<sup>\*,1</sup>, Hongxun Fang<sup>1</sup>, Yibin Sun<sup>1</sup>, Manvendra Sharma<sup>2</sup>,Xiaoyu Cao<sup>1</sup>, Zhongqun Tian<sup>1</sup>, Marcel Utz<sup>3</sup>, Zhong Chen<sup>1</sup><sup>1</sup>*Xiamen University*, <sup>2</sup>*University of Southampton*,<sup>3</sup>*School of Chemistry, University of Southampton*

Molecular assembly is crucial for improving our understanding of biological world and creating novel functional materials. How to realize controllable molecular assemblies is still challenging, and requires more propounding insights into the mechanism of assembly process, both thermodynamic and kinetic. However, the structures and components of assemblies are quite complex, which make their kinetic process extremely difficult to characterize using traditional NMR techniques.

We collaborated with Prof. Marcel Utz from the University of Southampton and using microfluidic-NMR spectroscopy to study the kinetics in molecular assembly. Kinetics of a multicomponent host-guest supramolecular system containing viologen derivatives,  $\beta$ -cyclodextrins and cucurbit[7]urils were studied by a PMMA based microfluidic chip combined with a dedicated transmission line probe for NMR detection.

By combining microfluidic technology with NMR spectroscopy, the amount of material required for a full kinetic study could be minimized. This is crucial in supramolecular chemistry, which often involves highly sophisticated and synthetically costly building blocks. The small size of the microfluidic structure is crucial in bringing the time scale for kinetic monitoring down to seconds. At the same time, the transmission line NMR probe provides sufficient sensitivity to work at low (2 mM) concentrations.

**Reference:** [1] Fang, H.; Sun, Y.; Wang, X.; Sharma, M.; Chen, Z.; Cao, X.; Utz, M.; Tian, Z., Probing the kinetics in supramolecular chemistry and molecular assembly by microfluidic-NMR spectroscopy. *Science China Chemistry* 2018, 61 (11), 1460-1464.

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**Intestigation of metabolite-protein interactions and competitive binding in complex biological fluids by high-resolution relaxometry**

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Metabolomics is the study of all metabolites in biological samples. These small molecules constitute a unique chemical fingerprint of the metabolic status, and are insightful markers for many diseases. Not only metabolites play important roles in their free forms, but also they bind to macromolecules to carry out their functions in signaling, regulation and other cellular processes. Characterization of metabolite-macromolecule interactions in complex biological fluids is an emerging subject in interactomics. Yet, the investigation of metabolite-protein interaction by systematic analytical techniques has been limited.

Here, we use high-resolution NMR relaxometry to identify the interactions of metabolites with macromolecules in biological fluids. The method is based on the measurement and analysis of longitudinal relaxation rates over orders of magnitude of magnetic field (here from 15 mT to 14.1 T) using a sample shuttle apparatus [1]. The relaxation dispersion profiles identify transient binding of small molecules to large biomolecular complexes. The analysis provides accurate estimates of the properties of the system, including the size of the complex.

This approach was first developed and validated on a model sample, then applied to biological fluids relevant for metabolomics studies: human blood plasma and serum. The size dispersion of macromolecules in human blood was estimated with Fast Field Cycling (FFC) NMR. Identified metabolites revealed different features in high-resolution relaxation dispersion profiles. The interactions of some metabolites with macromolecules were characterized. Furthermore, we show that relaxation dispersion profiles are sensitive to competitive binding between small molecules. High-resolution relaxometry is an efficient tool for the investigation of dynamic interactions in biological fluids, and provides valuable information complementary to conventional metabolomics approaches, which may pave the way for NMR in interactomics.

**Reference:** [1] Charlier, C.; Khan, S. N.; Marquardsen, T.; Pelupessy, P.; Reiss, V.; Sakellariou, D.; Bodenhausen, G.; Engelke, F.; Ferrage, F., J. Am. Chem. Soc., 135, 18665 (2013)

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Acidic Nature of “NMR-invisible” Tri-coordinated Framework Aluminum Species in Zeolite H-ZSM-5

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Unambiguous characterization of different acid sites in zeolites is of great importance in heterogeneous catalysis. In addition to various well-characterized extra-framework Al species, tri-coordinated framework aluminum species can also serve as Lewis acid site (LAS) in zeolites, which is however “NMR-invisible” due to its extremely distorted/asymmetrical local environment resulting in the considerable line broadening of its <sup>27</sup>Al NMR resonance (with quadrupolar interaction > 30 MHz). By using sensitivity-enhanced two-dimensional <sup>31</sup>P-<sup>27</sup>Al heteronuclear multiple-quantum correlation NMR experiments coupled with trimethylphosphine oxide (TMPO) probe molecules, we provide a feasible and reliable approach to elucidate the acidic nature of the tri-coordinated framework Al in dehydrated H-ZSM-5 zeolites for the first time. Two types of tri-coordinated framework Al sites have been unambiguously identified, which amount to 11.6% of the total Brønsted and Lewis acid sites. Moreover, the synergistic effect arising from the close spatial proximity between tri-coordinated framework Al sites and Brønsted acid sites (BAS) in zeolite framework is confirmed by two-dimensional <sup>27</sup>Al-<sup>27</sup>Al double-quantum single-quantum homonuclear correlation experiment and DFT calculations. The Brønsted/Lewis acid synergy leads to generation of superacidity (with acid strength stronger than 100% H<sub>2</sub>SO<sub>4</sub>) in the zeolite, as evidenced by the <sup>31</sup>P chemical shift of 85-88 ppm for adsorbed TMPO. These findings would be helpful for understanding the catalytic performance of zeolites and rational design of highly efficient zeolite catalysts.

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HYPERPOLARIZED <sup>129</sup> XENON NMR FOR PROBING  
SURFACE-FUNCTIONALIZED POROUS MATERIALS  
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Hyperpolarized <sup>129</sup> Xenon NMR spectroscopy provides a powerful method to investigate porous materials as has been shown on silica-based materials of MCM-41 structure in previous work in our group [1]. It offers insights to pore sizes, pore geometries and diffusivity. We are planning to apply hyperpolarized Xenon NMR for probing surface-functionalized porous materials based on controlled pore glass (CPG) with well defined structures in the micro- and mesoporous range. Until now, surface functionalization of silica-based materials is not yet fully understood.

The application of such materials is widely spread, for example it provides an advantageous alternative membrane material in Redox-flow batteries used for renewable energy storage [2]. It is planned to apply Ultrafast Laplace NMR experiments on hyperpolarized Xenon to obtain complementary information about the surface functionalization from those relaxation and diffusion measurements [3]. Ultrafast Laplace NMR replaces conventionally time-consuming two-dimensional experiments by an alternative approach based on spatial encoding. That leads to the reduction of experimental time requirement by several magnitudes. It is planned to apply this type of experiment to hyperpolarized Xenon.

**References:** [1] J. Hollenbach et al. (2017), JPCC 121, 15804-15814. [2] H. Mögelin et al. (2018), J. Appl. Electrochem. 48, 651–662. [3] O. Mankinen et al. (2018), Microporous Mesoporous Mater. 269, 75–78.

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**P501**

**An efficient scheme for two-dimensional  $^{13}\text{C}$ - $^{13}\text{C}$  correlation spectroscopy under an ultrafast MAS spinning rate**

Sungsool Wi<sup>\*1</sup>, Lucio Frydman<sup>2</sup>

<sup>1</sup>NHMF, <sup>2</sup>Weizmann Institute of Science

We introduce an efficient, yet easy to use, homonuclear dipolar recoupling scheme by employing a chirp pulse or a train of chirp pulses. It operates well at any MAS spinning rates, but is especially superior to other methods known to date at an ultrahigh MAS spinning rate ( $\Omega_r = 60$  kHz). This method is useful for generating cross peaks between any sites that possess small, medium, or large chemical shift differences because of possessing a broad offset frequency profile of magnetization transfer. Our mixing scheme is also very tolerant to the dipolar truncation effect, and also has an advantage of minimizing the RF heating effect because this method employs a weak RF pulse strength for mixing. Various aspects in utilizing this mixing scheme are described in detail, including the optimization of the chirp pulse parameters, such as dwell time ( $\Delta t$ ), sweep bandwidth (BW), and total pulse duration (tp). Experiments were demonstrated on a model peptide system, U- $^{13}\text{C}$ -labeled Tyrosine, and on a protein sample system, U- $^{13}\text{C}$ -labeled Barstar.

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Protein-nucleotide contacts in the motor  
protein DnaB probed by <sup>31</sup>P- and <sup>1</sup>H-detected MAS spectroscopy  
Thomas Wiegand<sup>\*,1</sup>, Maarten Schledorn<sup>1</sup>, Alexander A. Malär<sup>1</sup>,  
Riccardo Cadalbert<sup>1</sup>, Laurent Terradot<sup>2</sup>, Beat H. Meier<sup>1</sup>, Anja Böckmann<sup>2</sup>  
<sup>1</sup>ETH Zürich, <sup>2</sup>IBCP Lyon

Non-covalent interactions, such as hydrogen bonds, van-der-Waals or ionic interactions are of key importance in catalytic processes and play an important role in molecular recognition. We present a combined approach based on <sup>1</sup>H- and <sup>31</sup>P-detected Magic-Angle Spinning (MAS) experiments to identify protein-nucleotide contacts in the bacterial DnaB helicase from *Helicobacter pylori*, an oligomeric motor protein with a molecular mass of 708 kDa.

As a first step, we discuss the feasibility of proton-detected experiments at fast MAS for such a large protein. The different contributions to the <sup>1</sup>H line-widths for fully-protonated and deuterated (100% back-exchanged) protein samples were determined. Proton resonance assignment was achieved on a fully-protonated sample by a sequential assignment approach which directly provides H<sup>N</sup> and H<sup>A</sup> chemical-shift values.

This is the foundation for the second step, in which we use the sensitivity of <sup>1</sup>H chemical-shift values for hydrogen bonding to probe protein-nucleotide binding modes. In combination with <sup>31</sup>P-detected heteronuclear correlation experiments, which yield spatial connectivities but no direct information about the chemical bonding state, we identified two key residues of DnaB participating in DNA binding. One is a lysine forming via its sidechain a salt bridge to one of the DNA phosphate groups, the other one is an arginine which contacts via the sidechain the thymidine base edge by forming a hydrogen bond. Our study illustrates how NMR allows to distinguish in an interaction-specific manner between different protein-DNA binding modes yielding new insights into molecular recognition processes important in both fields, biology and materials sciences.

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3D MR Microscopy with 6D Diffusion-Relaxation Distributions

Linn Winsnes Thrane\*, Alexis Reymbaut, Hong Jiang,  
João P. de Almeida Martins, Daniel Topgaard  
Lund University

Most materials are heterogeneous on the millimeter resolution of magnetic resonance imaging (MRI) in the sense that they are comprised of multiple microscopic domains with different chemical and diffusional properties. Consequently, the measured MRI signal on the voxel scale is highly unspecific as it originates from the average of the signals arising from these domains. However, multidimensional relaxation rate and diffusion tensor correlation experiments can tease apart the various microscopic contributions of interest [1, 2].

Here, we introduce a 6D diffusion relaxation correlation MRI sequence which encodes the signal in a 6D sampling space of echo time TE, recovery time TR, and the magnitude  $b$ , anisotropy  $b_{\Delta}$ , and orientation  $(\theta, \Pi)$  of the encoding b-tensor. In turn, Laplace inversion of the signal results in distributions in the 6D space of the relaxation rates  $R_1$  and  $R_2$ , isotropic diffusion  $D_{iso}$ , diffusion anisotropy  $D_{\Delta}$ , and diffusion tensor orientation  $(\theta, \varphi)$ , which combined yield reliable scenarios of intra-voxel heterogeneity. Implementation of the pulse sequence was done on a Bruker 11.7 T microimaging system with in-plane spatial resolution of  $0.3 \times 0.3 \text{ mm}^2$  and slice thickness of 0.6 mm. The signal inversion is performed with our unregularized Monte Carlo inversion algorithm [1-3] and the 6 physical parameters extracted from the inversion are reported in correlation maps.

The pulse sequence used to execute the signal encoding is a diffusion-weighted spin echo RARE sequence where the trapezoidal gradient pulses have been replaced by free gradient waveforms [2]. Experiments were performed and validated on materials with known diffusion and relaxation properties. The results show great potential for identifying intra-voxel chemical and structural micro-environments in various heterogeneous materials.

**References:** [1] Martins, Sci. Rep. (2018). [2] Topgaard, NMR Biomed. (2019). [3] Martins, Phys. Rev. (2016).

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## P504

## Validating conformation generators with NMR solution data

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The quality of models for prediction of molecular properties in solution relevant to drug discovery

is critically dependent on the quality of the conformations used to build those models. Unfortunately conformation generators have, historically, been validated against conformations found in the solid state.

We have therefore built a framework to validate and compare conformation generators using solution NMR data. This framework comprises input NMR conformational data (NOEs and scalar couplings), a conformation ensemble generator and a method to identify those conformations from the ensemble that best match the NMR data as well as their probabilities. Part of this framework is the new tool NMR\_FIT, which is a user-friendly and faster reimplementaion of the well-established NAMFIS code. We have validated NMR\_FIT against the reference implementation on a variety of molecules, and used it to compare different methods of conformation generation in solution.

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## P505

 **$^{13}\text{C}$  and  $^1\text{H}$  NMR for purity determination of certified reference materials for quantitative measurements**

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NMR is a primary method of measurement that provides quantitative results without the need of a standard of the same measurand. Amongst different procedures for quantification of organic compounds by NMR, the International Bureau of Weights and Measures (BIPM) encourages the use of internal standard (IS) NMR as it presents less sources of errors and smaller uncertainties. The use of a certified reference material (CRM) as the IS is essential to produce metrological traceable results with high accuracy. This work focused on the certification of ISs for NMR.  $^{13}\text{C}$  and  $^1\text{H}$  NMR were compared to the mass balance approach, a well-established method for purity characterization of CRMs based on the determination of impurities. Despite the advantages of the  $^1\text{H}$  nucleus such as high sensitivity, short  $T_1$  and widespread distribution in organic compounds, some molecules present only labile H atoms or  $^1\text{H}$  peaks overlapped with impurities. With a new scheme named CHORAD, based on adiabatic pulses, this study showed that  $^{13}\text{C}\{^1\text{H}\}$  NMR achieved bias of 1.8% and expanded uncertainty of 0.62% (confidence level 95%) for purity determination. This meant a 1.2% bias reduction over the ordinary  $90^\circ$  hard pulse with WALTZ-16  $^1\text{H}$  decoupling. For the same substance, purity was obtained by  $^1\text{H}$  NMR with 0.2% bias and 0.15% expanded uncertainty. The better performance of  $^1\text{H}$  nucleus over  $^{13}\text{C}\{^1\text{H}\}$  prompted its use for CRM characterization in comparison to the reference mass balance method (expanded uncertainty 0.06%). A suite of high-purity CRMs (maleic acid, potassium hydrogen phthalate, dimethyl sulfone and dimethyl terephthalate) was developed to be used in NMR quantifications of substances of different polarities and chemical shifts. When the sample does not present suitable  $^1\text{H}$  nuclei, a protocol for  $^{13}\text{C}$  NMR purity determination was proposed with a correction for the heretofore unreported contribution of the  $^{13}\text{C}$  natural non-statistic isotopic fractionation.

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## P506

## Neural network processing of DEER with exchange coupling

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Deep neural networks (DNNs) offer a powerful alternative to Tikhonov regularised analysis procedures for standard DEER data. DNNs can be trained on libraries of synthetic DEER traces, allowing them to learn a simulated reality which can include carefully modelled distortions and noise – we have shown that DNNs trained in this manner generalise well to real experimental data. [1]

The well-established Tikhonov methods involve a regularised fitting that relies on a simplified DEER kernel which neglects the exchange interaction ( $J$ ) completely. In many situations this assumption is reasonable due to the distances and structure between spin-labels. However, the presence of a significant exchange coupling will complicate the interpretation of data under these regimes. It is also often desirable to be able to quantify the exchange coupling, this may present a significant challenge beyond the simpler cases. [2]

In this communication we present new functionality in DEERNet for analysis of DEER in the presence of exchange coupling. By training feed-forward DNNs using data simulated via the full kernel for DEER including exchange coupling, we are able to extract confident estimates for both the inter-spin distance distribution and the exchange interaction.

**References:** [1] S. G. Worswick, J. A. Spencer, G. Jeschke, I. Kuprov, *Science Advances*, 2018, 4, 1-7. [2] S. Richert et al., *Physical Chemistry Chemical Physics*, 2017, 19, 16057-16061.

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NMR Study of Star Block Polypeptide Hydrogel: Influence of Core Size on Chain Dynamics and Water Diffusion

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Synthetic polymeric hydrogel have attracted huge attention due to their unique properties as a 3D crosslinked network as well as their various applications in tissue engineering scaffolds and bio-sensors. Among all the synthetic polymeric hydrogels, polypeptides shows excellent suitability for hydrogelating materials as they possess a number of desirable properties. Besides their side chain functionalities, conformations as well as degradation degree can be systematically tailored, these hydrogel networks prepared from biomimetic polypeptides also display similar physico-chemical properties to these extra-cellular matrix (ECM). Recently, we have reported the design of a series of hydrogelating scaffolds from star block polypeptides tethered to a dendritic core, which is a nano-sized, radially symmetric molecules with well defined, homogeneous and monodisperse structure consisting of tree-like arms or branch. Based on a combination of long glutamic acid block and valine block, these hydrogelators can form a hydrogel directly through hydrophobic interaction stemming from the self-assembly of the valine domains which are known to cluster in  $\beta$ -motif arrangements. The importance of the star-shaped structure can be underpinned by the fact that the linear equivalents of the block copolypeptide do not form hydrogels. It would appear that the covalent point-like junctions of the star polypeptide core crucially contribute to the stable network formation. Hence it would be of great interest for material scientists to understand the impact of dendritic core on the physico-chemical properties of the hydrogel system. In this study, we use NMR diffusometry and relaxometry to analyze the water dynamics as well as the polymer chain dynamics inside these star block hydrogel networks. It is found that the morphology of the hydrogel changed when dendritic core becomes too large. The water self-diffusion coefficient is no longer proportional to the hydrogelator's Mw. These results are further correlated to SAXS analyses on these systems.

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Conformational changes in the guanidine-II riboswitch observed with PELDOR  
Christine Wuebben, Maria F. Vicino, Marcel Mueller, Senada Nozinovic, Olav Schiemann

Riboswitches are functional RNAs acting as cis regulatory genetic elements. The binding of a specific ligand at the aptamer domain stabilizes a certain conformation or causes conformational changes that influence the gene expression. One of these riboswitches is represented by the guanidine-II riboswitch (mini-ykkC) [1]. It consists of two conserved stem-loops P1 and P2 connected via a linker of variable length. Crystal structures were obtained of the separated hairpins P1 and P2 and showed for both a kissing loop homo-dimerization with one guanidine bound to each loop [2, 3]. Based on these structures a switching model was proposed in which the binding of guanidine leads to a P1-P2 hetero-interaction within the same RNA strand and subsequently to the exposure of the Shine-Dalgarno Sequence leading to promotion of translation of the associated genes [4].

Here, we show with SDSL and PELDOR that the isolated P1 and P2 hairpins form also in solution the head-to-head dimers in the presence of guanidine. In addition, we found with SDSL/PELDOR and NMR that both hairpins change their structure in dependence of the annealing procedure and divalent ions. Interestingly, these conformational changes can be performed in a cyclic manner, where adding guanidine does lead in each case to the homo-dimerization. Finally, we can show with SDSL/PELDOR that P1 and P2 form a hetero-dimer within the full-length riboswitch.

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**Probing Host-guest interactions in zeolites by solid state NMR spectroscopy**Jun Xu<sup>\*1</sup>, Wang Chao<sup>2</sup>, Wang Qiang<sup>2</sup>, Qi Guodong<sup>2</sup>, Deng Feng<sup>2</sup><sup>1</sup>National Center for Magnetic Resonance in Wuhan, Wuhan Institute of Physics and Mathematics, CAS, <sup>2</sup>National Center for Magnetic Resonance in Wuhan, Wuhan Institute of Physics and Mathematics, CAS

Host-guest interactions between adsorbed molecules and active sites on catalyst strongly influence catalytic performances (activity and selectivity) because they play essential roles in the reactant adsorption, intermediate formation, and product desorption. The methanol-to-olefins (MTO) conversion on acidic zeolite catalysts provides an alternative to produce lower olefins from decreasing crude oil and thus has attracted significant attention in both industry and academia. Due to the complex network involved in the MTO reaction, mechanistic understanding of the formation of olefins remains as a topic of great challenge in the field of heterogeneous catalysis. We experimentally demonstrated the formation of supramolecular reaction centers composed of organic hydrocarbon species and inorganic zeolite framework in H-ZSM-5 zeolite by advanced <sup>13</sup>C-<sup>27</sup>Al double resonance solid-state NMR spectroscopy. Such host-guest interactions were also revealed in zeolites with different topology. It was found that methylbenzenes and cyclic carbocations located closely to Brønsted acid/base sites form the supramolecular reaction centers in the zeolite channel. The product shape selectivity of zeolites greatly influences the catalytic performance which can be linked to the nature of hydrocarbon species and the host-guest interactions. The analysis of the host-guest interactions show insights into the distribution of the carbonaceous species in deactivated catalysts. We also showed that the host-guest interactions lead to the formation of extra-framework Al bound surface methoxy species in the MTO reaction on H-ZSM-5 zeolite, which was determined to be the key intermediate for ethene generation in the early reaction stage.

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**P510**

**Accurate Determination of  $^1\text{H}$ - $^{15}\text{N}$  Dipolar Couplings Using Inaccurate Settings of the Magic Angle in Solid-State NMR Spectroscopy**

Kai Xue<sup>\*,1</sup>, Salvatore Mamone<sup>1</sup>, Riddhiman Sarkar<sup>2</sup>, Bernd Reif<sup>3</sup>

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<sup>2</sup>*TU München,* <sup>3</sup>*Technical University of Munich*

Magic-angle spinning (MAS) is an essential ingredient in a wide variety of solid-state NMR experiments. The standard procedures to adjust the rotor angle are not highly accurate, resulting in a slight misadjustment of the rotor from the magic angle ( $\sim 54.74^\circ$ ) on the order of a few millidegrees. This small missetting has no significant impact on the overall spectral resolution, but is sufficient to reintroduce anisotropic interactions. Shown here is that site-specific  $^1\text{H}$ - $^{15}\text{N}$  dipolar couplings can be accurately measured in a heavily deuterated protein. This method can be applied at arbitrarily high MAS frequencies, since neither rotor synchronization nor particularly high radiofrequency field strengths are required. The off-MAS method allows the quantification of order parameters for very dynamic residues, which often escape an analysis using existing methods.

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Intra-cerebral hemorrhage rehabilitates treatment  
rat models brain metabolite concentration change.

SEUNGMAN YU\*

Gimcheon University of Korea

If the intracerebral hemorrhage (ICH) rehabilitation animal models studies have included processes related to imaging diagnosis, not only interim evaluation of treatment effects, but also objectification of treatment effects would have been possible. The purpose of this study was to examine the rehabilitate treatment effect bio-marker by Magnetic resonance imaging/spectroscopy in animal intra cerebral hemorrhage model.

Two groups of rats were used in this study: 1) rehabilitation treatment group, 6-weeksold SpragueDawley 12 rats with experimental hemorrhage received rehabilitation; and 2) control group, 12 rats with experimental hemorrhage received no intervention. Training rehabilitation was implemented 15 min daily for 2 weeks with 55 to 85 percent of VO2 max. We conducted MRI/MRS scan before and after ICH rat modeling to evaluate brain metabolite concentration changes. Signal intensity of T2WI was measured for the site of ICH and the opposite side which had almost the same area as ICH. Integrating areas under peaks were measured for cerebral metabolite concentration.

The difference in mean T2WI-SI ratios measured at two weeks after ICH induction was not statistically significant ( $p = 0.514$ ) between the control group and the experimental group. However, brain metabolite tCho/tCr ratio in the control group was significantly lower than that in the experimental group at 2 weeks after ICH induction ( $0.243 \pm 0.044$  vs.  $0.326 \pm 0.061$ ,  $p = 0.007$ ). The tCho/tCr might be used as a biomarker to evaluate the effect of rehabilitation treatment for ICH.

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Lipid proton concentration in an obesity fatty liver  
SEUNGMAN YU\*  
SEUNGMAN

The purpose of this study is to investigate the lipid proton concentration in an obesity fatty liver so that the more accurate quantification of fat deposition can be performed by applying different lipid proton (LP) concentration changes using the multi-interference DIXON technique.

A high fat diet 60% was used to feed eight Sprague-Dawley rats in order to cause obesity fatty liver. Baseline Magnetic Resonance Imaging (MRI)/Spectroscopy data (Control Group) were obtained prior to the introduction of the high fat diet, and data acquisition experiments were performed after eight weeks with identical procedures as those used for the base-line. All MRI/Spectroscopy were performed using a 3.0 Tesla MRI with a four-channel receive animal coil. The six lipid proton metabolites (methyl protons, methylene proton, allylic protons,  $\alpha$ -methylene protons to carboxyl, diallylic protons, and methane protons) were calculated using LCModel software. The methylene proton showed the highest increase in the LP, but it took only 71.86% of total LP concentration. The methylene proton plays a leading role in fat accumulation in liver parenchyma, but other LP concentrations also need to be applied in order to express relatively accurate fat content.

The multi-interference DIXON technique has to perform by applying this LP concentration change on obesity fatty liver for more accurate fat percentage expression.

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The smokers/non-smokers brain insular cortex connectivity with fMRI and metabolites concentration with MRS evaluation.

SEUNGMAN YU\*

Gimcheon University of Korea

The purpose of the experiment was to investigate differences in the connectivity between the insular cortex out of the brain areas and other brain areas and the concentration.

A total of 20 experimental subjects consisting of 10 smokers and 10 non-smokers were selected from among males in their 20s. The mean age of the experimental subjects was 23.2±3 years. All MRS experiments were conducted using a 3.0T 3.0Tesla MRI scanner. The 1H-MRS images of the insular areas on both sides were separately obtained through point-resolved spectroscopy (PRESS) sequences under the conditions of TR = 1,500 msec, TE = 35 msec, NEX = 32, and 20×20×38 mm3 voxel. The fMRI images and a total of 3096 functional images were obtained under the conditions of axial, mode = 2D, scan timing: TE = 30 ms, TR = 2200 ms, flip angle = 90, matrix = 64 × 64, slice thickness = 5 mm, 36 slices.

The concentrations of the eight metabolites in the right and left insular cortex areas of smokers were analyzed. However, in the case of non-smokers, the concentrations of Cr, GPC, GPC+Pch, and Cr+PCr were shown to be statistically significantly different between the left and right insular cortex areas.

As for differences in metabolite concentrations in the left and right insular cortex areas between smokers and non-smokers, the Cr concentration in the left insular cortex area was identified to be statistically significantly higher among non-smokers. The smokers had stronger connectivity between the right insular cortex area and Lingual Gyrus Right, Occipital Fusiform Gurus Right, Vermis 4 5, cerebellum 6 right, vermis 6, cerebellum Crus 1 right, Cingulate Gurus posterior division, precuneous cortex, and cerebellum 4 5 right.

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## P514

### Nuclear Spin Singlet States in Photoactive Molecules: From Fluorescence/NMR Bimodality to a Bimolecular Spin Singlet State Switch

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Louis-S. Bouchard<sup>2</sup>, Stefan Glöggler<sup>1</sup>

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Biophysical Chemistry, <sup>2</sup>Department of Chemistry and Biochemistry,  
University of California Los Angeles, <sup>3</sup>Max Planck Institute for Biophysical Chemistry

Photoactive chemicals have received considerable attention because of its interesting applications[1]. Singlet state NMR properties as response to light induced processes have attracted our attention. Nuclear spin singlet states consist of two spin-1/2 nuclei coupling into a resulting spin-0 state[2]. The key feature of singlet states is they are immune to intramolecular dipole-dipole. As a result, the lifetime of nuclear spin singlet order (Ts) may be extended beyond the spin lattice relaxation time (T1). Thus, contrast agents with long-lived singlet state lifetime offers great potential for the development of magnetic resonance imaging.

Herein, two different molecules are presented to investigate the concept of photoactive molecules with nuclear spin singlet states[3]: firstly, a bimodal fluorescence/nuclear singlet state contrast agent, <sup>13</sup>C2-tetraphenylethylene, has a long-lived singlet state in organic solvent, while it shortens upon addition of water. Simultaneously, we found its fluorescence intensity enhances greatly due to the aggregation-induced emission (AIE) effect. Secondly, we are demonstrating a concept that nuclear spin singlet states can be populated in a bimolecular photoswitch, which is based on the 3-2H-coumarin. In the monomeric form, a singlet state cannot be populated due to the existence of an isolated proton. Upon UV-light exposure a dimer forms and a coupling in the dimer between two previously isolated protons is generated and then nuclear spin singlet state can be populated. Excitation with a wavelength of 254 nm results in partial ring cleavage of the molecule back to its monomer. These studies will pave a way to design new contrast agents for the study of monitoring bio-distribution of photo-pharmaceuticals.

**References:** [1] A. A. Beharry, G. A. Woolley, (2011) Chem. Soc. Rev. 40, 4422-443. [2] M. H. Levitt, (2012) Annu. Rev. Phys. Chem. 63, 89-105. [3] S. Yang, J. McCormick, S. Mamone, L.-S. Bouchard, S. Glöggler, (2019) Angew. Chem. Int. Ed. 58, 2879-2883.

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Solution Heteronuclear NMR Spectroscopy of 15N-enriched Proteins Encapsulated in Lipidic Cubic Phase

Thomas Meikle<sup>1</sup>, Ashish Sethi<sup>2</sup>, David Keizer<sup>2</sup>, Jeffrey Babon<sup>3</sup>, Frances Separovic<sup>2</sup>, Paul Gooley<sup>2</sup>, Charlotte Conn<sup>1</sup>, Shenggen Yao<sup>\*,2</sup>

<sup>1</sup>RMIT University, <sup>2</sup>The University of Melbourne,

<sup>3</sup>The Walter & Eliza Hall Institute of Medical Research

Lipidic cubic phases, which form spontaneously via the self-assembly of certain lipids in an aqueous environment, are highly prospective nanomaterials with applications in membrane protein X-ray crystallography and drug delivery. We report 1H-15N heteronuclear single/multiple quantum coherence (HSQC, HMQC) spectra of 15N enriched proteins encapsulated in lipidic bicontinuous cubic phases obtained on a standard commercial high resolution NMR spectrometer at ambient temperature. Features of 15N-enriched proteins encapsulated in lipidic cubic phase, including: (i) significantly reduced solvent chemical exchange of backbone amides, and (ii) additional spectral sensitivity gain with the use of BEST RF sequence, will be discussed.

Lipid cubic phases could also be used to study the hydration of encapsulated proteins. The importance of interactions between water and biomolecules in protein-protein interactions and subsequent functions in biological systems are well recognized [1]. However, experimental quantification of such protein hydration dynamics, at least at the atomic level, remains poorly characterized, largely due to limited resources available for such studies. Solution NMR has been long considered as a promising means for gaining insight into site-specific protein hydration dynamics via measurement of the nuclear Overhauser effect (NOE) [2]. Quantification of protein hydration dynamics in aqueous solution has proven to be challenging due largely to the presence of excessive bulk water. Reduced solvent chemical exchange of backbone amides for proteins encapsulated in lipidic cubic phases potentially provides a novel alternative, for the quantification of residue-specific hydration dynamics of proteins by NMR, to reverse micelles [3] which involve the use of elevated pressure for protein encapsulation and dedicated low-viscosity fluids for sample preparation.

**References:** [1] B. Halle, Philos T R Soc B 2004, 359, 1207-1223. [2] G. Otting, E. Liepinsh, K. Wuthrich, Science 1991, 254, 974-980. [3] N. V. Nucci, M. S. Pometun, A. J. Wand, Nat Struct Mol Biol 2011, 18, 245-249.

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P516

Identifying Spin-Dependent Recombination and Hopping Processes in Organic Solar Cells  
Ula Yasin\*, Felix Kraffert, Jan Behrends

Berlin Joint EPR Laboratory, Fachbereich Physik, Freie Universität Berlin

With the recent record power conversion efficiency of 17.3% [1], organic solar cells have become serious contenders among third generation photovoltaics. This success is related amongst other things to efforts in overcoming the drawbacks of organic semiconductors, such as their low exciton-dissociation yield and charge-carrier mobility, as they limit device performance.

Studying light-induced charge-carrier dynamics under working conditions can help develop new ideas for improving device operation.

As the transport in organic semiconductors occurs by hopping of charge carriers from and to localized states, the spin degree of freedom plays a role in the allowed hops according to the Pauli principle. Thus the spin provides a probe for the identification of transport-limiting processes and the type of spin species they involve. At this stage, the spin-sensitive techniques Electron Paramagnetic Resonance (EPR) and Electrically Detected Magnetic Resonance (EDMR) spectroscopy become conducive. The detection of light-induced EPR signals in a time-resolved mode allows us to follow the evolution of photo-generated charge carriers. By simultaneously measuring time-resolved EDMR signals, the contribution of the detected spin species to the photocurrent is elucidated. This enables us to correlate the state after photo-excitation, being a charge-transfer state or free charge carriers, and its influence on the photocurrent. Furthermore, we show that the biasing conditions (charge-carrier injection or extraction) directly affect the dynamics of the current-influencing paramagnetic species.

We present results obtained from bias-dependent transient (tr)EPR and trEDMR measurements on poly(4,4-diocetylthieno(3,2-b:2',3'-d)silole)-2,6-diyl-alt-(2,1,3-benzothiadiazole)-4,7-diyl (PSBTBT-8) blended with [6,6]-phenyl-C61-butyric acid methyl ester (PCBM) bulk-heterojunction solar cells and show that the resonant signals observed at low-temperature, attributed to positive polarons in the polymer and negative polarons in the fullerene phase, are involved in different spin-dependent processes. We will report on the possible spin-dependent mechanisms the polarons undergo at specific times after optical excitation.

Reference: [1] L. Meng et al., Science, 2018, 361, 1094.

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## P517

**Probing the NBD-TMD cross-talk of the bacterial ABC transporter MsbA by solid-state NMR**

Phoebe (Sin Yeng) Ye<sup>\*1</sup>, Hundee Kaur<sup>2</sup>, Johanna Becker-Baldus<sup>1</sup>,  
Andrea Lakatos-Karoly, Roberta Spadaccini, Clemens Glaubitz<sup>1</sup>

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ATP-binding cassette (ABC) transporters regulate the human biological systems through the trafficking of endogenous components (e.g. lipids, vitamins, steroids, and metabolites) and exogenous compounds (e.g. drugs). They are known for their critical role in multidrug resistance (MDR) in human cell derivatives and diseases (e.g. cancer, Alzheimer's disease, atherosclerosis), as well as in the antibiotic resistance of bacteria, made possible by their highly abundant distribution across different species.

The bacterial homologue MsbA is an excellent candidate to study the structural importance of this protein family. Its well-known MDR behaviour to export amphiphilic substrates across the membrane in Gram-negative bacteria, is mediated by ATP-hydrolysis and thereby coupled to a catalytic cycle in the nucleotide binding domains (NBDs). However, the cross-talk between the NBDs and the transmembrane domains (TMDs) during substrate translocation remains unresolved. It is suggested that the coupling helices (CHs) located between the NBD and TMD are responsible for this process. To this end, special attention is devoted to the coupling helices of MsbA with the aim to understand the NBD-TMD cross-talk and to shed light upon the structural conformation and dynamics.

Previous studies by solid-state NMR from our lab focussed on the catalytic cycle by real-time <sup>31</sup>P MAS NMR and DNP revealing a so far unexplored reverse adenylate kinase mechanism connected with ATP hydrolysis (Kaur et al Nat Commun. 2016, Kaur et al. J. Am. Chem. Soc. 2018). Here, we use multidimensional <sup>13</sup>C-<sup>15</sup>N MAS-NMR to explore conformational changes within the MsbA coupling helices during the catalytic cycle by trapping intermediate states (i.e. apo state, ADP·BeF<sub>3</sub> emulated prehydrolysis state, ADP·VO<sub>4</sub> emulated transition state, ADP·VO<sub>4</sub> + ADP·βS emulated ATP-ADP bound state, ADP·VO<sub>4</sub> + AMP emulated ATP-AMP bound state), in absence and in presence of substrate.

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Metabolic Profiling of Complex II-Energized Mitochondria

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University of Iowa

We recently reported a previously unrecognized mitochondrial respiratory phenomenon. When ADP concentration was held constant (“clamped”) at sequentially increasing concentrations in succinate-energized muscle mitochondria in the absence of rotenone (commonly used to block complex I), we observed a biphasic, increasing and then decreasing, respiratory response, while membrane potential ( $\Delta\psi$ ) continues to decrease with increasing ADP levels.

Here we investigated the mechanism involved in this response by using NMR spectroscopy to determine the metabolic profile through assessing oxaloacetate (OAA), malate, fumarate, and citrate concentrations in isolated succinate-respiring mitochondria. When these mitochondria were incubated at varying ADP concentrations, we found that a decrease in respiration at high ADP levels was associated with OAA accumulation. Moreover, a low pyruvate concentration, that alone was not sufficient to drive respiration, could help metabolize OAA to citrate and completely reverse the loss of succinate-supported respiration at high ADP concentrations. Furthermore, chemical or genetic inhibition of pyruvate uptake prevented OAA clearance and preservation of respiration. These results, along with measures of mitochondrial inner membrane potential ( $\Delta\psi$ ), NADH/NAD<sup>+</sup>, superoxide, and H<sub>2</sub>O<sub>2</sub> (a marker of reverse electron transport from complex II to I), supported our proposed mechanism that involves the interplay of these measured parameters in an ADP concentration-dependent manner.

In summary, we have shown that succinate-energized respiration is initially increased by ADP concentration-dependent effects on membrane potential, but then decreased at higher ADP levels by OAA-mediated inhibition of succinate dehydrogenase.

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## P519

## Methyl-bearing side-chain motions at pico- to nanosecond timescales

Milan Zachrdla<sup>\*,1</sup>, Pavel Kadeřávek<sup>2</sup>, Nicolas Bolik-Coulon<sup>1</sup>, Philippe Pelupessy<sup>3</sup>,  
Guillaume Bouvignies<sup>3</sup>, Thorsten Marquardsen<sup>4</sup>, Jean-Max Tyburn<sup>5</sup>, Damien Laage<sup>1</sup>,  
Guillaume Stirnemann<sup>6</sup>, Fabio Sterpone<sup>6</sup>, Fabien Ferrage<sup>1</sup>

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Protein side-chain motions are essential for protein function. Methyl-bearing side chains have proven to be very useful probes of molecular motions [1]. Recently, we have combined high-resolution relaxometry and molecular dynamics simulations to study motions of isoleucine side chains with relaxation measurement of carbon-13 of  $\delta_1$  methyl groups [2]. This novel approach gives us access to hitherto inaccessible slow nanosecond motions.

In this study, we further increase the number of probes with specific  $^{13}\text{C}^2\text{H}_2^1\text{H}$  labelling of Leu<sup>proS</sup> and Val<sup>proS</sup> methyl groups in ubiquitin. Twenty methyl groups in total enable us to probe most of the protein core as well as parts of the protein surface.

Using high-resolution relaxometry, we acquired relaxation rates at twelve magnetic fields from 8 T down to 0.33 T. These rates were complemented by standard high-field relaxation rates measured at 11.7 T, 14.1 T, 18.8 T, and 22.3 T. Influence of cross-relaxation, effective when the sample is outside the high-field probe, was taken into account by simulating the experiments at every step of the fit of local dynamics parameters using the newly developed program MINOTAUR. We identified several residues that undergo slow chemical exchange that would otherwise bias standard analysis of high-field relaxation only [2].

Overall, we provide an extensive description of fast side-chain motions, ranging from few picoseconds to a few nanoseconds.

**References:** [1] Kasinath V, et al., *J. Am. Chem. Soc.*, 2013, 135, 40, 15092-15100 [2] Cousin SF, et al., *J. Am. Chem. Soc.*, 2018, 140, 41, 13456-13465

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MAGNETIC RESONANCE THERMOMETRY OF ADIPOSE TISSUE  
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Joshua Stoll<sup>2</sup>, Zbigniew Celinski<sup>2</sup>, Janusz Hankiewicz<sup>2</sup>, Stefan Jurga<sup>1</sup>  
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Temperature monitoring is critical in MRI guided ablations to deliver necessary thermal energy to kill tumor cells while preventing damage to healthy adjacent organ tissue. The most popular tMRI technique, proton resonance frequency (PRF) based on the 0.01 ppm/°C change in chemical shift of protons in water, fails in areas near or within adipose tissue. A minimally invasive MRI thermometry (tMRI) that produces high thermal, spatial and temporal resolution temperature maps superimposed on anatomical images within the targeted organ would address these efficiency and safety requirements.

In this project, we explore a novel contrast agent for temperature measurement using MRI in fat or in tissues with significant fat content. We hypothesize that magnetic particles embedded in the fatty tissue create a local dipole magnetic field that deteriorate the homogeneity of the main static magnetic field of the MRI scanner, shortening the nuclear  $T_2^*$  relaxation time of protons in the fat. Consequently, MR images weighted by  $T_2^*$  should exhibit temperature dependent variations in intensity that can be correlated with temperature.

Measurements of relaxation times of phantoms embedded with different concentrations of ferrite particles in field 14.1T allowed us to determine the temperature dependence of PRF shift and relaxivities ( $R_1$ ,  $R_2$  and  $R_2^*$ ) between 5-45 °C. Analysis of acyl-chain methylene protons line at 1.5 ppm confirmed only a weak PRF thermal shift (0.003 ppm/°C). While  $R_1$  and  $R_2$  in pure fat and fat doped with particles exhibit the same thermal dependence,  $R_2^*$  drops significantly due to a decrease of particles' magnetization with temperature.  $R_2^*$  results are correlated with intensities of images obtained with gradient echo  $T_2^*$  weighted MRI at 9.4 T.

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### Relaxation properties of theranostic liposomes prepared by microfluidic and thin lipid film hydration method

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Objective of the study was to compare relaxation properties of theranostic paramagnetic liposomes prepared by two distinct preparation methods, that is thin lipid hydration (TLH) and microfluidic method (MM). Liposomal formulations were prepared with Zn-Phthalocyanine (ZnPc) a highly hydrophobic model photosensitizer, incorporated within lipid membrane with or without PEG moieties, composed of POPC, PG and a lipid bearing head group modified with MRI contrast agent, PE-DTPA(Gd). Liposomes were obtained by lipid film hydration followed by filter-extrusion or microfluidic method under various flow parameters. Content of ZnPc and PE-DTPA(Gd) in vesicles was determined by UV/vis spectrophotometry and NMR spectroscopy, respectively. Moreover liposome size was determined by dynamic light scattering showing the vesicles diameters of 30 nm and 60 nm prepared by MM and TLH method respectively. NMR relaxivity parameters  $r_1$  and  $r_2$  measured for different liposomes were obtained at low (16.5 MHz) and high (400 MHz) magnetic fields at room temperature and at 37 °C. In both cases,  $T_1$  and  $T_2$  proton's relaxation times measured for dispersion of liposomes (obtained by different methods) in saline solution showed influence of preparation methods on the content of PE-DTPA(Gd) incorporated into liposomes, vesicles size and consequently on NMR relaxation processes of water protons.

#### Acknowledgments

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Enhancing resolution in NMR spectroscopy by chemical shift upscaling

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Nuclear magnetic resonance (NMR) spectroscopy serves as a versatile analytical tool. Resolution is an essential challenge in NMR spectroscopy. Due to the limited range of chemical shifts and extensive signal splittings due to scalar couplings, signal congestion and even overlap are common in NMR spectra, especially in the widely used proton spectra. One major approach to enhance spectral resolution lies in increasing magnetic field strength. At present, the highest magnetic field strength used in commercial NMR spectrometers corresponds to proton resonance frequency of ~1 GHz. The higher the magnetic field strength is, the harder it is for it to increase.

A number of experimental methods and data processing approaches have been developed to improve spectral resolution in NMR spectroscopy. Among them, pure shift technique is one of the most promising approaches. Multiplets are collapsed into singlets through homodecoupling and spectral congestion is greatly alleviated. However, pure shift is usually achieved at the cost of reduced sensitivity. Another drawback is that scalar coupling information is removed in pure shift spectra.

Here, we propose a chemical shift upscaling method to enhance spectral resolution. A pure shift evolution period is added in the indirect dimension to enhance chemical shift evolution. Signals are rearranged by data chunking as used in pure shift experiments. Resulting spectra are characterized by enlarged chemical shifts which are typically available in much higher magnetic field strength. Chemical shifts are amplified by a factor of  $\lambda$ . With upscaled chemical shift, signal dispersion is improved and hence it is possible to separate overlapped signals. In chemical shift upscaled spectra, patterns of these multiplets can be unraveled and integral of each multiplet can be easily obtained for quantitative analysis.

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## P523

## Interpretation of NMR relaxation data of flexible molecules via a hybrid approach

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Slowly relaxing molecular motions, from nanoseconds up, strongly affect molecular properties, even modifying their chemical functions. Nuclear magnetic relaxation (NMR) data is sensitive to these time-scales and therefore its interpretation provides information on the structural and dynamics characteristics of molecules.

Simple approaches allow extracting information easily for rigid molecules from NMR relaxation experiments. Complex methods are required for flexible molecules, if decoupling of global and local motion is not invoked. Molecular dynamics (MD) simulations are used to simulate dynamic observables (i.e. spectral densities) from which NMR data are evaluated. However, efficient sampling of long MD runs is an important, somewhat neglected, issue.

We propose a hybrid approach based on a partition of molecular degrees of freedom (dof) into two sets: one directly entering the spectral densities from which the NMR data are calculated (relevant dof) and another indirectly coupled (irrelevant dof) [1]. Short (a few ns) standard or biased MD simulations are employed to evaluate the dependence of free energy surfaces from relevant dof. Hydrodynamic methods are then employed to evaluate dissipative properties of the system. Finally, a Fokker-Planck equation, from which the irrelevant dof have been projected out, is employed to describe a stochastic long time dynamics in the relevant dof only.

Here, we apply the approach to interpret NMR relaxation data of oligosaccharides [2]. Global/internal dynamics decoupling, comparison with standard MD simulations, and predictivity are discussed. New perspectives are then introduced about a generalized protocol for macromolecules with a large number of degrees of freedom obtained from rigorous, first-principles treatment [3].

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Investigating the RNA unwinding mechanism of  
DEAD-Box helicase RhlB with NMR Spectroscopy  
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RNA helicases are enzymes that unwind double stranded RNA in an ATP dependent reaction. They have shown to be important players in RNA metabolism in both eukaryotes and prokaryotes. RhlB is a DEAD-Box RNA helicase from E.coli. It constitutes an essential part of the RNA catabolizing enzyme complex degradosome. Its ATP dependent unwinding reaction, which features a strong bend in the RNA, is stimulated by the presence of the interaction partner RNase E. Since it is still unknown, how the allosteric binding of RNase E to RhlB leads to a boost in both ATP hydrolysis and RNA unwinding activity of the helicase, we are using a wide range of NMR techniques to both map the RNAs conformational landscape and ATP turnover during the reaction cycle. This allows us to decipher how different domains of RNase E contribute to individual steps in the unwinding mechanism.

Here, we show how by the use of high-resolution experiments it is possible to decipher RhlB's preferences of RNA binding and the according changes in conformational dynamics. Further, we present <sup>31</sup>P real-time NMR data that were recorded by the application of in situ mixing techniques. The obtained structural as well as kinetic information revealed how RNase E works as both an allosteric activator as well as a product trap. In particular, we are able to demonstrate that RNase E increases RhlB's affinity for RNA substrates by locking the helicase into a more binding competent conformation and what effect that has on the bound RNA substrate.

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## P525

**A general 2D J-resolved NMR method for high-resolution measurements on complex samples under inhomogeneous fields**

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2D J-resolved NMR spectroscopy, separating chemical shifts and J couplings along two independent dimensions, serves as a promising tool for studying molecular structures and compositions. In practice, the classic homo-nuclear 2D J-resolved spectroscopy [1], generally suffers from phase-twist lineshape and field inhomogeneity effects, thus rendering its potential applications limited. Here, a general NMR approach, termed as HRHC-2DJ (High-Resolution and High-Clean 2D J-resolved spectroscopy), is proposed to suppress these effects.

The HRHC-2DJ is designed via the combination of the Zangger-Sterk [2] and echo-train J-acquisition [3] modules, to deliver the orthogonal 2D J-resolved spectra. Then, the summation of the initial and its F1-reversed spectra is preformed to remove dispersive components. The HRHC-2DJ remains insensitive to field inhomogeneity along the chemical-shift dimension because of the reduced effective sample volume per signal [4].

The HRHC-2DJ bypasses the field-inhomogeneity effect and recovers high-quality spectral information from inhomogeneous fields. The HRHC-2DJ naturally delivers orthogonal phase-sensitive spectra, free of distorted lineshapes stemming from the absolute-value mode and spectra shearing, thus offering satisfactory resolution enhancement. The HRHC-2DJ also awards a straightforward and high-resolution detection on heterogeneous tissues (e.g., table fruit and excised biological tissues). In conclusion, the HRHC-2DJ allows the high-resolution extraction of chemical shifts and J couplings on complex samples with crowded and overlapped resonances under inhomogeneous magnetic fields, and may be a promising choice for detecting heterogeneous biological tissues.

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Assessment of the Lung Compliance Changes caused by  
Fibrosis using Hyperpolarized Xenon MRI

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Pulmonary compliance is an important index for evaluating the function of the lung, which could reflect the pulmonary ability to stretch and expand. Hyperpolarized  $^{129}\text{Xe}$  MR is a powerful tool in quantifying the microstructure and function of the lung in vivo, and it has unique advantages in measuring the gas volume in the lung. In this study, a proof-of-concept method was proposed to measure lung compliance via  $^{129}\text{Xe}$  ventilation MRI, and the changes in lung compliance caused by fibrosis were quantitatively evaluated.

Ten male Sprague-Dawley rats were divided into two groups ( $n = 5$ ) randomly. The experimental group was treated with 0.4 ml solution of bleomycin (2.5 U/kg body weight), while the control group treated with an equivalent amount (0.4 ml) of normal saline. Pulmonary function tests and MRI experiments were performed on all the rats. Hyperpolarized  $^{129}\text{Xe}$  ventilation images with different lung pressure were acquired using multi-slice FLASH sequence, and the lung compliance was determined as the slope of the pressure-volume curve.

A significant difference ( $p < 0.001$ ) was found in the measured quasi-static compliance between the healthy rats and fibrosis rats, and the mean values of measured lung compliance were  $1.27 \pm 0.15$  and  $0.67 \pm 0.12$  ml/cm  $\text{H}_2\text{O}$ , respectively. Additionally, the lung compliance derived from xenon MRI was found decreased obviously ( $p = 0.003$ ) from  $0.40 \pm 0.05$  ml/cm  $\text{H}_2\text{O}$  to  $0.25 \pm 0.06$  ml/cm  $\text{H}_2\text{O}$  in fibrosis rats. The lung compliance measured by two different methods has a strong correlation ( $r = 0.89$ ,  $p = 0.001$ ).

Our study demonstrated the feasibility of hyperpolarized  $^{129}\text{Xe}$  ventilation MRI in measuring lung compliance in vivo, which extends the potential applications of gas MRI in pulmonary functional evaluation.

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Quantitatively Evaluation of the Lung Injury Caused by  
PM2.5 using Hyperpolarized Gas Magnetic Resonance

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Fine particulate matter with an aerodynamic diameter less than 2.5  $\mu\text{M}$  ( $\text{PM}_{2.5}$ ) is one of the most important air contaminants. The exposure to  $\text{PM}_{2.5}$  could cause pulmonary oxidative stress, inflammation, and thickening of the alveolar wall. The conventional methods including bronchoalveolar lavage fluid (BALF), synchrotron radiation X-ray fluorescence, and histopathological sections, are difficult to evaluate pulmonary gas exchange function in the early stage of lung injury caused by  $\text{PM}_{2.5}$ . In this study, we tried to demonstrate the feasibility of hyperpolarized  $^{129}\text{Xe}$  MR in quantifying the lung structure and functional changes caused by  $\text{PM}_{2.5}$  exposure in vivo.

$\text{PM}_{2.5}$  sample was collected on quartz fiber filters from the air, then extracted and suspended in normal saline. Twelve Sprague-Dawley rats were randomly divided into two groups, experimental group (PM group,  $n = 6$ ) treated with  $\text{PM}_{2.5}$  by intratracheal instillation, and control group (NS group,  $n = 6$ ) received an equivalent volume of normal saline. Hyperpolarized  $^{129}\text{Xe}$  experiments were performed on the Bruker 7 T animal MRI scanner. Chemical shift saturation recovery (CSSR) and multi-b-value diffusion-weighted imaging (DWI) were utilized to obtain quantitative function and structural parameters of the lung.

The exchange time constant (T) and septal wall thickness (d) derived from CSSR data were found increased significantly in PM cohort, while the physiological parameters extracted from DWI data have no obvious difference. The possible reason is that  $\text{PM}_{2.5}$  animal model in our study is mild and the lung injury caused by  $\text{PM}_{2.5}$  is in the very early stage, in which the structural changes are not detectable.

In this study, we demonstrated the feasibility of hyperpolarized xenon MR in evaluating the  $\text{PM}_{2.5}$  induced pulmonary physiological changes in the early stage, which may offer a new method for quantifying the lung injury caused by fine particulate matter.

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Large Conformation Rearrangement in Transmembrane Histidine Kinase Activation

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MPI-BPC

CitA is a citrate sensing, membrane-bound histidine kinase. Despite significant progress in characterizing the structural and binding properties of its periplasmic citrate binding domain (PASP)[1][2][3], the processes involved in transferring the citrate binding signal to the cytosolic domain (PASC) remain obscure. Crystal structures and COLD (Cryogenic Optical Localization in 3D)[4] measurements of functional point mutants of PASc show dramatic conformational changes. Here, by combining solution-state and magic angle spinning (MAS) NMR data, we propose a model for CitA signaling where the PASc undergoes an antiparallel to parallel dimer transition upon citrate binding. In solution, Chemical Exchange Saturation Transfer (CEST) and double-edited NOESY experiments proved the existence of sampling of different conformations in the isolated PASc. For probing the relevant signaling states of CitA, we used the CitApc fragment (34kDa), comprising PASp, the two transmembrane helices (TM1 and TM2) and the PASc. This allows investigation of the relevant signaling states of CitApc in liposome with <sup>1</sup>H-detected MAS NMR. Solvent paramagnetic relaxation enhancement (sPRE) and deuterium exchange experiments were conducted on the CitApc fragment to probe PASc structure and dynamics changes during citrate signaling. Together, we show that CitA PASc can adopt different structures that were previously not observed, and that these changes are relevant to the signaling mechanism in the context of the TM helices. This work offers insight into transmembrane signaling and shows how technical challenges have been overcome in studying membrane proteins larger than 30 kDa with MAS NMR.

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P529

15-N photo-CIDNP in the cysteine-lacking LOV domain of  
phototropin in solid-state NMR

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University Leipzig

Chemically induced dynamic nuclei polarization(CIDNP) is one of the hyperpolarization methods allowing to enhance sensitivity of NMR. The CIDNP effect, which occurs in photochemical reaction, is called photo-CIDNP effect. In this research flavin-binding LOV domain, which is a blue light receptor, can receive an electron from a short distance(appr. 11Å) tryptophan. This electron is the core of the photo-CIDNP effect. In the chromophore, FMN forms a covalent bond with a short distance cysteine after absorb a blue light photon and lost the electron absorptive capacity. Thus the cysteine was mutated to serine, which can not bond with FMN. And a glutamine is mutated to tryptophan, which provides electron. Both position are located near to FMN. Three sample(CS, QW, CSQW) was measured through 15-N solid-state NMR. The photo-CIDNP was observed on sample CSQW.

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## P530

**Parahydrogen-induced polarization with metal-free catalytic systems**Vladimir V. Zhivonitko<sup>\*1</sup>, Konstantin Chernichenko<sup>2</sup>, Kristina Sorochkina<sup>2</sup>,Jonas Bresien<sup>3</sup>, Ville-Veikko Telkki<sup>1</sup>, Axel Schulz<sup>4</sup>, Igor V. Koptug<sup>5</sup>, Timo Repo<sup>2</sup><sup>1</sup>NMR Research Unit, University of Oulu, <sup>2</sup>Department of Chemistry,University of Helsinki, <sup>3</sup>Institute of Chemistry, University of Rostock, <sup>4</sup>Leibniz-Institut für  
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Chemical activation of parahydrogen molecules plays a key role in parahydrogen-based hyperpolarization techniques. Commonly, metal-containing activators/catalysts (complexes, nanoparticles, oxides) are employed to mediate such activations and produce hyperpolarization in parahydrogen-induced polarization (PHIP) as well as in signal amplification by reversible exchange (SABRE). Signal enhancements provided by the hyperpolarization in homogeneous and heterogeneous chemical processes has been used for boosting sensitivity in NMR, metabolic imaging and mechanistic studies of chemical reactions. At the same time, more biogenic main group chemical systems have been documented as efficient metal-free activators for molecular hydrogen. Herein, an overview of current results about PHIP and SABRE with metal-free systems is presented.[1-5]

We show that unimolecular pairs of sterically separated ('frustrated') Lewis acids and bases (FLPs) are promising metal-free parahydrogen activators that provide hyperpolarization of protons and heteronuclei in FLP-H<sub>2</sub> adducts and in free FLP molecules. We present results on efficient N-B based FLP systems and P-P biradicaloids.[1-5] Role of kinetic parameters, dihydrogen bonding and structural features are discussed in the context of the hyperpolarization effects. We also present new alternative P-B pair based metal-free systems for PHIP and SABRE.

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**Influence of the N-terminal domain on dynamic processes and structural stability of the human prion protein**

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The prion protein (PrP) is responsible for several neuropathies belonged to Transmissible spongiform encephalopathies (TSE). The TSE connected with changes of the PrP structure from nontoxic cellular (PrPC) to protease resisted (PrPSc) forms. The transition from PrPC to PrPSc provide to formation fibrils contained  $\beta$ -strands conformation. The cellular form of the PrP protein demonstrated two domains where the C-terminal domain (residues 125–231) presented as three  $\alpha$ -helices together with two short antiparallel  $\beta$ -strands. The N-terminal segment (residues 23–124) is highly disorder. The influence of N-terminal domain on of molecular dynamic processes were reported, but still not understood in detail [1].

In our study we perform analysis of molecular dynamic processes in two constructs of human PrPC (huPrPC) protein. The information about influence N-terminal domain to the structure and dynamics of wild-type huPrPC we analyzing <sup>15</sup>N relaxation data (R1, R2, 1H-<sup>15</sup>N NOE and Relaxation Dispersion) recorded for 90-231 and 23-231 variants of huPrPC at various temperatures using Reduce Spectral Density Mapping approach [2]. The existence long nonstructural domain on N-termini of huPrPC substantially decrease the translational and rotation diffusion in solution. So, recorded <sup>15</sup>N relaxation data were supplemented by DOSY experiments performed at various conditions. The obtained results were compared with conformational alterations in folded C-terminal domain observed at various pH. The <sup>15</sup>N relaxation measurements were performed at two magnetic fields – 14.7 and 18.8 T – on Agilent DDR2 600 and DDR2 800 NMR spectrometers installed at NanoBioMedical Centre (Poznan, Poland).

**Acknowledgments**

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## P532

**Elucidation of the exchange interaction sign in rigid D-X-A dyads:  
dominance of minor channel in CIDNP formation**

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Results of CIDNP studies of a series of rigidly linked electron donor/electron acceptor dyads (D-X-A) are presented consisting of triarylamine (TAA) and naphthalene diimide (NDI) moieties with spacers X. After photoexcitation charge separation occurs, where the TAA unit serves as electron donor and the NDI unit as the acceptor. Variation of the magnetic field allowed us to obtain a pronounced maximum of CIDNP showing enhanced absorption for all nuclei in the NMR spectrum disregarding the sign of HFCs in the transient biradicals. Since the total spin z-projection is conserved in singlet-triplet transitions, the nuclear polarization sign allows to determine the sign of exchange interaction J when the multiplicity of the precursor is known. Considering that the biradicals are formed predominantly in their singlet state[1], the observed enhanced absorption would correspond to  $S\beta \rightarrow T\alpha$  transitions and a negative sign of J. However, the predicted CIDNP enhancement for such a mechanism appeared to be much smaller than the experimentally detected one. Moreover, it was shown<sup>1</sup> that there is no effective triplet state decay process except triplet  $\rightarrow$  singlet interconversion for these dyads. In this case, the net nuclear polarization is proportional to the fraction of biradicals which are formed in the triplet state, even if one is much lower than that of the singlet state. Therefore, the nuclear polarization is determined by the minor ( $T\beta \rightarrow S\alpha$ ) transition channel corresponding to a positive sign of J. A theoretical approach combining semiclassical and quantum mechanical treatment for the simulation of <sup>1</sup>H and <sup>13</sup>C CIDNP field dependences has been developed. It enables to model the electron spin dynamics in the presence of up to 24 magnetic nuclei, allowing to determine the isotropic HFC values for all <sup>1</sup>H and <sup>13</sup>C nuclei and to estimate the anisotropy of <sup>13</sup>C HFC.

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## P533

**Analysis molecular dynamic processes on base  
15N relaxation data sets acquired at one magnetic field.**

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Recently, widespread N6-methyladenosine (m6A) modification of messenger RNA (mRNA) and non-coding RNA was discovered in yeast, flies, mammals and plants [1]. At the functional level m6A plays an important role in many biological processes [2]. In mammals two methyltransferases: METTL3 and METTL14, together with the adaptor protein WTAP are required for m6A methylation [3]. In plants MTA and FIP37, identified as homologs of METTL3 and WTAP, respectively [3].

Our studies were focus on 120 a.a. fragment of MTA protein. To the time being, we solve the high-resolution 3D structure (will be published elsewhere). The analyzed domain exhibit folded in a very stable structure which gave us possibility to acquire high quality 15N relaxation data using Varian Inova 500 NMR spectrometer. Usually collected R1, R2 and 1H-15N NOE data were supplemented measurements R1rho (with adiabatic ramp) [4] and DD/CSA cross-relaxation rates [5]. In our poster we will present the analysis molecular dynamic processes in MTA fragment on base five independent experimental data measured at one (11.7 T) magnetic field according ModelFree approach.

#### Acknowledgments

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NMR benchmarking of force fields for proteins containing structured and intrinsically disordered regions

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NMR spectroscopy and molecular dynamics are ideal tools to investigate intrinsically disordered proteins (IDPs), difficult to study by other structural methods. Recent literature documents that force-fields developed for well ordered proteins fail to simulate molecular dynamics of intrinsically disordered proteins (IDPs) realistically. In particular, parameters of the water model need to be modified in order to improve applicability of the force fields to both ordered and disordered proteins. Development of new force fields relies on thorough benchmarking. NMR is very well suited for this purpose. Here, we present comparison of performance of force fields recommended for IDPs in MD simulations of three proteins differing in the content of ordered and disordered regions: Two tested proteins (delta subunit of bacterial RNA polymerase and regulatory domain of human tyrosine hydroxylase) consisted of a well-structured domain and of a disordered region with and without a transient helical motif, one protein (fragment of microtubule associated protein 2c) was disordered but contained a region of increased helical propensity. The obtained molecular dynamics trajectories were used to predict measurable NMR parameters including chemical shifts, residual dipolar couplings, paramagnetic relaxation enhancement, and N-15 relaxation data. Comparison of the simulated and experimental data showed that the NMR relaxation parameters, rarely used for benchmarking, are particularly sensitive to the choice of force field parameters, especially of those defining the water model. Therefore, the benchmarking protocol used in our study, being more sensitive to the imperfections than the commonly used tests, is well suited to evaluate performance of newly developed force fields. This work was supported by the Czech Science Foundation grant No. 19-12956S.

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Structural Model of the Tail-Tube of the  
Bacteriophage SPP1 and its decorating FN3 Domain

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Bacteriophages are re-attracting interest as tools for combating pathogenic bacteria as anti-  
biotics increasingly face the fate of resistance. The bacteriophage SPP1 belongs to the family  
of *Siphoviridae*, of which, to date, no tail assembly atomic structure is known. Viral tail  
structures play important roles in the infection process, since they conduct DNA transport  
into the host cell. Here we present a structural model of the major tail protein gp17.1 within  
the tube assembly of SPP1. The hybrid structure is based on the integration of structural  
restraints from solid-state NMR, such as torsion angles and long-distance restraints, and an  
electron density map from single-particle cryo EM, which on its own does not suffice for  
modeling. The structure features hexameric rings that form a  $\beta$ -barrel motif. These rings  
are stacked onto each other and interconnected by linker regions creating a hollow tube.  
Relaxation measurements suggest that the tail fiber displays a vast dynamic landscape,  
which might explain the low resolved cryo EM maps. This is a major difference to other  
phage tail-systems that show rigid, readily “cryo EM-accessible” structures. Additionally,  
we present the solution NMR structure of a decorating FN3 domain. These proteins are  
found on the tail surface *in vivo* and facilitate the adsorption of the phage particle to the  
cell wall of the host.

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Investigating mRNA-ribosome complexes during translation initiation

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A large ribonucleoprotein complex called the ribosome is responsible for several steps of protein synthesis in all organisms. In bacteria, regulation of translation begins at initiation. Despite the availability of structural information, we still lack a clear understanding of how the ribosome encounters folded mRNA structures during translation initiation.

A general mechanism for initiation could involve a ribosome standby binding to single-stranded regions of the mRNA in the vicinity of the ribosome binding site, representing the first docking step and thus providing a general mechanism for initiation. This standby model describes the paradox of high translation rates from highly structured mRNAs with a sequestered RBS. Until now, little attention has been paid to standby complexes, particularly those of structured mRNAs.

Riboswitches are structured cis-acting RNA elements located in the 5'-untranslated region of mRNA that regulate gene-expression at the level of transcription, RNA cleavage and translation in response to binding of a cognate ligand.

The direct regulation by riboswitches operates by interfering with the formation of translation initiation complexes.

However, a structural description of the interaction between riboswitches and ribosomes during translation initiation is currently missing. It therefore has remained elusive how further structured elements can be fully accommodated within the initiation complex. We investigate the influence of the 30S ribosome on the function of the adenine-sensing riboswitch from *Vibrio vulnificus* by NMR spectroscopy. Surprisingly, the adenine-induced allosteric switch leading to an opening of the RBS is insufficient for efficient translation initiation. Additional stable structured elements around the initiation region prevent mRNA accommodation in the ribosome decoding channel.

Our results show that the full activity of the riboswitch is only obtained upon concerted interaction with adenine and ribosomal proteins. The RNA chaperone activity of the ribosomal protein S1 is needed for melting of secondary structures that would otherwise preclude complex formation.

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ssNake; A cross-platform open-source NMR data processing and fitting application  
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For the processing and analysis of solid-state NMR data or unconventional experiments, only a limited number of up to date processing and simulation software packages are available. Therefore, we have developed ssNake\*, an NMR processing program which provides both interactive and script-based processing tools. ssNake is mainly aimed at solid-state NMR experiments, but can also be used for liquid-state experiments. It can read various data formats, including those from all major spectrometer vendors. ssNake offers a wide variety of processing tools, from basic processing involving zerofilling, apodization, etc. to advanced operations such as NUS processing, linear prediction, and hypercomplex processing. It also provides extensive fitting capabilities, which can be used for spectral deconvolution and analysis of line shapes. ssNake provides the rather unique feature of being able to fit multiple spectra (or curves) simultaneously, where some or all of the fitting parameters are shared. This method can be used, for example, to fit spectra of quadrupolar nuclei at various magnetic fields simultaneously. This makes it possible to accurately determine quadrupolar and chemical shift parameters. ssNake furthermore provides a convenient method for fitting data using external simulation packages, such as SIMPSON. This makes fitting very versatile, as it brings together experimental data and simulation software. ssNake is freely available under the GNU General Public License (<https://www.ru.nl/science/magneticresonance/software/ssnake/>)

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**Rapid-melt DNP; Solid-state DNP enhancements for liquid-state multidimensional and heteronuclear NMR experiments**

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Over the years Dynamic Nuclear Polarization (DNP) has developed into a powerful method for sensitivity enhancement of NMR. At high magnetic fields, DNP in the solid-state is particularly effective. This has led to the successful implementation of cryo-MAS DNP for studying proteins and materials and dissolution DNP in a clinical setting. However, the application of DNP to chemical analysis of complex mixtures is so far limited. In our lab a 400 MHz Rapid-melt DNP probe has been developed in which solid-state enhancements can be transferred to the liquid state. The probe uses a stripline NMR detector, which detects signals from hundreds of nanoliter sample volumes contained in a fused silica capillary. This capillary can travel to different positions in the probe using a linear motor. One region contains liquid nitrogen for freezing the sample containing stable radicals while simultaneously irradiating it with microwaves. After the spin system has been hyperpolarized, the sample is shuttled to a heating region, melting the sample within 100 ms due to its small volume. Finally, the sample is moved up to the stripline detector, where a high-resolution liquid-state spectrum is recorded of the hyperpolarized sample. Since the sample composition does not change during the experiment, it is possible to repeat this cycle of DNP, melting, and NMR detection. This makes signal averaging and/or the recording of multidimensional experiments possible with high sensitivity. The time required for a single cycle is of the order of seconds. The stripline detector of the probe is double-resonant for protons and carbons, which allows for heteronuclear experiments. The capillary has been constructed in such a way that it can be used for stopped-flow experiments. All these features make Rapid-Melt DNP a powerful and versatile method for recording liquid-state NMR spectra with high sensitivity.

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The conduction pathway of potassium channels is water-free under physiological conditions

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Potassium (K<sup>+</sup>) selective channels allow for the selective and efficient conduction of K<sup>+</sup> ions across the cell membrane. They are involved in numerous physiological and pathophysiological processes. K<sup>+</sup> channels have been characterized in great detail by numerous studies using electrophysiology, X-ray crystallography, molecular dynamics (MD) simulations, and spectroscopic approaches. A selectivity filter (SF), comprising four K<sup>+</sup> binding sites, is formed in the homo-tetrameric arrangement by segments of the four subunits with the signature sequence TVGYG. This narrow pore allows for high selectivity and near diffusion-limited conduction rates. Despite these achievements, some elementary properties of the K<sup>+</sup> channel remain under debate. E.g. it was proposed early on that water molecules are co-transported with K<sup>+</sup> ions and that the conduction through the SF occurs via alternating water molecules and ions in a mechanism referred to as ‘water-mediated’ knock-on. This had been the accepted model in the field until 2014 when a study appeared that suggested that ions are in direct contact with each other and that no water molecules are involved in the conduction process. The proposed ‘direct’ knock-on mechanism was first observed in computational electrophysiology simulations and furthermore supported by reanalysis of X-ray crystallographic data, indicating that also under crystallographic conditions, adjacent binding sites are simultaneously occupied by K<sup>+</sup> ions. Using proton detected solid-state NMR techniques tailored to characterize the interaction between water molecules and the ion channel in combination with MD simulations we show that the selectivity filter of a potassium channel is free of water under physiological conditions. Our results are fully consistent with the ‘direct’ knock-on mechanism of ion conduction but contradict the previously proposed ‘water-mediated’ knock-on mechanism.

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Dynamics of the phase-position ensemble in magnetic resonance

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We consider the behaviour of precessional phase carried by molecules of a diffusing specimen under magnetic fields typical of magnetic resonance experiments. A time evolution equation for the ensemble of particles is constructed, which treats the phase as well as the position of the molecules as random variables. We refer to the associated probability density function  $p(\vec{x}, \varphi | t)$  as the `_local phase distribution_` (LPD). It is shown to encode solutions to a `_family_` of Bloch-Torrey equations (BTE) for transverse magnetization density. This is a consequence of the fact that LPD is a more fundamental quantity from which magnetization density emerges upon averaging:  $m(\vec{x}, t) = \int_{\varphi}^d \backslash, e^{i\varphi} p(\vec{x}, \varphi | t)$ . In studying the dynamics of such systems, the present paradigm represents a conceptual advantage over the BTE, since the LPD is a true probability density subject to Markovian dynamics, rather than an aggregate magnetization density whose evolution is less intuitive. We work out the obvious special cases which admit an analytical solution; namely free homogeneous diffusion and diffusion inside harmonic confinement, both under uniform magnetic field gradient.

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# Optical detection of nuclear magnetization using nuclear spin-induced circular dichroism

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NMR signal is usually measured using induction in a coil around the sample. However, it has been shown that nuclear magnetization can also be detected using optical methods sensitive to the polarization of light [1]. Such methods might, for example, employ nuclear spin-induced circular dichroism (NSCD), one of new theoretically predicted nuclear magneto-optic effects [2].

In its essence, NSCD optically detects nuclear magnetization by observing its influence on the electron cloud during an electron excitation by a beam of light. As has been theoretically shown [2], NSCD arises due to presence of excited states and localized nuclear quantum mechanical operators. Thus, NSCD uniquely combines information about electron excitations with a highly localized properties originating from atomic nuclei, similarly to NMR.

We have investigated the underlying principles of NSCD by implementing a computational protocol based on quadratic response function formalism, which allows us to look at NSCD signal for different nuclei for each excitation [3]. The results show that a strong NSCD signal for a particular combination of nucleus and excitation can arise only if the nucleus is a part of the excited chromophore. This finding shows that NSCD could be used as an experimental tool for optical detection of nuclear magnetization, and that it offers insight into location and spatial extent of excited states within a molecule with the resolution down to that of individual atoms.

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# Approach to Fully Resolved Structure and Composition of Complex Lubricant Base Oil by Multidimensional NMR Spectroscopy – Molecular Refining

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The functions of lubricant base oil are largely correlated to its structure and composition, which are however very difficult to be measured simultaneously by current analytical methods. Mass spectroscopy could get quantitative group composition of base oil, but it is deficient in characterization of structures with branches. On the other hand, conventional NMR method is not only suffered from seriously overlapped signals, but also, it could not distinguish paraffins and cycloalkanes, the two important components in lubricant base oil.

In the present study, we report our progress approach to fully resolved structure and composition of a lubricant base oil by NMR. Mainly relying on a modified three-dimensional HSQC-TOCSY which significantly improves detection sensitivity and data quality, we could resolve most molecules in the lubricant oil by their different <sup>1</sup>H TOCSY signals. When a 10% (w/v) refined lubricant base oil was used, signals from six different molecules could be assigned, which accounted for 75% of the total carbons. When the concentration was increased to 30% (w/v), around 90% of the total carbons were determined. To our surprise, we could not find clear signal of cycloalkanes as suggested by mass spectroscopy. Evidences from experiments and databases indicated that the 3D NMR data could undoubtedly distinguish paraffins and cycloalkanes, raising a question about mass spectroscopy on the measurement of cycloalkanes by ASTM standard for lubricant base oil.

In our opinion, it is the first time that a three-dimensional NMR spectroscopy was used to resolve the structure and composition of mineral-based lubricant base oil, suggesting that molecular refining in petrochemical industry is highly possible. In addition, the present study also provides a NMR method dedicated to distinguishing paraffins and cycloalkanes.

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Combining NMR and integrative methods to study recognition of clustered RNA elements by the multidomain RNA-binding protein IMP3

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Recently it has emerged that multidomain RNA-binding proteins recognise their specific target sequences, based on a combinatorial code, that is central in providing specificity and avidity in vivo for a diverse range of natural sequences. However, how such multivalent interactions are achieved in the crowded intracellular environment has remained elusive as no established systematic approaches have been put forward. We focus on a prototypical multidomain RNA-binding protein, IMP3 (also called IGF2BP3), which contains six RNA-binding domains (RBDs): four KH and two RRM domains, to identify how combinatorial interactions can achieve both specificity and avidity.

Using NMR relaxation and titration experiments, SAXS data and homology modelling we identified that the RRM tandem domains adopt a compact arrangement, suggesting that RNA is directly recognized by RRM1, while the potential RNA binding surface of RRM2 contributes allosterically. Furthermore, show that the individual domains in the KH1-2 tandem have unique binding preference for CA-rich and GGC RNA motifs, respectively. Comprehensive analysis of SELEX data and additional biophysical experiments, reveal that the RNA-binding specificity of IMP3 comprising six RNA binding domains (RRM1-2, KH1-4) is achieved through cooperative recognition of a cluster of appropriately spaced CA-rich and GGC RNA motifs. Our approach provides an elementary model of how both specificity and flexibility of IMP3-RNA recognition is achieved, thus providing a paradigm for the function of multivalent interactions with multidomain RNA-binding proteins in gene regulation.

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**Combining MAS and oriented solid-state NMR to reveal structure, oligomerization and topology of polypeptides in membranes**

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Dennis W Juhl, Maria Kardash, Morane Lointier, Arnaud Marquette,  
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In view of the world-wide emergence of pathogens being resistant to a large range of commonly used antibiotics finding pharmaceuticals with novel mechanisms of action has become an urgent need. Antimicrobial peptides are abundant in the plant and animal kingdom, have escaped resistance development over millions of years and are therefore considered valuable templates to develop novel drugs. In order to reveal how these compounds selectively kill bacteria and fungi but not human cells we study their membrane interactions using solid-state NMR spectroscopy.

On the one hand, MAS approaches are used to obtain intra- and intermolecular distance information as well as to count the number of peptides that form oligomers within lipid bilayers. On the other hand, the peptides are reconstituted into uniaxially oriented bilayers. Angular constraints are measured from orientation-dependent chemical shifts, dipolar and quadrupolar splittings which provide not only information on the secondary structure but also on the membrane topology of the polypeptide domains as well as rotational diffusion and size. Importantly, the tilt and rotational pitch angle angular constraints are analyzed by simulating motions of the polypeptide in liquid crystalline bilayers. Furthermore, we have evaluated the systematic errors that can arise that arise from uncertainties in the description of the NMR interactions, e.g. the description of chemical shift tensors.

Examples of our investigation include polypeptides of different length, some predominantly hydrophobic others being cationic and amphipathic. Despite the marked differences in sequence composition most of them are prone to undergo considerably dynamics where their alignment equilibria are affected by lipid composition, hydration and other environmental factors.

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Structural characterization of the PHD5-C5HCH tandem domains of NSD family as epigenetic readers of H3K27me3 and interactors of Nizp1-C2HR

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The NSD methyltransferases (NSD1, NSD2, NSD3) contain several chromatin-related modules (a catalytic SET domain, two PWWP and six PHD domains), all implicated in developmental diseases and cancer. The PHD tandem domain (PHDv-C5HCH) plays a crucial but diversified role in the three NSDs members. In the NUP98-NSD1 fusion protein it is required for the recruitment the protein to HoxA gene promoter in Acute Myeloid Leukaemia (AML), in NSD2 it contributes to NSD2-dependent tumour cell proliferation in multiple myeloma, and in NSD3 it is supposed to contribute to protein recruitment to chromatin through histone H3 interactions. In my home group, I have started a systematic structural/functional investigation of the PHDv-C5HCH tandem domain of the three NSD family members. Our study shows that despite high sequence identity (~60%), the NSD PHDv-C5HCH domains have a divergent role in histones recognition and in protein-protein interactions. On one hand, the PHDv-C5HCH domain of NSD1 does not interact with histone H3 peptides, whereas it binds specifically with micromolar affinity to the C2HR domain of Nizp1 (Nizp1-C2HR), a co-repressor regulating NSD1 transcriptional activity in AML. On the other hand, the NSD2 and NSD3 tandem domains act as classical histone readers but interact with low affinity with Nizp1-C2HR. We attribute these differences to small but crucial differences in aminoacidic sequence located on the interaction surfaces. Intriguingly, we demonstrate that NSD2 and NSD3 PHDv-C5HCH tandem domains specifically recognize H3K27me3, via the inter-domain interface. Methylation of H3K27 is usually associated to repressive chromatin, we thus hypothesize that PHDv-C5HCH of both NSD2 and NSD3 contribute to recruitment of NSD2/3 to repressed chromatin, to facilitate gene activation through methylation of H3K36 via the catalytic NSD-SET domain. In conclusion, our data propose a regulative scenario in which the same NSD tandem domain can differently regulate the recruitment of cofactors/epigenetic modifications necessary for gene transcription.

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**Insights into the exfoliation process of  $V_2O_5 \cdot nH_2O$  nanosheets formation using real-time  $^{51}V$  NMR**

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Nanostructured hydrated vanadium oxides ( $V_2O_5 \cdot nH_2O$ ) are actively being researched for applications in energy storage, catalysis, and gas sensors. Recently, a one-step exfoliation technique for fabricating  $V_2O_5 \cdot nH_2O$  nanosheets in aqueous media was reported. This simple exfoliation process is advantageous in comparison to other synthesis methods based on an ion-exchange or sol-gel route. However, to further optimise the exfoliation, we need to understand the underlying mechanism. Herein, we followed the synthesis of  $V_2O_5 \cdot nH_2O$  nanosheets from the  $V_2O_5$  and  $VO_2$  precursors in real-time using solution- and solid-state  $^{51}V$  NMR. Solution-state  $^{51}V$  NMR showed that the aqueous solution contained mostly the decavanadate anion  $[H_2V_{10}O_{28}]^{4-}$  and the hydrated dioxovanadate cation  $[VO_2 \cdot 4H_2O]^+$  and, during the exfoliation process, decavanadate was formed while the amount of  $[VO_2 \cdot 4H_2O]^+$  remained constant. The conversion of the solid precursor  $V_2O_5$ , which was monitored with solid-state  $^{51}V$  NMR, was only initiated when  $VO_2$  was in its monoclinic form. We hypothesize that both  $V_2O_5$  and  $VO_2$  had been dissolved and  $VO_2$  formed as  $[V^{4+}O \cdot 5H_2O]^{2+}$  cations, which were oligomerized with  $[V^{5+}O_2 \cdot 4H_2O]^+$  species from  $V_2O_5$  and then polymerized further. Magnetic susceptibility data confirmed that the dried  $V_2O_5 \cdot nH_2O$  nanosheets were weakly paramagnetic, due to a minor content of isolated  $V^{4+}$ , which is in agreement with our ESR results. This study demonstrates the use of real-time NMR techniques as a powerful analysis tool for the exfoliation of bulk materials into nanosheets. A deeper understanding of this process will pave the way to tailor these important materials.

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Simplifying Measurement of Zero- to Ultralow-Field Nuclear Magnetic Resonance with Commercial Atomic Magnetometers

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Zero- to ultralow-field nuclear magnetic resonance (ZULF NMR) is an emerging alternative magnetic resonance modality where measurements are performed in the absence of an applied magnetic field [1]. By eliminating the need for a large magnetic field to encode chemical information in the form of chemical shifts, ZULF NMR avoids some problems encountered by conventional NMR, such as broadening from susceptibility gradients in complex materials, limited RF penetration into conductive samples, and truncation of nuclear spin interactions that do not commute with the Zeeman interaction. The ability to detect nuclear spin magnetization in sub- $\mu$ T fields is also appealing for the development and optimization of hyperpolarization techniques like SABRE-SHEATH (Signal Amplification by Reversible Exchange of parahydrogen in SHield Enables Alignment-to-magnetization Transfer to Heteronuclei).

Because of the comparatively low frequencies associated with nuclear spin-spin couplings, direct detection of ZULF NMR requires the use of sensitive non-inductive detectors, such as atomic magnetometers [2]. This has unfortunately raised a barrier to many researchers who might be interested in ZULF NMR, but have limited experience with atomic physics. Recently, however, a number of companies [3] have developed standalone “plug-and-play” optically pumped atomic magnetometers with magnetic-field sensitivity within an order of magnitude of that achieved with state-of-the-art instrumentation.

We have demonstrated that these commercial sensors can be used for the detection of ZULF NMR signals. We will report on the measurement of hydrogenative PHIP (parahydrogen-induced polarization) and SABRE hyperpolarized reaction products in situ using ZULF NMR, and on our progress toward zero-field magnetic resonance imaging. We will also describe an open-source spectrometer design that will allow the NMR community broader access to ZULF-NMR techniques.

**References:** [1] J.W. Blanchard and D. Budker. Zero- to Ultralow-Field NMR. *eMagRes*, 2016, 5, 1395. [2] M.C.D. Tayler, et al. *Rev. Sci. Instrum.* **88** (9), 091101 (2017). [3] For example, QuSpin Inc.: <https://quspin.com/>.

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**Biocompatible spin labels for in-cell EPR at physiological concentrations**Enrica Bordignon<sup>\*1</sup>, Tufa Assafa<sup>1</sup>, Stephanie Bleicken<sup>1</sup>, Laura Galazzo<sup>1</sup>, Gunnar Jeschke<sup>2</sup>, Svetlana Kucher<sup>1</sup>, Andrzej Rajca<sup>3</sup>, Markus Teucher<sup>1</sup><sup>1</sup>Ruhr University Bochum, <sup>2</sup>ETH Zurich, <sup>3</sup>University of Nebraska

Gadolinium is the most promising spin type for in cell studies of spin-labeled proteins. However, the availability of other bioresistant spin labels could aid the development of structural studies of biomolecules in a cellular context. The relatively small and flexible nitroxide label could offer many advantages in this area of research. We characterized iodoacetamide- and maleimide-functionalized nitroxide spin labels based on the gem-diethyl pyrroline structure using cw and DEER experiments [1]. We compared their chemical resistance to reducing agents in ascorbate solutions, oocytes, E. coli cells and mammalian cells and found a remarkably different behavior in different cells at different concentrations.

Spin/protein concentration is an important issue for in-cell EPR. In fact, low micromolar concentrations may already be the upper limit for physiologically relevant concentrations of most proteins in cells. We therefore also compared the concentration sensitivity of Q-band DEER for Gd- and NO-labeled proteins, to identify the lowest concentrations at which reliable DEER data can be still extracted in vitro and in cell. We found that submicromolar concentrations are accessible for DEER measurements in cellular context.

We address the potential of biocompatible orthogonal labels using the proapoptotic protein Bax labeled with Gd or gem-diethyl NO labels. The use of Gd-labeled nanobodies recognizing specific epitopes in ABC transporters will also be addressed as possible tool for measurements in cellular context.

Despite many challenges are still ahead, in-cell DEER with orthogonal labels is within reach.

Reference: [1] Bleicken et al., 2019, ChemistryOpen, accepted.

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Design of Heterogeneous Catalysts with Improved Activity,  
Pairwise Selectivity, and Yield for Parahydrogen Enhanced NMR  
Clifford Bowers<sup>\*,1</sup>, Tommy Zhao<sup>1</sup>, Yong Du<sup>1</sup>, Minda Chen<sup>2</sup>, Yuchen Pei<sup>2</sup>,  
Diana Choi<sup>1</sup>, Bochuan Song<sup>1</sup>, Helena Hagelin-Weaver<sup>1</sup>, Wenyu Huang<sup>2</sup>  
<sup>1</sup>University of Florida, <sup>2</sup>Iowa State University

Heterogeneous catalysis offers intrinsic benefits for parahydrogen induced hyperpolarization (PHIP). In addition to the ease of separation of the catalyst and compatibility with continuous-flow production of hyperpolarized fluids, supported transition metal nanoparticle catalyst materials are generally more robust and recyclable than dissolved transition metal complexes. However, the down-side is that the monometallic (e.g. Pd, Pt, Rh, Ir) supported metal nanoparticles, which mediate hydrogenation by the Horiuti-Polanyi mechanism, are inefficient PHIP catalysts due to the low pairwise selectivity (i.e. fraction of adduct molecules formed by addition of both protons from the same H<sub>2</sub> molecule) of hydrogenation over these catalysts. The combination of fast H ad-atom diffusion and step-wise H ad-atom transfer conspire to randomize the parahydrogen singlet proton spin state during transfer to the substrate. This presentation will demonstrate several novel strategies for synthesizing supported metal catalysts with surface properties that mitigate spin state randomization, resulting in high pairwise selectivity and yield (i.e. the product of selectivity and activity). In experiments with both liquid and gaseous substrates, the novel catalytic nanomaterials afford significantly higher het-PHIP performance than conventional supported monometallic nanoparticles.

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## Advancement of the SWAMP Technique

Clifford Bowers<sup>\*,1</sup>, Minda Chen<sup>2</sup>, Yong Du<sup>1</sup>, Tommy Zhao<sup>1</sup>,Evan Zhao<sup>1</sup>, Raghu Maligal-Ganesh<sup>2</sup>, Huang Wenyu<sup>2</sup><sup>1</sup>University of Florida, <sup>2</sup>Iowa State University

In the SWAMP effect (Surface Waters Are Magnetized by Parahydrogen), hyperpolarization of the protons of liquid water, methanol or ethanol is mediated by mesoporous silica-encapsulated Pt<sub>3</sub>Sn intermetallic nanoparticles (iNPs).[1] Hydroxy protons exhibit stimulated emission NMR signals after a few seconds of bubbling of para-enriched H<sub>2</sub> through a suspension of the iNPs at low magnetic field (ca. 0.5 G). Nonexchangeable methyl or methylene protons of the alcohols are also hyperpolarized, an observation which is key to the interpretation of the mechanism and spin-dynamics of SWAMP. The SWAMP catalyst is non-toxic and insoluble, which allows it to be completely separated from the hyperpolarized liquid. SWAMP could enable low-field MRI without superconducting magnets, providing access to inexpensive MRI. New insights into the mechanism as well as improvements in the SWAMP catalyst and experimental protocol will be presented. Funded by NSF CHE-1808239 and CHE-1507230.

Reference: [1] EW Zhao, R Maligal-Ganesh, Y Du, TY Zhao, J Collins... - Chem, 2018.

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Feasibility of functional MRI at ultralow magnetic field via changes in cerebral blood volume

Kai Buckenmaier<sup>\*1</sup>, Anders Pedersen<sup>2</sup>, Paul SanGiorgio<sup>2</sup>,  
Klaus Scheffler<sup>1</sup>, John Clarke<sup>2</sup>, Ben Inglis<sup>3</sup>

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<sup>3</sup>Henry H. Wheeler, Jr. Brain Imaging Center, University of California, Berkeley

We investigate the feasibility of performing functional MRI (fMRI) at ultralow field (ULF) with a Superconducting QUantum Interference Device (SQUID), as used for detecting magnetoencephalography (MEG) signals from the human head. While there is negligible magnetic susceptibility variation to produce blood oxygenation level-dependent (BOLD) contrast at ULF, changes in cerebral blood volume (CBV) may be a sensitive mechanism for fMRI given the five-fold spread in spin-lattice relaxation time ( $T_1$ ) values across the constituents of the human brain. We undertook simulations of functional signal strength for a simplified brain model involving activation of a primary cortical region in a manner consistent with a blocked task experiment. Our simulations involve measured values of  $T_1$  at ULF (130  $\mu$ T) and experimental parameters for the performance of an ULFMRI scanner with a noise level of 0.1 fT/Hz<sup>-1/2</sup> and a prepolarizing field of 200 mT. Under ideal experimental conditions we predict a functional signal-to-noise ratio of between 3.1 and 7.1 for an imaging time of 30 min, or between 1.5 and 3.5 for a blocked task experiment lasting 7.5 min. Our simulations suggest it may be feasible but challenging to perform fMRI using a ULFMRI system designed to perform MRI and MEG in situ.

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### Advantages of combining nuclear magnetic hyperpolarization and ultralow-field magnetic resonance

Kai Buckenmaier<sup>\*1</sup>, Matthias Rudolph<sup>2</sup>, Andrey Pravdivtsev<sup>3</sup>, Paul Fehling<sup>4</sup>, Theodor Steffen<sup>1</sup>, Christoph Back<sup>5</sup>, Rebekka Bernard<sup>1</sup>, Rolf Pohmann<sup>1</sup>, Johannes Bernarding<sup>6</sup>, Reinhold Kleiner<sup>5</sup>, Dieter Koelle<sup>5</sup>, Jan Hövener<sup>7</sup>, Klaus Scheffler<sup>1</sup>, Markus Plaumann<sup>6</sup>

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Ultralow-field (ULF) nuclear magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) are promising methods allowing for, e.g., the simultaneous detection of multiple nuclei or imaging in the vicinity of metals. To overcome the inherently low signal-to-noise ratio that usually hampers a wider application, we present an alternative approach to prepolarized ULF MRS, employing hyperpolarization techniques like signal amplification by reversible exchange (SABRE) or Overhauser dynamic nuclear polarization (ODNP). Both techniques allow continuous hyperpolarization of <sup>1</sup>H and other MR-active nuclei. For implementation, a superconducting quantum interference device (SQUID)-based ULF MRS/MRI detection scheme was constructed. Due to their very low intrinsic noise level, SQUIDS are superior to conventional Faraday detection coils at ULFs. The noise level of the here presented system is  $\sim 1 \text{ fT/Hz}^{1/2}$ . Additionally, the broadband characteristics of SQUIDS enable them to simultaneously detect the MR signal of different nuclei such as <sup>13</sup>C, <sup>19</sup>F, or <sup>1</sup>H. The MR signal can be measured in absolute units, which allows quantitative investigations of the hyperpolarization techniques without a reference. The detection field can be changed without the need of tuning or matching the SQUID-based detection coil. The whole system was constructed with the goal of providing an easily accessible sample volume. Therefore, the setup sits inside a three-layer shielding chamber consisting of two layers of mu metal for shielding DC and low-frequency magnetic field noise and one layer of aluminum for shielding high-frequency noise.

As a demonstration of the performance of the system, first quantitative two-dimensional NMR results from SABRE-enhanced samples are presented. These measurements enabled us to understand the underlying physical mechanisms of SABRE in so far unprecedented detail (effects of solvent and heteronuclei). In addition ODNP-enhanced MRI was performed on a dead rat. 3D images with an isotropic resolution in the mm range could be acquired with this method.

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What can NMR analysis reveal about Acyl Glucuronides and their relative stability to inform on potential DILI risk?

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<sup>1</sup>Merck and Co., Inc., <sup>2</sup>Merck and Co, Inc., <sup>3</sup>Merck and Co., Inc

Acyl Glucuronides (AGs) and their respective degradation products are one of the most common drug metabolites. They have been implicated in the cause of drug-induced liver injuries (DILI) – a major factor in the attrition of drug candidates. NMR and other analytical methods have previously been applied to study these degradation products and their transformations, though mainly to the purpose of evaluating the rates of AG degradation. It is clear that a better understanding of the processes of AG transformation, specifically the AG rearrangement, would be of high benefit. Here we show that by using NMR spectroscopy, one can study both the kinetic mechanism of AG transformation and the structure of intermediates and degradation products. We present an example of such an in-depth analysis for Ibufenac AG. We show that the combination of kinetic and structural NMR data analysis can provide unprecedented clarity of details regarding the mechanism of AG transformations. These capabilities are of great help to propose new hypothesis and design new experiments which should ultimately lead to a better understanding of the mechanism of DILI and its prevention.

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Conformational changes in the neurotensin peptide upon binding  
neurotensin receptor 1 may drive receptor activation

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Alice Whitehead<sup>4</sup>, Xuan Tan<sup>5</sup>, Joshua Ziarek<sup>1</sup>, Margaret Johnson<sup>5</sup>, Ross Bathgate<sup>6</sup>, David  
Chalmers<sup>2</sup>, Daniel Scott<sup>6</sup>, Paul Gooley<sup>3</sup>

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<sup>4</sup>The Florey Institute of Neuroscience and Mental Health, <sup>5</sup>The University of  
Alabama at Birmingham, <sup>6</sup>University of Melbourne

Neurotensin (NT) is a 13 amino acid peptide expressed in the central nervous, gastro-in-  
testinal and cardiovascular systems where it acts as a neuromodulator of classical neu-  
rotransmitters such as dopamine and glutamate, primarily through activation of neuroten-  
sin receptor 1 (NTS1), a G Protein-Coupled Receptor (GPCR). Peptide ligands of GPCRs  
bind through complex, and possibly multiple modes for which there are few representative  
crystal structures. In the case of NTS1, eight inactive-state crystal structures of thermosta-  
bilized variants have been solved in complex with the high affinity 8-13 fragment of NT  
(NT8-13, RRPYIL). In all crystal structures, NT8-13 adopts an identical extended conforma-  
tion with the C-terminal four residues (PYIL) buried deeply into the orthosteric site of the  
receptor. But the role of ligand conformational dynamics while bound to the orthosteric site  
is unclear. We used competition saturation transfer difference (STD) NMR to investigate the  
binding pose of a low-affinity neurotensin peptide fragment (NT10-13) to a thermostabi-  
lized, signaling-competent NTS1 variant solubilized in detergent micelles. Epitope mapping  
of NT10-13 suggests that tyrosine 11 (Y11) binds the NTS1 orthosteric site in an alternate  
conformation than previously observed in crystal structures. Using molecular dynamics  
(MD) simulations, we, and others, showed that Y11 can adopt two conformations on NTS1  
binding. Furthermore, <sup>13</sup>C HSQC spectra of a [U-<sup>13</sup>C,<sup>15</sup>N]-NT8-13:[U-<sup>2</sup>H,<sup>12</sup>C]-NTS1 com-  
plex revealed that the Y11 sidechain indeed exists in two conformations. Two states were  
also observed for the free peptide but at different populations compared to the complex.  
Exchange rates between the two conformers are currently being measured. Taken together,  
our integrative approach has captured a peptide conformation not evident from crystal  
structures thus highlighting the importance of NT peptide dynamics for NTS1 activation.

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ACERT 95 GHz MKII ESR SPECTROMETER  
Curt Dunnam<sup>\*,1</sup>, Boris Dzikovski<sup>2</sup>, John Franck<sup>3</sup>, Jack Freed<sup>2</sup>  
<sup>1</sup>Cornell University, <sup>2</sup>Cornell Univ., <sup>3</sup>Syracuse Univ.

Development of new ESR instrumentation is an important priority of the Cornell University National Biomedical Center for Advanced ESR Technology (ACERT) at Cornell University. The ACERT high power (1.2kW) 95 GHz broadband spectrometer[1] is currently the only system available to the scientific community allowing for 2D-ELDOR studies on biologically relevant aqueous samples at room temperature. In this presentation we report on recent “MKII” updates of the system, including:

- Specification, fabrication and installation of a new, fully heterodyne transceiver with significantly improved noise figure, wideband AWG modulation capability and gated pulse widths as short as 3ns.
- Almost doubling the effective B1 $\pi$  value (from 17G to nearly 30G) due to dielectric enhancement of the microwave field at the sample.
- Improvements in the design of the spectrometer quasioptical system resulting in substantially better sensitivity and reduced deadtimes of ~20ns, even with aqueous samples.

These improvements have been made with the aid of the full 3D wave simulator HFSS, which helped in determining electric and magnetic field distributions, and reduction of cross-polarization anomalies due to mode conversion. Unwanted reflections and, consequently, spectrometer dead time have been lowered. We further demonstrate use of the new AWG capability for improving spectral coverage of nitroxide radicals and for enhancing EDNMR on this system.

Near-term planned improvements include further increasing B1 at the Fabry-Pérot resonator and halving of deadtime to ca 10ns with AWG compensation.

Reference: [1] Hofbauer et al. Rev. Sci Instr. 75, 1194 (2004).

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Realization of Universal Quantum Gates with Spin-Qudits in Colloidal Quantum Dots

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<sup>1</sup>University of Milano-Bicocca, <sup>2</sup>Liverpool John Moores University,

<sup>3</sup>University of Lincoln, <sup>4</sup>University of Nottingham

Potential building blocks for multi-level qubits or qudits are intensively investigated because of the multi-dimensional Hilbert space ( $d > 2$ ) for encoding several bits per unit, reduced number of units and hardware size, and robustness against noise and error rates. These advantages over simple qubits could lead to novel scalable and downsized quantum computer architectures.

Although this type of qudit has been studied in transition metal (TM) ions and rare earth (RE) elements embedded in bulk solid state[1] and molecular systems,[2] its experimental implementation in universal quantum gates has not yet been achieved in quantum dots (QDs) doped with TM ions.[3]

We demonstrate that hyperfine interactions in isolated Mn-ion confined in colloidal QDs can be exploited to probe an arbitrary superposition of states between selected hyperfine energy level pairs by using electron double resonance detected nuclear magnetic resonance (EDNMR). This enables the observation of Rabi oscillations and the experimental realization of NOT and  $\sqrt{\text{SWAP}}$  universal quantum gates that are robust against decoherence. We propose a protocol for cyclical preparation, manipulation and read-out of logic gates that could be implemented in QDs integrated in scalable quantum circuit architectures beyond solid state electron spin qubits.

**References:** [1] a) S. Bertaina, et al. Phys. Rev. B 2017, 96, 024428; b) E. Baibekov, et al. J. Mag. Res. 2011, 209, 61–68. [2] C. Godfrin, et al. Phys. Rev. Lett. 2017, 119, 187702; J. Kobak, et al., Nature Commun. 2014, 5, 3191. [3] S. T. Ochsenbein and D. R. Gamelin, Nature Nanotech. 2011, 6, 111; F. Moro, L. Turyanska, J. Wilman, A. J. Fielding, M. W. Fay, J. Granwehr, and A. Patanè, Sci. Rep. 2015, 5, 10855.

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CONFIRMATION OF THE IMIDAZOLE-IMIDAZOLIUM  
HYDROGEN BONDS IN HISTIDINE TETRAD OF FULL LENGTH  
M2 PROTON CHANNEL BY SOLID-STATE NMR

Riqiang Fu, Yimin Miao, Huajun Qin, Timothy Cross

The integral membrane M2 protein is a 97-residue membrane protein that assembles as a tetramer to conduct protons at a slow rate ( $10^2$ - $10^3$ /s) when activated by low pH. The proton conductance mechanism has been extensively debated in the literature, but it is accepted that the proton conductance is facilitated by hydrogen bonds involving the His37 residues. However, the hydrogen bonding partnership remains unresolved. Here, we use a variety of solid-state NMR experiments to obtain convincing evidence that differentiates two hydrogen bonding schemes leading to an understanding of the M2 proton channel conductance. In particular, we measured  $^{15}\text{N}$ - $^{15}\text{N}$  J-couplings of  $^{15}\text{N}$  His37-labeled full length M2 (M2FL) protein from Influenza A virus embedded in synthetic liquid crystalline lipid bilayers using two-dimensional J-resolved NMR spectroscopy and observed the hydrogen-bond mediated J-couplings between Nd1 and Ne2 of adjacent His37 imidazole rings, providing direct evidence for the existence of various imidazolium-imidazole hydrogen-bonding geometries in the histidine tetrad at low pH, thus validating the proton conductance mechanism in the M2FL protein by which protons are transferred through the breaking and reforming of the hydrogen-bonds between pairs of His37 residues.

ACKNOWLEDDgements

This work was supported by NIH grants AI023007 and AI119178. All NMR experiments were performed at the NHMFL supported by the NSF DMR-1644779 and the State of Florida.

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**P559****Leucine methyl labeling in eukaryotic and cell-free expression systems**

Haribabu Arthanari<sup>1</sup>, Sandeep Chhabra<sup>1</sup>, Abhinav Dubey<sup>1</sup>,  
 Shantha Elter<sup>2</sup>, Manuel Etzkorn, Vladimir Gelev<sup>\*,4</sup>, Nikola Burdzhiev<sup>4</sup>,  
 Ognyan Petrov<sup>4</sup>, Nikolay Stoyanov<sup>5</sup>, Thibault Viennet<sup>1</sup>

<sup>1</sup>*Dana-Farber Cancer Institute*, <sup>2</sup>*Heinrich-Heine-Universität Düsseldorf*,

<sup>4</sup>*Sofia University*, <sup>5</sup>*FB Reagents Ltd*

A relatively affordable synthesis of leucine that is labeled stereo-selectively <sup>13</sup>CH<sub>3</sub>/d<sub>3</sub> at the two methyl groups is described. Use of this leucine in otherwise natural abundance medium provides “local” deuteration that significantly improves <sup>13</sup>C/<sup>1</sup>H<sub>3</sub> observation of proteins expressed in insect, mammalian, and cell-free systems.

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Small molecule-based targeting of TTD-A dimerization to control TFIID transcriptional activity represents a potential strategy for anticancer therapy

Virginie Gervais<sup>\*1</sup>, Isabelle Muller<sup>1</sup>, Pierre-Olivier Mari<sup>2</sup>, Pascal Ramos<sup>1</sup>, Julien Marcoux<sup>1</sup>, Valérie Guillet<sup>1</sup>, Georges Czaplicki<sup>1</sup>, Odile Burlet-Schiltz<sup>1</sup>, Ambra Giglia-Mari<sup>2</sup>, Alain Milon<sup>1</sup>

<sup>1</sup>IPBS CNRS Université Paul Sabatier, <sup>2</sup>Neuromyogene institute

The TFIID transcription factor is a huge complex composed of 10 subunits that form an intricate network of protein-protein interactions that are critical for regulating transcriptional and DNA repair activities of TFIID. The smallest subunit (TTD-A/p8) displays dimerization properties allowing to shift from a homodimeric state in the absence of a functional partner, to a heterodimeric structure with the p52 TFIID subunit, enabling dynamic binding to TFIID. Recruitment of p8 at TFIID stabilizes the overall architecture of the complex, whereas p8's absence reduces its cellular steady-state concentration and consequently decreases basal transcription, highlighting that p8 dimerization may be an attractive target for down-regulating transcription in cancer cells

We considered the dimerization interface of p8 as a “druggable” target. With the aim to design molecules that destabilize the homodimer structure of p8 and alter its recruitment into TFIID, we applied a fragment-screening strategy based on virtual, biophysical, and NMR screening. We have identified two compounds that bind to and may react with the dimerization motif of p8, provoking destabilization of its protein-protein interface and its precipitation. Using quantitative imaging in living cells, we found that these two compounds have a noticeable effect on concentration and transcription activity of TFIID.

In conclusion, our study offers offers p8 as an alternative pharmacological target that could be considered for an effective modulation of transcription. In a more general context where tumor cells require high levels of transcription for proliferation and survival, our work demonstrates the potential of small molecules in targeting protein-protein interactions that are critical for basal transcription machinery, opening new perspectives to design modulators of TFIID-associated transcription.

**Reference:** Gervais, V. et al.(2018) Small molecule-based targeting of TTD-A dimerization to control TFIID transcriptional activity represents a potential strategy for anticancer therapy. J Biol Chem 28, 14974-14988

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Analyzing motional dynamics of spin-labeled proteins at  
sub-microsecond time scales via 2D ELDOR at 95 GHz

Pranav Gupta, Zhichun Liang, Boris Dzikovski, Curt Dunnam, Jack Freed

Two-Dimensional Electron Double Resonance (2D-ELDOR) studies at high frequencies such as 95GHz can offer unique information on molecular motions and dynamics given its better g-tensor resolution. This spectroscopic technique has much potential, since it extends the time-scale of ESR spectroscopy toward slower processes (e.g. conformational changes, chemical exchange, etc), which can be observed by the development of exchange cross-peaks. 2D-ELDOR has been successfully applied at lower frequencies to study phase equilibria in complex membranes, but further use for biological systems, in particular at higher frequencies, was so far limited by several difficulties. One of these lies in spectral analysis, since the usual methods of spectral simulation in the slow motional region are limited for 2D-ELDOR and break down for the case of slow motional 95GHz spectra. ACERT has in the past pioneered the development of computational tools to simulate and interpret dynamic ESR spectra. Here we report on our latest improvement in computational analysis compared to earlier versions of our programs, since it offers greater stability in the very slow motional regime than the Lanczos algorithm used to diagonalize Stochastic Liouville matrices. With our new numerical approach we are able to simulate experimental 2D-ELDOR at slow motions, all the way to rigid limit. We generate slow motional 2D-ELDOR spectra corresponding to a prospective biological application of 2D-ELDOR, the conformational exchange in biomolecules. We investigate the effects of the exchange rate, of the difference in populations and EPR parameters between the exchanging states, as well as the effects of global and local molecular dynamics on the diagonal and non-diagonal features of the corresponding very slow-motional 2D-ELDOR spectra. Combining the latest theoretical developments with upcoming dead time improvements in our 95GHz 2D-ELDOR ACERT spectrometer, we are now well-equipped to obtain key insights into the conformational dynamics of biomolecules at sub-microsecond time scales.

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Assesment of Mixtures by Spectral Superposition.  
An Approach in the Field of Metabolomics  
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*University of Hamburg*

The aim of a metabolic study is the differentiation of biological populations by their metabolite composition. Even in a simple model the assessment of large numbers of samples is mandatory to produce statistically meaningful data. This requirement becomes even larger in the analysis of metabolite progression, for example in disease development, or mixtures of commodities, for example the dilution of expensive products by inferior or low-priced goods. Particularly in large-scale studies, the number of intermediate states or mixtures tends to expand significantly and in practice, a manual analysis is extremely difficult if not nearly impossible.

In the first part, 262 hazelnut samples from five Eurasian countries (France, Germany, Georgia, Italy, and Turkey) have been investigated by <sup>1</sup>H NMR regarding their geographic origin. The samples were classified using a machine learning algorithm (subspace random classifier). The training of this classifier with 2/3 of the total number of samples derived a five-class classifier model capable of assigning the origin of samples. Overall, the prediction achieved an accuracy of 92% and 96% for the training and test set, respectively.

The high prediction accuracy of this model motivated us to use this data set for the analysis of hazelnut samples with mixed geographic origin, in the second part. Due to the high number of possible combinations we calculated the mixture spectra by superposition of spectra from the corresponding pure samples. Considering several mixture ratios, we obtained more than 160000 NMR spectra which were used for the calculation of assessment curves, estimating the extend to recognize admixtures within a multivariate classification model. To validate this model, we prepared a subset, several hundred mixtures, in the laboratory and measured them. The calculated spectra are very similar to the acquired data and the resulting deviations are on a similar scale to the errors of current metabolomic measurements.

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High concentration solutions of monoclonal anti-bodies studied by EPR spectroscopy:  
Perspectives and challenges

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Monoclonal antibodies (mAbs) are among modern bio-therapeutics which are often needed and applied in high concentrations (>100mg/ml), especially for subcutaneous injections. EPR spectroscopy is among the few methods which can be applied directly at such conditions, without making concentration approximations. To the best of our knowledge for the first time, EPR spectroscopy is used to study the high concentration solutions of mAbs and their effect on co-solvated small molecules. Concentrations up to 200 mg/ml of two mAbs formulated in pure water and citrate buffer were considered, utilizing a spin probing assay.

We used TEMPO as a neutral spin probe, positively charged CAT1 and designed a new citrate spin probe bearing negative charges (named CITPRO). We could obtain information about the surrounding environments of mAbs in their high concentration and were able to compare EPR obtained microscopic viscosities (scaled by rotational correlation times) to macroscopic viscosities measured through rheology. It is found that up to concentrations of 50 mg/ml, the mAb-spin probe systems have similar micro- and macro- viscosity trends, using mixtures of glycerol-water-spin probes as a model system. The results show that the charged spin probes sense rather unchanged aqueous solution even at very high concentrations, which in turn indicates the existence of large solvent regions that despite their proximity to large mAbs, essentially offer pure water reservoirs for co-solvated small charged spin probes.

We show the challenges of the common spin labeling methods and their applicability on mAbs and the possible new strategies and report the first results of double electron-electron resonance(DEER) measurements on high concentration solutions of mAbs and problems arising by data analysis of such solutions.

**References:** [1] H.H.Haeri, J.Blaffert, F.A.Schöffmann, M.Blech, J.Hartl, P. Garidel, and D. Hinderberger. 2019, submitted. [2] J. Blaffert, H. H. Haeri, M.Blech, D.Hinderberger and P. Garidel. 2018, Anal. Biochem. 561–562, 70–88.

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## P565

Observation of the Cage Effect and Cooperative Motion in Bulk TEA-BF<sub>4</sub>/ACN Electrolytes Using the Pulsed Field Gradient NMR and Molecular Dynamics Simulations

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Organic electrolytes, such as tetraethylammonium tetrafluoroborate in acetonitrile (TEA-BF<sub>4</sub>/ACN) are used in most commercial supercapacitors [1]. Studies on the aqueous electrolytes reported the presence of hydration cages around the ions and provided the evidence for the interaction of ions beyond their first hydration shell [2, 3]. This work is aimed at the investigation of ion dynamics in the bulk TEA-BF<sub>4</sub>/ACN. Pulsed field gradient (PFG) NMR spectroscopy enabled determination of the ionic self-diffusion coefficients in 1.0 M, 0.5 M, 0.25 M and 0.13 M electrolyte solutions on the 10 ms timescale at 253–298 K. The self-diffusion coefficients were also calculated under the same conditions via the molecular dynamics simulations (MDS), however, on 100 ns timescale. Obtained from PFG NMR experiments ionic self-diffusion coefficients increased with the decrease of the electrolyte concentrations and increase in temperature. The same trend was obtained by the MDS. The effective ionic radii were estimated based on the experimentally obtained diffusion coefficients and the Stokes-Einstein equation. The values of ionic radii increased with the decrease in concentration. To investigate this finding, distances between ions, their trajectories and the average lifetimes of the solvent molecules in the solvation shell of the ions were analyzed. Finally, combination of the two methods allowed to shed a light on the ionic interactions and the “cage”-like dynamics in the bulk electrolyte.

**References:** [1] J. Lang et al., J. Power Sources 423 (2019) 271–279. [2] G. Schwaab et al., Angew. Chem. Int. Ed. 58 (2019) 3000–3013. [3] P. Schienbein et al., J. Phys. Chem. Lett. 8 (2017) 2373–2380.

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**NMReDATA, an Initiative promoting a new file format for  
NMR-extracted parameters of small molecules**

Damien Jeannerat\*

*University of Geneva*

An open initiative involving some of the major players of computer-assisted structure elucidation (CASE), including methodology specialists, software and database developers and the editorial board of Magnetic Resonance in Chemistry, is addressing the old problem of reporting and sharing the assignment of 1D and 2D NMR spectra.

We introduced a data format to associate the data extracted from the spectra (chemical shifts, coupling, list of 2D correlations) and the structure of the identified compound. The file uses the SD format, a type of files that is compatible with .mol files (quite a common format used to draw chemical structures). These .sdf files including NMReDATA can in principle be generated by any computer-assisted structure elucidation routine, available in most NMR related software and have multiple roles

- 1) They make the link between the atoms of the structure and the signals found in the spectra (assignment).
- 2) They list, for each 1D and 2D spectrum, the spectral parameters in a defined format (chemical shifts, couplings, integral, 2D correlations)
- 3) They combine the data extracted from the spectra into an aggregated table (list of chemical shifts, coupling network, etc.).
- 4) They include links from the spectral data to the original files of the spectra (located in a local files folder or linked to a database).

**Reference:** [1] M. Pupier, J.-M. Nuzillard, J. Wist, N. E. Schlörer, S. Kuhn, M. Erdelyi, C. Steinbeck, A. J. Williams, C. Butts, T. D. W. Claridge, B. Mikhova, W. Robien, H. Dashti, H. R. Eghbalian, C. Farès, C. Adam, P. Kessler, F. Moriaud, M. Elyashberg, D. Argyropoulos, M. Pérez, P. Giraudeau, R. R. Gil, P. Trevorrow and D. Jeannerat, "NMReDATA, a standard to report the NMR assignment and parameters of organic compounds", *Magn. Reson. Chem.* **2018**, 56, 703-715, DOI: 10.1002/mrc.4737

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Perspectives after 20 years of developments of  
automated processing and analysis of 2D NMR spectra

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If pulse sequence developments are essential and academically rewarding, the processing and analysis of NMR spectra are no less important to provide the end user of NMR spectroscopy with a quick and reliable access the chemically relevant information.

We observed that methods developed to improve the resolution, reduce the coupling structure and process the spectra of COSY, J-resolved (and related experiments), HSQC and HMBC experiments are not sufficiently exploited in the context of small molecules structure determination. This is in part because of lack of integration of modernization of processing and analysis of 2D spectra.

We should present a selection of processing tools used and, in some cases, improved in the Jeannerat Group during the last 20 years. We will mention lineshape deconvolution, phase correction, peak picking and how spectral quality driven by SAN (Signal-artifact-noise) analysis can be used to generate a flux of processed data instead of static spectra. At the next stage, the data can be analysed using peak picking and multiplet structure analysis in a manner that simultaneously integrate all the spectra - taking advantage of multiplet separation of 2D spectra and the presence of resolved multiplet in high-resolution DQF-COSY and J-resolved/DIAG spectra. A strong synergy is expected when combining this classical bottom-up method with a top-down approach taking the available information about the compound and use of NMR parameter prediction and spectral simulation. This should result in a rigorous validation of structure determination and produce high-quality NMR parameters obtained after refinement of chemical shifts and coupling constants. Automatic analysis of complex mixtures will also strongly benefit from such a bidirectional analysis of NMR spectra.

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## P568

## Studies towards the influence of spacer groups on the thermoresponsive properties of sidechain-modified homopolyglutamate-based alignment media

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Elucidation of the spatial structure of small molecules based on NMR spectroscopy is possible either by the use of conventional methods in isotropic solution (scalar coupling, NOE) or via anisotropic parameters for example in the form of residual dipolar couplings (RDCs).[1] These anisotropic parameters are accessible through a diverse range of alignment media. In the past two decades more and more alignment media on the basis of lyotropic liquid crystals were published for this purpose.[2] However, until now the interaction between alignment medium solvent and analyte is not really understood.

To further establish the RDC approach a better understanding of the alignment process itself is necessary.

Recently, we presented poly-*p*-biphenylmethyl-glutamate (PBPMG) as a new enantiodifferentiating alignment medium with temperature tunable properties.[3] To obtain deeper understanding of the reasons of this thermoresponsive behavior we want to investigate the influence of the sidechain and its flexibility towards the alignment process. Therefore, we decided to introduce spacer groups with different lengths between the polymer backbone and the sidechain mesogen. Possible changes in the thermoresponsive behavior can be related to the increasing flexibility as a consequence of the spacer groups and a possible decoupling of the sidechain mesogen from the mainchain mesogen. The herein presented temperature dependent measurements of these newly synthesized polymers utilizing the RDC approach possibly allow for new insights on the influence of sidechains to the alignment process in general.

**References:** [1] C. M. Thiele, *Concepts in Magnetic Resonance Part A* **2007**, 30A (2), 65-80. [2] V. Schmidts, *Magn. Reson. Chem.* **2017**, 55, 54-60. [3] S. Jeziorowski, C. M. Thiele, *Chem. Eur. J.* **2018**, 24, 15631-15637.

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**BRCA2 phosphorylation by Plk1: regulation of mitosis and impact of variants found in breast cancer patients**

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BRCA2 is a 3000 residues long protein that serves as a platform for proteins involved in genomic stability and mitosis. These functions are regulated by kinases phosphorylating BRCA2 unfolded regions. Among these kinases, Plk1 has the most remarkable impacts on BRCA2. Plk1 is involved in cell cycle progression and mitosis regulation and it is found overexpressed in several cancers, including breast cancers. The N-terminal region of BRCA2 is highly phosphorylated by Plk1 at the entry of mitosis and is suggested to regulate both chromosome segregation and cytokinesis. Our study aims primarily at identifying BRCA2 key residues in order to classify breast cancer mutants.

Here, we followed BRCA2 phosphorylation by Plk1 using solution NMR. We confirmed that the N-terminal region of BRCA2 is disordered. Then, we identified BRCA2 residues phosphorylated by Plk1 and monitored their phosphorylation kinetics using the usual 1H-15N-HSQC and the 13Cα-13Co method implemented at the lab. We also discovered that Plk1 creates its own docking site on BRCA2 upon phosphorylation, an interaction that we later detailed using ITC and XR crystallography. This provided substantial basis to explain the impact of breast cancer BRCA2 variants on Plk1 activity.

Second, the cell-cycle kinase Cdk also creates a Plk1 docking site on BRCA2 before mitosis. In our hands, phosphomimetics proved to be irrelevant to characterize this priming phospho-mechanism. Thus, we switched to BRCA2 pre-phosphorylation by Cdk and the use of native chemical ligation, which permits the precise quantification of Cdk activity on BRCA2 phosphorylation by Plk1.

Finally, these data were integrated into a biological model, supported by cell biology experiments. In this model, during mitosis, BRCA2 recruits both Plk1 and the spindle assembly factor BubR1 to favor phosphorylation of BubR1 by Plk1, thus contributing to the regulation of chromosome segregation.

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**Pressure response of the Ras protein in solution and single crystals studied by high pressure NMR spectroscopy and high pressure x-ray crystallography.**

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High-pressure NMR spectroscopy (HPNMR) represents a powerful method for characterizing conformational equilibria in biological macromolecules thermodynamically in solution [1]. High-pressure x-ray crystallography (HPX) is an alternative method to observe pressure induced conformational changes with high structural precision [2]. Ras protein is a central element of the cellular signal transduction involved in proliferation and cellular differentiation. It is activated by guanine exchange factors (GEFs) that exchange the normally bound GDP by GTP and deactivated by GTPase activating proteins (GAPs) that lead to the hydrolysis of GTP to GDP. The analysis of the pressure dependent chemical shift changes of activated Ras allowed the identification and thermodynamic characterisation of four different conformational states that coexist at ambient pressure. The conformational equilibrium is shifted by pressure in the accessible pressure range between 0.1 and 300 MPa. We have recorded x-ray data of Ras single crystals at various pressures up to 900 MPa. The high structural precision of the x-ray data is used to interpret the observed chemical shift changes in structural terms and to separate simple compression effects from structural transitions.

**References:** [1] Kalbitzer, H. R. (2015) High Pressure NMR Methods for Characterizing Functional Substates of Proteins. In "High Pressure Bioscience - Basic Concepts, Applications and Frontiers" (K. Akasaka and H. Matsuki, eds, pp. 179-198), Springer, Heidelberg, Germany. [2] Fourme, R., Girard, E., Akasaka, K. (2012) High-pressure macromolecular crystallography and NMR: status, achievements and prospects. *Curr Opin Struct Biol* 22(5):636-642. [3] Kalbitzer, H. R., Rosnizeck, I. C., Munte, C. E., Puthenpurackal Narayanan, S., Kropf, V., Spoerner, M. (2013) Intrinsic Allosteric Inhibition of Signaling Proteins by Targeting Rare Interaction States Detected by High-Pressure NMR Spectroscopy. *Angew. Chem. Int. Ed.* 52, 14242 -14246.

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NMR studies of membrane protein insertase machineries  
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There are two major types of integral membrane proteins:  $\alpha$ -helical membrane proteins and  $\beta$ -barrel outer membrane proteins (OMPs). While OMPs are found exclusively in the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts,  $\alpha$ -helical membrane proteins reside in the inner membranes of various cellular organelles. Proper folding into the correct biological membrane is carried out by a dedicated set of essential protein insertases. These are the evolutionarily conserved BAM-complex for OMPs and the YidC/Oxa1p/Alb3 family and the SEC translocon for inner membrane proteins. Although apo-structures of these insertases have been determined by crystallization, intermediate states that occur along the insertion pathways are not known at the atomic level. We aim to fill this gap by employing high resolution NMR spectroscopy and complementary biophysical techniques.

Extensive solution NMR studies of BamA showed that the  $\beta$ -barrel in solution populates a slow exchange equilibrium of various conformations due to the dynamic gated region between the first and the last  $\beta$ -strand. Based on sequence-specific resonance assignments, we now aim to stabilize various intermediate states from the observed dynamic ensemble in NMR. Here, we report the successful application of solution NMR spectroscopy for screening of nanobodies to shortlist the targets for crystallization. As a result, the three crystal structures of BamA-nanobody complex obtained correspond to BamA stabilized in two different conformations, i.e., open and closed gate.

Towards understanding the mechanism of client insertion into the inner membrane, we aim to understand the YidC-only insertase activity. Several single- or double-helical clients such as M13, Pf3, F0C have been characterized as the clients of YidC. We aim to monitor the insertase process at the atomic level by stabilizing various intermediate states of client(s) bound to YidC in membrane environment. Here we present the first steps of sample preparation optimization of YidC and one of its clients.

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**Solid-State NMR and DNP Spectroscopy Study of Room Temperature CO Oxidation catalyzed by Supported Pt Nanoparticles**

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A series of 1 and 2 nm sized platinum nanoparticles (Pt-NPs) deposited on different support materials, namely  $\gamma$ -alumina ( $\gamma$ -Al<sub>2</sub>O<sub>3</sub>), titanium dioxide (TiO<sub>2</sub>), silicon dioxide (SiO<sub>2</sub>) and fumed silica are investigated by solid-state NMR and Dynamic Nuclear Polarization Enhanced NMR Spectroscopy (DNP). DNP signal enhancement factors up to 170 enable to gain deeper insight on the Pt-NPs surface chemistry. Carbon monoxide is used as probe molecule to analyze the adsorption process and the surface chemistry on the supported Pt-NPs. The studied systems show significant catalytic activity in carbon monoxide oxidation on their surface at room temperature. The surface carbonate and bicarbonate species are formed only on the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> surface, which indirectly indicates CO oxidation reaction. The underlying catalytic mechanism is the water-gas shift reaction. Similar carbonate formation is also observed when physical mixtures of neat alumina with silica, fumed silica and titania supported Pt-NPs are studied. Natural abundance carbonate species on bulk  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> were detected which originated from carbon dioxide adsorbed from atmosphere. This impressively demonstrates the potential of DNP to study the surface chemistry on supported metal NPs using non labeled reactants.

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Protein instability as a virtue: High pressure relaxation dispersion NMR reveals partially folded dynamic intermediates in Cadherin-11 dimerization

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Classical Cadherins are calcium-dependent cell-cell adhesion proteins which comprise type I and type II Cadherins. Of the five extracellular domains EC1-EC5, EC1 dimerizes as part of the adhesion process. A number of beta-strand residues of the two interacting EC1 domains are swapped. The strand-swapping region includes residues Trp2 in all classical Cadherins and Trp4 in type II Cadherins, which can insert into the hydrophobic pocket of the interacting protein. The role of strand-exposed intermediates in type II Cadherin is still elusive in the context of N-domain (N>2) constructs.

Cadherin-11 is mostly dimerized at concentrations relevant for NMR. High pressure experiments can generally increase the population of states with a smaller partial volume and, more specifically, dissociate dimers. We gained experimental access to various conformational, including monomer, states using high pressure and temperature titrations, in combination with customized relaxation dispersion (RD) experiments. Multiple peaks per residue as well as peak broadening are encountered frequently. RD experiments confirm slow, but also intermediate/slow and intermediate/fast exchange processes. In addition to an unfolded state appearing at >1 kbar, we find partially folded intermediate states as part of the multi-site kinetics of Cadherin II dimerization, in both EC1 and EC1-EC2 constructs. Our recent theoretical work on multi-site RD experiments assists in data analysis (Palmer AG, Koss H, Methods Enzymol. 2019;615:177).

Our research shifts the focus of research to the Trp binding pocket, which is partially unfolded in intermediate states. Mutant construct data reveal that Trp2 and Trp4 can insert independently into their binding pockets. We also discuss whether partial unfolding of this binding pocket is facilitating dimer formation or dissociation. The observation of cold denaturation under various conditions, in conjunction with a large binding interface which is characteristic for type II Cadherins, suggests that partial protein instability is regulating type II Cadherin dimerization kinetics.

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**Novel alignment media for the measurement of RDC to  
natural products and organic molecules**

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Residual dipolar couplings (RDCs) have proven to be an invaluable anisotropic NMR parameter for the structural elucidation of complex biomolecules and organic molecules. However, a remaining bottleneck limiting its wider use by organic and natural product chemists is the lack of a range of easily applicable aligning media for diverse organic solvents. In recent years, we have developed three type media for the RDC measurement in organic solvent. The first media graphene oxide (GO) liquid crystals (LCs) and polymer brush grafted GO LC were developed to induce partial orientation of organic molecules to allow RDC measurements in polar organic solvents; the second alignment medium Oligo peptide AAKLVFF liquid crystal also was developed for the medium-polarity solvent MeOH; the third medium is the novel helical polyisocyanopeptide (L,L-PIAF-OBn) which showed the low critical concentration in aploar solvent chloroform. Those developed medium offer versatile and robust methods for RDC measurements that may routinize the RDC-based structure determination of organic molecules.

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**Phosphorylation of BAF, a small protein at the interface between nucleoskeleton and DNA**

Agathe Marcelot, Agathe Petitalot, Camille Samson, Florian Celli, François-Xavier Theillet, Sophie Zinn-Justin

Barrier-to-auto integration factor (BAF) is a 10-kDa abundant DNA binding protein essential for development in metazoans. It is involved in mitosis, nuclear assembly, viral infection and epigenetic regulation (Jamin & Wiebe, Curr. Opin. Cell Biol. 2015). The atomic structure of the BAF dimer is known. One dimer exhibits two binding sites for double-stranded DNA. BAF's ability to bridge distant DNA sites is essential for guiding membranes to form a single nucleus at the end of mitosis (Samwer et al., Cell 2017). Moreover, BAF is able to interact simultaneously with LEM-domain of proteins anchored at the nuclear membrane and lamins that form the nucleoskeleton (Samson et al., Nucleic Acids Res. 2018). A defect in BAF/lamin binding is observed in several progeroid diseases.

BAF function is regulated by vaccinia-related kinases (Nichols et al., Mol. Cell. Biol. 2006). BAF phosphorylation by VRK1 abrogates its interaction with DNA in vitro and reduces its association with the nuclear chromatin / matrix in cells. Deletion of VRK1 leads to BAF retention on mitotic chromosomes and abnormal nuclear assembly (Molitor et al., Mol. Biol. Cell. 2014).

We here present new data highlighting how BAF and several variants interact with double-stranded DNA. We reveal, using Switchsense, ITC and NMR, that phosphomimetic BAF mutant S4E and phosphorylated BAF weakly bind to DNA, but exhibit significant differences in their conformations and capacities to bind to the lamin A/C. Based on these results, we discuss the mechanisms of BAF function regulation by vaccinia-related kinases.

**Reference:** Ref. from the lab: Samson et al., Nucleic Acids Res. 46, 10460-10473 (2018).

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**P577****Interfaces in drug loaded nanosized Metal-Organic Frameworks**Marianna Porcino<sup>1</sup>, Ioanna Christodoulou<sup>2</sup>, Ruxandra Gref<sup>2</sup>, Charlotte Martineau-Corcoss\*<sup>3</sup><sup>1</sup>CEMHTI-CNRS, UPR 3079, <sup>2</sup>ISMO-UMR CNRS 8214,<sup>3</sup>ILV-UMR CNRS 8180 & CEMHTI-CNRS UP 3079

Iron-based nanoscale metal-organic frameworks (nanoMOFs) have demonstrated their medical applications owing to the versatility of their structural features, low toxicity, stability, and tailored functionality. Among them, nanoMOF MIL-100(Fe) is a promising candidate, which properties can be tailored by coating the particle surface with cyclodextrin phosphate (CD-P) and which can accommodate large amount of drug. If the medical applications of the coated nanoMOFs have been studied, little information is known about the atomic level interactions between the CD-P coating and the nanoparticle (NP) surface sites, or about the drug/MOF interactions. As a model of the paramagnetic nanoMIL-100(Fe) compound, we have chosen to focus on its diamagnetic analogue, namely nanoMIL-100(Al). The MIL-100(Al) topology is similar to that of MIL-100(Fe), it was shown that large amount of drugs can be incorporated, and that the surface can also be efficiently coated by CD-P. The main challenges to address in this investigation were i) the complexity of the system, which yields broad overlapping <sup>1</sup>H MAS NMR spectra even at high field and fast-MAS, and ii) the low quantity of the interface species.

We took benefit from the heteroatoms present: <sup>27</sup>Al, arising solely from the nanoMOF and <sup>31</sup>P, arising solely from the coated CD-P or the drug to investigate both the inner and outer surfaces of CD-P coated nanoMIL-100(Al) using a set of dipolar-based homo- and hetero-nuclear recoupling NMR experiments. In particular, the spatial proximity between the <sup>27</sup>Al and <sup>31</sup>P nuclei were evidenced, providing for the first time the signature of the Al surface species present in nanoMIL-100(Al) and linked to the CD-P. We then pushed further our <sup>27</sup>Al-<sup>31</sup>P ssNMR methodology to probe the interactions between nanoMIL-100(Al) and a loaded drug, adenosine triphosphate. We provide here a general NMR methodology to study external and internal interfaces in these nanoMOFs systems.

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Optimization of side-arm parahydrogen-induced hyperpolarization of [1-13C] pyruvate based on long-range heteronuclear J-coupling networks in esters and its application to 13C metabolic MRI.

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Pyruvate is a hub metabolite of glucose and its metabolic flux is highly sensitive to tumor microenvironment. Success in clinical trials of hyperpolarized <sup>13</sup>C MRI of pyruvate metabolism using dissolution dynamic nuclear polarization (dDNP) have reignited interest in parahydrogen induced polarization (PHIP) as a low-cost alternative means to prepare hyperpolarized <sup>13</sup>C-tracers. In side arm hydrogenation (SAH) PHIP, an ester precursor of pyruvate with unsaturated alcohol is prepared, hyperpolarization of <sup>1</sup>H is induced by parahydrogenation of the unsaturated alcohol side arm and the <sup>1</sup>H spin order can be transferred to the carboxyl <sup>13</sup>C nuclei by magnetic field cycling (MFC), followed by hydrolysis of the side arm to generate hyperpolarized pyruvate. The timing and field strength of MFC required to obtain the maximum <sup>13</sup>C polarization can be theoretically simulated if the spin-spin coupling (J-coupling) network of the <sup>13</sup>C labeled tracer is known.

In this study, we determined long-range heteronuclear J-coupling values of acetate and pyruvate precursors experimentally on a Bruker 800 MHz NMR spectrometer using the CLIP-HSQMBC technique for 2/3-bond J<sub>HC</sub> couplings and the sel-HSQMBC-TOCSY approach for 4/5-bond J<sub>HC</sub> <0.5 Hz, and numerically using DFT simulations. The timing of magnetic field change for MFC was optimized by density matrix simulations using the J<sub>HC</sub>-values determined. Preparation of hyperpolarized [1-<sup>13</sup>C]pyruvate was conducted either manually or using an automated PHIP hyperpolarizer consisting of a parahydrogenation reactor controlled by electromagnetic valves and placed at the center of zero-field chamber at <0.1 μT and a sweep coil for MFC control. <sup>13</sup>C NMR signal enhancement > of 30,000x was observed at 1.5T. Production of <sup>13</sup>C-lactate was observed by <sup>13</sup>C MRS when hyperpolarized [1-<sup>13</sup>C]pyruvate was mixed with tumor homogenate or intravenously injected into a mouse. Collectively, our results show that the PHIP-SAH is a promising cost-effective tool for hyperpolarized <sup>13</sup>C metabolic MRI studies.

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**P579****Molecular dynamics in ionic liquid/polymer gels investigated by relaxation, diffusion and double quantum low field NMR**Carlos Mattea\*, Linus Krämer, Siegfried Stapf  
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Molecular dynamics of imidazolium-based ionic liquids (ILs) with Br anion [1] combined with poly(vinyl alcohol) (PVOH), as well as structural features and conductivity in these gels is investigated using relaxation, diffusion and double quantum low field NMR.

The combination of ILs solvents with polymers offers the possibility of new applications in the field of material science in particular for energy storage (batteries, electrolytes, supercapacitors), and functional materials (electroresponsive gels for actuators). ILs have the clear advantage of replacing conventional organic solvents or water, for instance, in polymer electrolyte membrane fuel cells and batteries. However, how to confine or even immobilize ILs in polymer matrices while retaining transport and flexibility inside these systems remains still as an important challenge. On a microscopic level, the restriction in mobility of ILs and polymer chains, determined by the nature of the interactions between the monomers and the ions, can be assessed by NMR relaxation times. The anion of the IL in these systems determines whether the polymers form rigid structures where ILs are confined by retaining high translational dynamics [2, 3], or they form homogeneous gels where polymer and ILs have slow dynamics, giving rise to residual dipolar couplings (RDCs) probed by double quantum (DQ) NMR of chains. It is show in this presentation that different concentrations in PVOH-IL networks correlates with the degree of the amorphous part.

The results are discussed in terms of the relative interactions among PVOH, the anion and the cation, as well as what is the factor that regulates hydrogen bond formation between polymer chains and cations.

**References:** [1] A. Ordikhani-Seyedlar, S. Stapf, C. Mattea, J. Phys. Chem. B, 121, 5363-5373 (2017). [2] C. Mattea, B. Gizatullin, S. Stapf, Magn. Reson. Imaging, 56, 126-130 (2019). [3] A. Ordikhani Seyedlar, S. Stapf, C. Mattea, Magn. Reson. Chem., (2019) Doi: 10.1002/mrc.4852.

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**NMR exchange spectroscopy for quantification of  
xenon host molecules via binding kinetics**

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Hyperpolarized xenon (hpXe) has emerged as a powerful NMR probe for imaging and spectroscopy applications. Its ability to associate with hydrophobic molecular structures has enabled the design of biosensors as conjugates of synthetic xenon host molecules and target specific sensor molecules. Due to saturation transfer from host-bound to freely dissolved hpXe, biosensors can be detected with exceeding sensitivity at nanomolar to picomolar concentrations. A number of important biomarkers, e.g. metalloproteinase activity for cancer detection, cellular receptor recognition by antibodies, or peptide presentation by the MHC-complex, were successfully targeted with this type of biosensors and made amenable to a qualitative analysis by hpXe-NMR. However, the quantitative understanding of such processes, i.e. the determination of the concentration of biosensors possibly in complex with the target molecules, is much more difficult to achieve. The usual approach of qNMR, to obtain numerical results by the comparison of the signal intensities with those from a standard, fails here, as the saturation transfer depends not only on the amount of substance present in the solution but also in the host-xenon exchange kinetics. We thus set out to develop an experimental approach to accomplish a quantitative analysis of the xenon exchange process and thereby enable quantification of the biosensors. The exchange kinetics depends on the amount of freely dissolved xenon, and the power and duration of the saturation irradiation, all of which are under control of the experimenter. Various experimental schemes where these parameters are deliberately varied to numerically disentangle the exchange process, are discussed and convincing data are presented for their application to the well-known xenon host molecules cryptophane-A and cucurbit[6]uril. The work demonstrates the feasibility of quantitative evaluations of NMR biosensing studies using hpXe-based biosensors.

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Determination of structural composition of the  
inverse spinel ferrites using <sup>57</sup>Fe-Zero Field Nuclear Magnetic Resonance  
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<sup>1</sup>Bangalore University, <sup>2</sup>Indian Institute of Science

We have demonstrated <sup>57</sup>Fe Zero Field Nuclear Magnetic Resonance (ZFNMR) as a powerful tool in determining the structural composition of nickel-cadmium spinel ferrites of various compositions of Ni<sub>1-x</sub> Cd<sub>x</sub>Fe<sub>2</sub>O<sub>4</sub> from  $x = 0$  to 1, which are synthesized via one-step auto combustion technique. The XRD measurements confirm the phase purity of all the samples. Vibrating Sample Magnetometry (VSM) measurements show that saturation magnetization (MS) increases initially (up to  $x = 0.3$ ) and then decreases for higher concentrations of cadmium. The Fe<sup>3+</sup> ions in the inverted spinel ferrite distribute equally among two possible sites (tetrahedral A and octahedral B) with different hyperfine fields. Therefore for  $x = 0$  under the assumption that Ni enters B sites, <sup>57</sup>Fe NMR of Fe<sup>3+</sup> ions yield two signals of equal integral intensities in spectral lines corresponding to these sites. Thus, for the sample series Ni<sub>1-x</sub> Cd<sub>x</sub>Fe<sub>2</sub>O<sub>4</sub>, the contribution of Fe<sup>3+</sup> nuclei varies for A and B sub-spectra with the substitution of a non-magnetic Cd<sup>2+</sup> ion. By measuring the Fe<sup>3+</sup> distribution on A and B sites which is determined from relative spectral areas of A and B NMR sub-spectra the cation distribution is estimated and has been verified by the binomial distribution. Further, XRD Rietveld refinement results are also in good agreement with the composition estimated by NMR technique and the ideal composition. We have demonstrated the usefulness of NMR technique to quantify the accurate composition of the mixed spinel ferrite systems using Ni-Cd ferrite as a test case. Further, the estimated inversion parameter (at around  $x = 0.4$ ), for the studied system, obtained from ZFNMR, XRD, and VSM techniques are in excellent agreement with each other.

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**In-cell molecular structure and dynamics of  
photosynthetic membrane using solid-state NMR**

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Most of the structural and functional information about biological macromolecules is obtained from experiments in vitro, far from their physiological context. Elucidating the structure, dynamics, function and molecular interactions of such complex molecules in their cellular context is necessary to understand physiological processes. NMR spectroscopy is an upcoming technique to investigate structural and dynamical features of macromolecules at atomic resolution in living cells. To date, in-cell solution and solid-state NMR approaches have been successfully applied to both prokaryotes and eukaryotic cells to study the structure and dynamical behavior of macromolecules in their natural environment [1].

We use solid-state NMR in combination with biosynthetic uniformly <sup>13</sup>C isotope labeling to elucidate chemical profiles, molecular structure and dynamics of photosynthetic membranes in functional states in intact *Chlamydomonas reinhardtii* algae cells. Wild-type and mutant cells are used to follow structural-dynamic changes that correlate with physiological membrane responses in the cell. The morphology of cells and the oxygen production activity are checked after each NMR experiment. This unique approach enables us to gain more insight on the characteristics of thylakoid membrane by simultaneously detecting proteins and lipids and following their molecular dynamics directly inside the living cell.

**Reference:** [1] Luchinat, E.; Banci, L. IUCrJ 2017, 4, 108.

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### Estimation of Proton Transfer (PT) rates of Uracil derivative in an entrapped environment: A three site vs. two site exchange Model

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Pyrimidine base namely Uracil and its derivatives have immense biological relevance. The Proton Transfer (PT) rate in solution for these molecules determines the nature of solvation, cellular mobility and effect of solution pH on the biological activity. Therefore, quantification of PT rate has attracted attention over the years[1,2]. In the present study, an attempt has been made to avail quantitative description of PT of 5-fluorouracil in an entrapped environment[3]. 5-FU being an anti-cancer drug has been used as model system to understand behavior of drug nanocarriers. In this context, PLGA microsphere has been used as the encapsulating agent. A set of 1D NMR experiments involving shaped selective pulses has been employed to quantify the exchange dynamics observed in encapsulated 5-FU and further compared with that of the free state of the molecule. To evaluate the relevant exchange rates, a two site exchange model in case of entrapped environment (microsphere) and a three site exchange model in case of the free state of 5-FU have been considered. Non-linear least squared fitting procedure based on the analytical solution of the relevant Bloch-McConnell equation has been opted for extracting the exchange rate in both the cases. 1D selective NOESY and 1D multiply selective inversion recovery experiments were found to be suitable for the free and the encapsulated state of 5-FU respectively. The PT rates extracted for imino protons within the microsphere exhibited several fold increase in exchange rate indicating highly hydrated interior. Appropriateness of the experiments employed, effect of derivetization and the exchange model chosen are under investigation.

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Microfluidic NMR perfusion system for liver slice culture

Bishnubrata Patra<sup>\*</sup>, Manvendra Sharma, Marcel Utz  
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An integrated microfluidic perfusion system that allows in-situ metabolomic observation by NMR of liver tissue has been developed. Ex-vivo culture of the precision-cut liver slice (PCLS) can be a preferable alternative to animal models to study the effect of external factors like drugs and toxicity on living systems due to the requirement of significantly less resources [1]. Recent advances in the integration of microfluidic lab-on-a-chip technology with high-resolution NMR spectroscopy enable metabolic observation of biological process at  $\mu\text{l}$  sample volume [2]. Combination of the ex-vivo culture of PCLS with NMR spectroscopy presents an excellent opportunity to probe the effects of external factors (drugs, metabolites) in real-time.

The main challenge for this research was to prepare a microfluidic device culturing a tissue slice inside the NMR magnet with the supply of oxygen (80%) and carbon dioxide (5%) at  $37^{\circ}\text{C}$  and obtain the high-quality NMR spectra from  $\mu\text{l}$  sample volume. We obtained the proton NMR spectra of the perfusate, averaged over the duration of the entire five hours of the experiment. This spectrum gives an indication of the ultimate quality of the spectral information. At least 20 different metabolites are clearly apparent from this spectrum; most prominently, glucose, lactic acid, alanine, glutamine, glutamate, and lipophilic amino acids such as valine, leucine, and isoleucine. ATP/protein is a widely accepted measure of the viability of the tissue slices. A PCLS is considered viable if it has ATP/protein value of  $6\text{ pmol}/\mu\text{g}$  {Units} or more. All the slices showed ATP/protein value of  $6\text{ pmol}/\mu\text{g}$  {Units} or more from the culture inside the NMR magnet. The results clearly indicate the soundness of the design to obtain high-quality NMR spectra and show that sufficient nutrients and oxygen was supplied to the tissue slices by this mode of operation.

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Pushing the Sensitivity Boundaries in Magic Angle Spinning NMR of Challenging Biological Assemblies with the New Triple-Resonance BioSolids CryoProbe

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Despite breakthroughs in MAS NMR hardware and experimental methodologies making large biological systems accessible for atomic-level characterization, sensitivity remains a major challenge. Here, we report dramatic, 3-4 fold, sensitivity enhancements, in heteronuclear-detected experiments using a novel CPMAS probe, where the sample coil and the electronics operate at cryogenic temperatures, while the sample is maintained at ambient temperatures (CPMAS CryoProbe<sup>TM</sup>). While this technology is mature for solution NMR applications, it has not been available for triple resonance MAS NMR experiments until very recently, with the introduction of the Bruker BioSolids CryoProbe<sup>TM</sup>. The benefits of BioSolids CryoProbe<sup>TM</sup>-based experiments are discussed for assemblies of the HIV-1 capsid protein and for kinesin/microtubule assemblies – systems that are challenging to study using conventional MAS NMR probes. The sensitivity gains afforded by this technology have permitted the acquisition of outstanding-quality 2D and 3D homo- and heteronuclear correlation spectra, as well as of single-scan 2D <sup>13</sup>C-<sup>13</sup>C correlation spectra. Multidimensional data of this sort is otherwise inaccessible for such complex systems, even at high magnetic fields, due to intrinsically low sensitivity and the resulting prohibitively long experiment times. Data sets acquired with the BioSolids CryoProbe<sup>TM</sup> contain signals that are not otherwise detectable and which enable resonance assignments for the systems studied. We present further analysis of the sensitivity and resolution in these data sets. We envision that this probe technology is applicable to a wide range of systems and particularly beneficial for large biological assemblies with intrinsically low sensitivity.

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Synthesis of deuterated n-Dodecyl- $\beta$ -D-Melibioside  
detergent for membrane protein solubilization

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Lipids and detergents used for solubilization of membrane proteins can be deuterium-enriched to improve the quality of protein NMR spectra. Alkyl glycoside detergents have been instrumental in the X-ray structure determination of a number of GPCRs, starting with the first structures of the beta-adrenergic receptor solubilized in dodecyl maltoside (DDM). DDMB is a promising newer detergent that forms significantly smaller micelles than its isomer DDM. Here we describe the synthesis and purification of d25-dodecyl melibioside (DDMB) deuterated at the C12 tail.

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**In situ structural and dynamic analysis of a membrane protein**

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Yersinia Adhesin A (YadA) is a protein found in the membrane of Yersinia Enterocolitica, which is involved in a number of food-borne diseases including enterocolitis, acute enteritis, diarrhea, and mesenteric lymphadenitis.

YadA plays an important role in the ability of Y. enterocolitica to colonize a host, by aiding in the autotransport of a head domain to the cell surface that can stick to host tissues. A structure of the YadA anchor domain region (YadAM) in the microcrystalline form, and we now aim to solve structure in the e. coli outer membrane, which is asymmetric in nature, to gain insights on lipid interactions and dynamics. Preliminary results were published in 2015, which included 150 unique assignments. By focusing heavily on both optimization of both the sample preparation and data acquisition we have over 800 unique assignments. We have now set up structure calculations, as well as begun dynamic measurements

This project, if successful, will provide the first in situ structure of a membrane protein, as well as its dynamics in the asymmetric outer membrane.

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## H-MAS at 170 kHz and other new probe technologies

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Availability of new materials, precision machining and good design software have facilitated the progress also in NMR probes, a key to successful experiment. Particularly interesting is a possibility to record  $^1\text{H}$ -MAS spectra at  $>170$  kHz providing a 0.05 ppm homogeneous resolution which may become an alternative to, even supersede, the present “high-resolution” NMR. Despite a very small, ca 100  $\mu\text{g}$  sample amount, H-MAS sensitivity is comparable  $^{13}\text{C}$ -MAS and even solutions, while not kDa limited. Sub-mm rotors offer also other interesting experimental options. We demonstrate speed ramp and an extended temperature range in-situ MAS, applicable in catalyses, hyperpolarization and biofermentation.

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<sup>13</sup>C-based experiments to measure very fast chemical exchange processes.

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Roberta Pierattelli, Isabella Caterina Felli  
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Exchange processes with the solvent may reveal useful information about intrinsically disordered proteins and their interactions. We introduce a new method to evaluate amide proton exchange rates that relies on the creation of a triple spin order state, i.e.  $4C_zN_zH_z$ . This spin state is left free to evolve in a variable mixing time during which it undergoes decorrelation by chemical exchange. The attenuation of the signal was monitored through a series of CON-based experiments, one of the most common experiments added to the NMR toolbox and plotted as a mono-exponential decay. Exploiting carbon-start carbon-detected experiments allows to avoid perturbing water magnetization and to monitor also some residues that are not visible in HN-HSQC spectra. This experiment is suitable for those protons that exchange very rapidly with solvent's protons and allows to monitor exchange rate constants up to  $10^3\text{ s}^{-1}$ . The method is tested both on a model IDP,  $\alpha$ -synuclein, as well as on a well-known globular protein, Ubiquitin, in different conditions (pH and T). The results are compared with the ones derived from different approaches.

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### Applying NMR spectroscopy to monitor modifications and glycosylation in protein biotherapeutics

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Glycosylation and spontaneous modifications are critical quality attributes of protein therapeutics that can impact drug efficacy and safety. Therefore they need to be tightly monitored during production, formulation and storage. Mass spectrometry and fluorescence-detected chromatography are currently the dominating methods for the analysis of post-translational modifications (PTMs) including glycans. However, identifying the exact chemical structures, the attachment sites and populations within proteins containing many different modification sites is still a challenge. Therefore the development of orthogonal methods that can be used e.g. for cross-validation is important.

Recently we introduced the use of 2D <sup>1</sup>H-<sup>13</sup>C NMR correlation spectra for the analysis of post-translational modifications in denatured proteins at natural abundance and detected a variety of glycan components of several commercially available proteins (Schubert et al. 2015) and the spontaneous modification succinimide (Grassi et al. 2017). Peng et al. from the FDA applied the protocol to fragments of therapeutic monoclonal to study the composition of the major glycan forms (Peng et al. 2018). Here we extend the application of our approach to full-length therapeutic mAbs that does not require any cleavage. Determining the random coil chemical shifts of additional PTMs increases the repertoire of modifications that can be identified and quantified by NMR spectroscopy.

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Schubert, F., et al. (2015). "Posttranslational Modifications of Intact Proteins Detected by NMR Spectroscopy: Application to Glycosylation." ANGEW. CHEM. INT. ED. 54(11): 7096-7100.

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**A Self-Resonant Micro-Helix for Studying nano-Liter Volume  
[FeFe]-Hydrogenase Single-Crystals using CW and Pulse EPR Techniques**

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Thomas Happe<sup>2</sup>, Alexander Schnegg<sup>1</sup>, Dieter Suter<sup>3</sup>, Wolfgang Lubitz<sup>1</sup>

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Electron Paramagnetic Resonance (EPR) in single crystals is used to study paramagnetic intermediates at the active-site of an enzyme to fully resolve the magnitudes and orientations of the g-, hyperfine, and quadrupole tensors. The electronic structure, when combined with X-ray crystallography, is related to a proposed molecular geometry to gain a better understanding of the catalytic mechanism. Previous EPR studies on paramagnetic intermediates of a [NiFe]-hydrogenase within a 2  $\mu\text{m} \times 0.5 \mu\text{m} \times 0.5 \mu\text{m}$  single crystal have been very successful in the past.[1-3] However, single-crystal experiments of [FeFe]-hydrogenase were not possible due to significantly reduced crystal dimensions ( $< 30 \mu\text{m}$ ). New technical advances were developed in order to study these limited sample-volumes. Here we present a novel microwave resonant structure, the self-resonant micro-helix, for nano-liter samples that can be implemented in a commercial X-band (9.5 GHz) EPR spectrometer.

The self-resonant micro-helix (6.5-turns, inner diameter 400  $\mu\text{m}$ , height 1.2  $\mu\text{m}$ ) provides a measured signal-to-noise improvement of approximately 30 with respect to the best available commercial EPR resonators. A resonator efficiency of  $3.2 \mu\text{T/W}^{1/2}$ , which corresponds to a  $20 \mu\text{m} \times \pi/2$ -pulse at only 20  $\mu\text{W}$ , was experimentally determined. The increase of EPR signal intensity, resonator bandwidth of 90 MHz, and the very high resonator efficiency make the self-resonant micro-helix advantageous for advanced pulse EPR experiments (e.g., ESEEM and HYSCORE).

This advancement in resonator design has allowed, for the first time, the collection of EPR data from a  $0.3 \mu\text{m} \times 0.1 \mu\text{m} \times 0.1 \mu\text{m}$  single crystal of [FeFe]-hydrogenase in the  $\text{H}_{\text{ox}}$  state from *Clostridium pasteurianum* (CpI). Additionally, the HYSCORE spectra collected are the first published results from a protein single-crystal with dimensions less than  $0.3 \mu\text{m}$ . Full g-tensor and preliminary  $^{14}\text{N}$  hyperfine tensor analyses of the active-site cofactor from the single-crystal data will be presented and discussed.

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**Fast-microsecond dynamics of the water network in the active site of an enzyme**Himanshu Singh<sup>\*,1</sup>, Suresh Vasa, Harish Jangra<sup>3</sup>,Petra Rovó<sup>3</sup>, Hendrik Zipse<sup>3</sup>, Rasmus Linser<sup>4</sup><sup>1</sup>TU Dortmund, <sup>3</sup>Ludwig Maximilians University, <sup>4</sup>Department of Chemistry and Chemical Biology, Technical University of Dortmund

Protein-water interactions have widespread effects on protein structure and dynamics. As such, the function of many bio-macromolecules can be directly related to the presence and exchange of water molecules. While the presence of structural water molecules can be easily detected by X-ray crystallography, the dynamics within functional water-protein networks is largely elusive. Here we use solid-state NMR relaxation dispersion measurements with a focus on those active-site residues in the enzyme human carbonic anhydrase II (hCAII) that constitute the evolutionarily conserved water pocket, key for CAs' enzymatic catalysis. Together with chemical shifts, peak broadening, and results of DFT shift calculation, the data suggest the presence of a collective fast- $\mu$ s-time scale dynamics range from 50 to 150 microseconds in the pocket linked together by the water network. This process is abrogated in the presence of an inhibitor which partially disrupts the water-network. The timescale of the water-pocket motion coincides both with the estimated residence time of bound water in the pocket as well as with the rate-limiting step of catalytic turnover. As such, the reorganization of water pocket:enzyme architecture might constitute an element of importance for enzymatic activity of this and possibly other proteins.

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**Structural investigation of protein aggregates at natural isotopic abundance enabled by fast-MAS dynamic nuclear polarization solid-state NMR**

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<sup>1</sup>CEA Grenoble, <sup>2</sup>University of Iceland, <sup>3</sup>Bruker BioSpin, <sup>4</sup>University of Groningen, <sup>5</sup>Science Institute, Department of Chemistry, University of Iceland

Protein aggregates are the hallmark for many incurable protein-misfolding disorders and continue to be challenging targets for structural studies. Magic angle spinning solid-state NMR (MAS ssNMR) has proved particularly adept at characterizing these types of self-assembled samples. Given the correlations between atomic structure of aggregate polymorphs and their toxicity, and the influences of the cellular milieu on aggregate formation, it is imperative to examine protein aggregates under native conditions. However, the reliance on multidimensional <sup>13</sup>C/<sup>15</sup>N correlation spectroscopy limits or prevents applications to protein aggregates that are hard or impossible to label, such as animal- or patient-derived samples. In addition, the amount of *ex-vivo* sample available from these sources is conceivably very small (i.e. the sub-mg range). Here, we report a method for determining the structural fingerprint, by DNP-enhanced ssNMR, of protein aggregates at natural isotopic abundance (NA). [1] We show that when using the newly developed polarizing agent, AsymPolPOK, it is feasible to obtain <sup>13</sup>C-<sup>13</sup>C correlation spectra and structural restraints of aggregates at NA with DNP-enhanced ssNMR under Fast-MAS (i.e. 40 kHz) with ~1 mg of sample.[1, 2, 3] In addition, the advantages of simplified spin dynamics in dilute spin systems at NA will be discussed, with an emphasis on obtaining structural restraints in this regime. The results presented here were obtained on neurotoxic polyglutamine aggregates formed by exon-1 of mutant huntingtin protein and a peptide-based model of its polyglutamine amyloid core, which are implicated in Huntington's disease.

**References:** [1] Smith A.N. et al. "Structural Fingerprinting of Protein Aggregates by Dynamic Nuclear Polarization-Enhanced Solid-State NMR at Natural Isotopic Abundance" *JACS*, 2018. [2] Mentink-Vigier F., et al. "Computationally Assisted Design of Polarizing Agents for Dynamic Nuclear Polarization Enhanced NMR: The AsymPol Family" *JACS*, 2018. [3] Smith A.N. et al. "Natural Isotopic Abundance Solid-State NMR Enabled by DNP Under Fast-MAS" *In Preparation*.

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**Phosphorylation orchestrates the structural ensemble of the intrinsically disordered protein HMGA1 and modulates its DNA binding to the NF[ $\kappa$ ]B promoter**

Bastian Kohl, Xueyin Zhong<sup>2</sup>, Christian Herrmann<sup>2</sup>, Raphael Stoll<sup>\*,2</sup>

<sup>2</sup>*Ruhr University of Bochum*

High Mobility Group Protein A1a (HMGA1a) is a highly abundant nuclear protein, which plays a crucial role during embryogenesis, cell differentiation, and neoplasia. Here, we present the first ever NMR-based structural ensemble of full length HMGA1a. Our results show that the protein is not completely random coil but adopts a compact structure consisting of transient long-range contacts, which is regulated by post-translational phosphorylation. The CK2-, cdc2-, and cdc2/CK2-phosphorylated forms of HMGA1a each exhibit a different binding affinity towards the PRD2 element of the NF[ $\kappa$ ]B promoter. Our study identifies connected regions between phosphorylation sites in the wildtype ensemble shifted considerably upon phosphorylation, indicating that these posttranslational modifications sites are part of an electrostatic contact network that alters the structural ensemble by shifting the conformational equilibrium. Moreover, ITC data reveal that the CK2-phosphorylated HMGA1a exhibits a different DNA promoter binding affinity for the PRD2 element. Furthermore, we present the first structural model for AT-hook 1 of HMGA1a that can adopt a transient [alpha]-helical structure, which might serve as an additional regulatory mechanism in HMAG1a. Our findings will help to develop new therapeutic strategies against HMGA1a-associated cancers by taking posttranslational modifications into consideration.

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**$\beta$ -Sheet Augmentation Is a Conserved Mechanism of  
Priming HECT E3 Ligases for Ubiquitin Ligation**  
Carsten Stollmaier<sup>\*,1</sup>, Magnus Jäckl, Silke Wiesner<sup>3</sup>

<sup>1</sup>Carsten, <sup>3</sup>Universität Regensburg

The post-translational modification of proteins with ubiquitin (Ub) is catalysed by Ubiquitin ligases (E3s) and plays a fundamental role in cellular homeostasis by being a signal for protein degradation as well as for receptor internalization.

HECT-type ubiquitin ligases accept an activated ubiquitin from a ubiquitin-conjugating enzyme (E2) in a trans-thiolation reaction and conjugate this donor ubiquitin via an isopeptide linkage to a lysine in a substrate or in a ubiquitin in a growing poly-ubiquitin chain.

Here, we combine NMR studies, x-ray crystallography and functional assays to gain insights into the catalytic mechanism of HECT-family E3s, of which remarkably little is known.

We solved the crystal structures of the HECT domain's C-terminal part (C-lobe) of the Huwe1 and Smurf2 ligases loaded with donor-ubiquitin. Although Huwe1 and Smurf2 have different poly-ubiquitin chain specificities (K48 versus K63), we observed in both cases an augmentation of the  $\beta$ -sheet in the C-lobe by an additional  $\beta$ -strand which is contributed from the donor-ubiquitin.

We introduced mutations that, while not disrupting the overall fold (as seen from little perturbation to the 1H,15N-TROSY spectra), impair this  $\beta$ -sheet augmentation. These mutations nearly abolish ubiquitin transfer activity of the Huwe1 and Smurf2 HECT-domains, confirming the functional importance of the interface.

Moreover, we used NMR chemical shift perturbation (CSP) studies to investigate the C-lobe:Ub interface in solution. Interestingly, in addition to the expected interface, we observed CSPs that map to the flexible residues at the HECT domains C-terminus, which play a role in catalysing isopeptide linkage formation. This provides the first experimental evidence that thioester formation leads to a conserved conformational rearrangement of the C-terminal residues.

In conclusion, our results show that the  $\beta$ -sheet augmentation is essential for the trans-thiolation of ubiquitin from an E2 to an HECT E3. In addition, it affects C-terminal residues important for isopeptide formation.

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**NMR, a molecular microscope to characterize the  
peroxiredoxin-Hsp70 interplay slowing down aging**

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Peroxioredoxins (PRDXs) are thiol active peroxidases responsible for the regulation of peroxides in the cell thanks to the presence of a peroxidatic cysteine within their active site. Upon elevated peroxides levels, they are subject to catalytic inactivation by hyperoxidation, which involves structural modifications and leads to the exhibition of chaperone function.

Recently, the major cytosolic PRDX in *S. cerevisiae*, Tsa1, was shown to recruit the molecular chaperone Ssa1 to aggregated proteins through an H<sub>2</sub>O<sub>2</sub>-specific redox switch. Interestingly, the sulfinylation of Tsa1 supports Ssa1 binding to damaged proteins accumulated in the aging process and its subsequent reduction triggers the disaggregation of misfolded and aggregated proteins. This molecular switch between peroxidase and chaperone functions seems to be a key aspect of this functional mechanism. However, besides the fact that Tsa1 and Ssa1 can physically interact, the structural and dynamical details of this interaction at the molecular level, as well as the functional consequences for both enzymes, remain elusive.

Using advanced high-resolution NMR-spectroscopy, we are studying the mechanism of the Tsa1-Ssa1 interaction, in order to characterize this complex in detail and to decipher the functional consequences for both enzymes. As classical solution state NMR-approaches are limited regarding the size of the system (300 kDa), two approaches are considered. First, the characterization of stable smaller Tsa1-variants using sire directed mutagenesis. Then, the application of methyl-TROSY NMR experiments in combination with methyl-specific isotopic labeling for the study of the larger wild-type protein complex. Finally, for the characterization of the chaperone Ssa1, a divide-and-conquer approach is implemented to study both sub-domains of Ssa1 separately. Here, we present the initial characterization of Tsa1-WT and Tsa1-S78D variant correlated with first NMR experiments on specific-methyl-labeled Tsa1, as well as preliminary results on Ssa1 subdomains.

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**$\alpha$ -Deuteration makes kinases more amenable to NMR studies**

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<sup>1</sup>I2BC-CNRS, <sup>2</sup>Cortecnet

Kinases are effectors of cell signaling and drive cell fate to a large extent, from cell cycle progression, proliferation, differentiation etc.. They are important drug targets with no doubts. However, they are not always easily amenable to structural investigations because of their dynamic, multiconformational behavior. Regarding NMR studies, they are relatively large proteins of ~35- 40kDa, which calls for advanced labeling strategies.

In this context, we have explored the potential benefits of  $\alpha$ -deuterated amino acid labeling.  $\alpha$ -protons are responsible for about half of dipolar relaxation. Homogeneous deuteration is beneficial but generates longer T1-relaxation, hence long interscan delays and low signal-to-noise per experimental time. Selective  $\alpha$ -deuteration would thus be attractive, but is subject to enzymatic back protonation during recombinant expression in living organisms.

Using <sup>15</sup>N-amino acids  $\alpha$ -deuterated<sup>1</sup> and an *E.coli* cell-free expression system, we have produced 2D $\alpha$ /<sup>15</sup>N-Ala/Ser labeled 40kDa MAP-kinases. These samples generates exploitable <sup>1</sup>H- <sup>15</sup>N SOFAST-HMQC spectra (with ~30% slower T2( <sup>1</sup>H)) that require 10 times less recording time than standard TROSY- HSQC approaches. This permits to distinguish kinases conformations and to detect drug interactions, which should be helpful for pharmacological investigations.

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**Phosphorylation of the oncoprotein Mdm2 in physiological conditions monitored using improved <sup>13</sup>C-NMR**

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Helena Kovacs<sup>2</sup>, Manon Julien<sup>1</sup>, Rania Ghouil<sup>1</sup>, Wolfgang Bermel<sup>3</sup>  
<sup>1</sup>I2BC-CNRS, <sup>2</sup>Bruker Biospin, Switzerland, <sup>3</sup>Bruker BioSpin GmbH

The ubiquitin-ligase Mdm2 is the principal regulator of the tumor suppressor p53. Mdm2 activity is driven to a great part by phosphorylation states of dozens of phosphosites in its large disordered regions. The underlying mechanisms are still to be elucidated. The kinases responsible of these phosphorylation states are either constitutively active, either stimulated by various stress conditions. Hence, this protein is modified in its native forms, before and after stress. Studying Mdm2 at the structural level requires prior phosphorylation.. Here, we present the characterization of Mdm2 phosphorylation by 3 important kinases, CK1, CK2 and the DNA-damage response kinase DNA-PK.

Of course, NMR spectroscopy is the only technique that provides residue-specific, quantitative information on these multiple phosphosites, together with their conformational consequences. It is however limited to non-physiological conditions when it comes to disordered proteins characterization, because of increased water-amide proton exchange at pH>7 and T>293K. Combining regular 2D <sup>1</sup>H- <sup>15</sup>N and in-house improved 2D <sup>13</sup>Ca- <sup>13</sup>CO pulse sequences, we could monitor Mdm2 phosphorylation in vitro and in nuclear cell extracts at pH7.5, where phosphorylated sites acquire their real electric charge (-2). Hence, we reveal novel phosphosites and surprising phosphorylation preferences. We will also show their consequences on Mdm2 interactions with cognate partners like p53 or ribosomal proteins.

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A compact-rf-to-light transducer for Electro-Mechano-Optical NMR

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Radio-frequency signals can be up-converted to the optical regime through electromechanical and optomechanical couplings [1]. Applying this technique, we reported on proof-of-principle demonstrations of Electro-Mechano-Optical (EMO) NMR [2, 3]. Towards realization of practical EMO NMR, we present here the design and fabrication of a compact rf-to-light transducer that can be installed inside the body of an NMR probe. The main body of the module is a palmtop vacuum chamber equipped with hermetically sealed SMA ports and an optical window. Inside the chamber is installed a metal-coated SiN membrane oscillator serving as both a capacitor electrode and a mirror of an optical cavity, so that the oscillating metal layer transcribes the profile of NMR signals onto the optical carrier. The module fits into a widebore superconducting magnet.

**References:** [1] T. Bagci, A. Simonsen, S. Schmid, L. G. Villanueva, E. Zeuthen, J. Appel, J. M. Taylor, A. Sørensen, K. Usami, A. Schliesser, E. S. Polzik, *Nature* 507, 81 (2014). [2] K. Takeda, K. Nagasaka, A. Noguchi, R. Yamazaki, Y. Nakamura, E. Iwase, J. M. Taylor, and K. Usami, *Optica* 5, 152 (2018). [3] Y. Tominaga, K. Nagasaka, K. Usami, and K. Takeda, *Journal of Magnetic Resonance* 298, 6 (2019).

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Conformational Ensemble Studies of Proteins

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Conformational ensembles of intrinsically disordered proteins (IDPs) are particularly challenging to characterize. One can obtain information about the entire conformational ensemble of the IDPs by Solid-State NMR in frozen solution. From the experimental side, we have studied the sparsely isotope labelled IDP  $\alpha$ -synuclein ( $\alpha$ -syn) in frozen solution by Dynamic Nuclear Polarization NMR (DNP-NMR) under different conditions. By using Val [1, 2] residues as local probes for the conformational ensemble of the protein, we evaluated the inhomogeneously broadened line-shapes of the Val C $\alpha$ /C $\beta$  cross peak. We could estimate the amount of disordered regions in fibrillar  $\alpha$ -syn and delineate the membrane binding regions of  $\alpha$ -syn in contact with membrane surfaces in different protein to lipid ratios [3] Furthermore, we found that secondary chemical shifts of neighboring amino acids tend to be correlated, indicative of formation of transient secondary structure elements. Our approach thus provides accurate quantitative information on the propensity to sample transient secondary structures in different functional states

We are also studying the conformational space sampled by Isoleucine side chains in globular proteins like GABARAP and SH3 domain, as well as in intrinsically disordered proteins. Spectra of denatured, well-ordered and intrinsically disordered proteins provide more complete information about conformational states of IDPs in general and may yield insight into protein folding and unfolding.

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### Elucidating Complex Biomolecular Mixtures using 3D Correlation of Diffusion and Relaxation Ordered $^{13}\text{C}$ NMR Spectroscopy

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Jan A. Delcour, Johan A. Martens, Francis Taulelle, Eric Breynaert  
*KULeuven*

Complex molecular mixtures of biological origin are difficult to analyze. Arabinoxylan oligosaccharides (AXOS), cereal derived dietary fibers, are a typical example.  $^1\text{H}$ -DOSY-NMR is frequently used for determining mixture composition by diffusional separation, but in the case of AXOS, it falls short because of severe overlap of signals due to extensive presence of J-couplings, and the anomeric equilibrium inherent to any sugar component.  $^{13}\text{C}$ -NMR offers a larger chemical shift range and a wider spread of relaxation properties as compared to  $^1\text{H}$ . Together with the use of proton decoupling during acquisition, this yields easy to interpret first order spectra. J-based polarization transfer from  $^1\text{H}$  to  $^{13}\text{C}$  can be used to enhance sensitivity. Such polarization-enhanced  $^{13}\text{C}$ -DOSY has never been applied on biomolecule mixtures. A complication to overcome is signal attenuation due to relaxation processes occurring on timescales similar to the diffusion period implemented during the DOSY experiment. Smart selection of pulse sequences allows to cope with this complexity. Both the relaxation properties of the  $^{13}\text{C}$  spins and the size of the molecules play a crucial role in the selection of the pulse sequence yielding optimal resolution in the diffusion dimension. For small, slowly relaxing molecules the highest signal to noise ratio was obtained using  $^{13}\text{C}$ -DOSY with SE refocusing and  $^{13}\text{C}$  detection. Stimulated spin-echo polarization-enhanced  $^{13}\text{C}$ -DOSY with  $^{13}\text{C}$  detection resulted in the highest signal to noise ratios in the analysis of AXOS mixtures. In case of larger molecules,  $^1\text{H}$ -DOSY with  $^{13}\text{C}$  detection is the preferred option, partly overcoming the limits of the field gradient hardware. Three dimensional plots displaying  $^{13}\text{C}$  diffusivity, relaxation and chemical shift parameters in combination with conventional 2D-NMR correlation experiments (HSQC, COSY) offer readily accessible information on chemical functions and molecular size of oligosaccharide mixtures. This was demonstrated successfully using AXOS, obtained from enzymatically hydrolyzed arabinoxylan.

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Establishing a high-throughput 19F-NMR screening protocol to assess the binding capabilities of second messenger binding riboswitches and other RNAs.

Albrecht Eduard Völklein\*, Oliver Binas, Tom Landgraf,  
Christian Richter, Sridhar Sreeramulu, Harald Schwalbe  
*Goethe University Frankfurt*

Riboswitches are non-coding RNAs binding a specific second-messenger, modulating transcriptional or translational efficiency of their corresponding genes. These regulatory mechanisms are found in a wide variety of bacteria and are often associated with lifestyle changes like biofilm formation and transition into anaerobic metabolism.[1-3] These lifestyle changes include pathways required for the pathogenesis of *Vibrio cholerae* and *Clostridium difficile*. Novel small molecules, which bind to these riboswitches, could provide new tools to target these germs.

Herein we investigate the binding of multiple RNAs, including constructs of this riboswitch class, to a library of 106 fluorine labeled fragments. Our high throughput experiment design allows screening of all fragments on a specific RNA sample in as little as 3-4 days including RNA preparation. Utilizing 19F-1D and 19F-CPMG experiments binding can be confirmed by chemical shift perturbation and T2-time variation due to longer rotational correlation time while docked. The initial screening phase yielded several binding fragments. These leads were followed up with titration experiments and competition experiments adding native ligand to further elucidate binding affinity and mode. Some fragments were able to distinguish riboswitches even in cases of similar secondary structure.

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Towards point-of-care diagnosis and monitoring of trauma-induced coagulopathy using magnetic resonance relaxometry

Lau Wai Hoe, Nathan J. White, Russell L. Gruen, Tsin Wen Yeo, Konstantin Pervushin

Trauma induced coagulopathy (TIC), a dysfunctional blood coagulation, is a fatal disorder through blood loss. TIC is caused by inflammation and oxidative stress associated with tissue damage. Conventional assays for plasma analysis could not discriminate the fibrinogen oxidative state associated with bleeding disorder. Recently, the advancement of miniaturization NMR systems utilizing small and mobile permanent magnets holds a promise to rapidly analyse the haemostasis state of whole blood via density and porosity analysis of the resulting clotted gels. We propose to use a portable MR device for rapid diagnosis of TIC using multidimensional T1/T2 and diffusion/T2 correlations. Fibrinogen oxidation is associated with impaired fibrin polymerization. Increasing oxidation of fibrinogen exhibited distinctive NMR signatures which can be observable in high-field NMR. The multi-dimensional NMR was used to simultaneously measure transverse relaxation time (T2), longitudinal relaxation time (T1), and diffusion coefficient (D) of the water signal in the fibrin gels with different levels of oxidation. The results show that with increasing oxidation levels in fibrin gels, water signal was shifted with longer T1 and lower D in D/T2 correlations, which leading to decreasing rate of relaxation and diffusion. Water signal with increasing oxidation levels was also moving to longer T1 relaxation in T1-/T2 correlation maps indicating the surface relaxation and wettability of fibrin gels is affected by oxidation. Our preliminary data have shown that water signal is a potential biomarker for TIC. The multi component relaxation rate of water signal can be captured by these multidimensional correlations. We suggest oxidation of fibrin gel decreased water diffusion and the rate of T1, T2 relaxation, which reflecting that the contracted fibrin clot is densely packed but small fibrils, reduced porosity and wettability, which could not stem bleeding. TIC attributed by oxidation of fibrinogen would significantly affect the relaxation properties of blood and clot contraction.

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## NMR Alignment Media Based on Helically Chiral Polymers

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The measurement of residual dipolar couplings (RDCs) in orienting media has become an important tool for solving conformational and configurational problems in structural analysis of small molecules[1,2].

The model-free analysis of configurational problems using the recently developed software *ConArch+*[3] benefits from measurements in as many as possible independent alignment media. Beyond that, we are interested in enantiodifferentiating orientation and therefore, we try to combine these goals by the development of helically chiral polyacetylenes[4] (PPAs) and polyisocyanides[5] (PICs).

We synthesized different PICs based on various amino acids to investigate the influence of the side chain in relation to the strength of alignment and the enantiodifferentiation. The LLC phases prepared yield RDCs in the range of up to  $\pm 40$  Hz and show excellent orienting properties for a broad range of analytes bearing various functional groups. Additionally, we observed outstanding enantiodifferentiation for alcohols, ketones and carboxylic acids.

Additionally, we developed a strategy to functionalise achiral grapheneoxide (GO), which was first introduced as alignment medium by LEI[6], with helically chiral PPA. Thus, we generated a new chiral alignment medium which combines the positive aspects of both media (GO + PPA).

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$\epsilon$ -Fe<sub>2</sub>O<sub>3</sub>/SiO<sub>2</sub> nanoparticles ESR in-situ study. Spatial stabilization and the size effect.

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During the last decades systems based on  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> nanoparticles attract particular and growing attention. The  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> phase, is the intermediate between  $\alpha$ - and  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>.  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> phase, which displays high (up to 20 kOe) coercivity at room temperature, two magnetic transitions in low and high temperature region, and is stable only in the form of nanoparticles, thanks to its low surface energy [1].

The majority of works on the  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> phase is dedicated to the investigation of the magnetic properties of this phase and the search for the routes to synthesize  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> in the form of stable and bulk particles (up to 200 nm). Most researches try to study magnetic properties of the  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> without taking into account size effects. However in some cases, such as catalysis, studying a few nanometers sized particles can be crucial. In the Boreskov Institute of Catalysis  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub>/SiO<sub>2</sub> nanoparticle system was created for the first time that has no other detectable iron-oxide polymorphs with narrow size distribution of  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub> supported nanoparticles [2].

ESR method in situ in comparison with HR TEM, XRD, Mossbauer spectroscopy, and magnetization measurements data were applied to investigate the initial stages of the  $\epsilon$ -Fe<sub>2</sub>O<sub>3</sub>/SiO<sub>2</sub> nanoparticles formation [3,4]. It was shown that the stabilization of the nanoparticles precursor on the silica support is the key factor to obtain the system clear from admixture of other iron oxide polymorphs.

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Conformational dynamics of a wildtype and an evolved G protein-coupled receptor

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G protein-coupled receptors (GPCRs) are membrane-integrated signaling proteins that translate extracellular stimuli into an intracellular response for processes ranging from vision, smell and taste to immunologic, neurologic and reproductive functions. Only 16% of the superfamily are clinically targeted – and yet these comprise nearly 35% of all marketed drugs. Despite their obvious therapeutic potential, the molecular details of signal transduction remain largely unknown. GPCRs are notoriously difficult to study due to their expansive conformational landscape and dynamic nature, which substantially reduces their long-term stability for biochemical, structural, and pharmacological studies. Although their intrinsic dynamics make them intriguing targets for solution-state measurements, instability and poor prokaryotic expression has limited studies to a small number of NMR-active probes (15N-Val, 15N-Met, 19F-BTFMA). Applying directed evolution to the neurotensin receptor, we produced a functional construct (enNTS1) with enhanced bacterial expression, detergent and thermal stability. Most importantly, E. coli expression opens the door to robust uniform and selective isotope labeling strategies in a perdeuterated background. Using 19F-NMR we are currently comparing the conformational dynamics of our evolved en2NTS1 construct to wildtype NTS1. We have collected spectra of both proteins in the apo form as well as the antagonist, agonist and G protein-bound states. Our preliminary data suggests that en2NTS1 can occupy equivalent conformational space but with slower exchange kinetics. Interesting, receptor evolution introduced mutations in key functional areas such as the toggle switch region and sodium ion binding site, but retained functional activity at ~75% wildtype. We are exploring the effect of these amino acid substitution on receptor function, structure and dynamics using aromatic stereo-array isotope label (SAIL) and 13C-methyl labeled receptor. Finally, we’re applying real-time NMR techniques to monitor phosphorylation of intracellular sites following receptor activation. Taken together, we aim to draw a complete picture of GPCR activation and signaling in solution.

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