

The Richard R. Ernst Prize Lecture: In-cell NMR: progresses and challenges

Professor Lucia Banci¹

¹CERM, University of Florence, Florence,

Opening Session, Lomond, SEC, July 9, 2023, 16:00 - 18:40

The characterization of functional processes and the understanding of molecular pathways in the cell require their description both at system (e.g. a cell) and at molecular level, (e.g. atomic-resolution characterization of biomolecules). This approach calls for the development of suitable methodologies, capable of addressing multiple, specific, and sometimes non-conventional, features and amenable to characterize functional processes in living cells. On this respect NMR spectroscopy is a unique tool not only for characterizing the structure and dynamics of biomolecules but also for describing transient interactions and functional events with atomic resolution, possibly in a cellular context. In particular, in-cell NMR (i.e. high-resolution NMR spectra of biomolecules in intact, living cells) through its combination of the atomic-level characterization of NMR with its application to a cellular context can provide crucial insights on cellular processes and drug efficacy with unprecedented level of detail. Through the use of NMR bioreactors, a key advantage of NMR applied to living cells/organisms is the possibility to study time-dependent phenomena over a broad range of timescales, thus adding the fourth dimension to the characterization.

Methodological aspects and innovations will be discussed and a few examples of the striking power of this approach for the characterization of functional processes, such as metal transport in the cell, will be presented. Particular focus will be on the cellular uptake and target binding in the cellular milieu of drugs and leads and on the meaningful differences observed between drug-target binding in living cells versus in vitro.

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The Richard R. Ernst Prize Lecture: ESR and Biophysics at ACERT

Dr Jack Freed¹

¹Department of Chemistry and Chemical Biology Cornell University, Ithaca, United States

Opening Session, Lomond, SEC, July 9, 2023, 16:00 - 18:40

I will present a short overview of examples of our recent research at ACERT, which focuses on biophysical studies using ESR. First I describe how ESR may be used to study viral attack on host membranes for a variety of dangerous viruses. We focus on their fusion peptides, which insert into the host membrane. Emphasis will be given to the coronaviruses SARS-CoV-1 and SARS-CoV-2 ("SARS-1" and "SARS-2"). Next I describe how ESR methods may be used to study Intrinsically Disordered Proteins using the example of Tau, which forms structures on membranes that are determined by the membrane size. Then I show how this IDP, in changing from structureless monomers to fibers, yields binding features that can be reliably recovered only by using the Srivastava-Freed SVD method of pulse dipolar analysis. Only subtle changes in distance distribution of a blue-light sensor protein occur between dark and light cases that require the SF-SVD method of analysis to distinguish. 2D-ESR can be an effective tool to study dynamics occurring at and below microsecond timescales in proteins labeled with nitroxides and also molecular exchange in nitroxides by the use of 2D-ELDOR at 95 GHz, as will be shown. This 2D method can recover motions in the range of ns. to μ s., filling a gap with 2D-IR at ps. and 2D-NMR at ms.

(218)

Raymond Andrew Prize Lecture: Spinlocked electrons: From electron-electron distance measurements to dynamic nuclear polarization

Dr Nino Wili¹

¹Aarhus University , Aarhus, Denmark

Opening Session, Lomond, SEC, July 9, 2023, 16:00 - 18:40

In this presentation, I will describe two seemingly disconnected topics from my PhD thesis. 1) The use of spinlocking, together with pulse sequences in the nutating frame, to measure electron-electron dipolar couplings. The spinlock decouples the electron spin from the surrounding nuclear spin bath, increasing its phase memory time and allowing for the measurement of longer distances. 2) Pulsed Dynamic Nuclear Polarization. The simplest pulsed DNP sequence (NOVEL) is also a spinlock sequence, where the irradiation strength matches the Nuclear Larmor frequency. In this case, the nuclear spin bath is “recoupled”, and the electron polarization is “lost” to the nuclei. While NMR spectroscopists seem generally happy about this, I will show experiments (at low field) where the nuclear polarization is transferred back to the electrons again. This way, one can investigate how the polarization on nuclei close to the unpaired electrons decays and diffuses away. Hopefully, this opens a new way to do electron-nuclear double resonance (ENDOR) experiments.

During the talk, I will also have the chance to highlight how other people contributed, either with ideas, with samples, or with teaching me theory and experiments.

Plenary Abstracts

PL001 - Using parahydrogen to sensitise MR detection

Professor Simon Duckett¹

¹Centre for Magnetic Resonance, Department of Chemistry, University Of York, York, United Kingdom
Plenary 1 & 2, Lomond, July 10, 2023, 08:45 - 10:15

Hyperpolarisation techniques, where a Boltzmann spin state population is disturbed from its equilibrium position, are used to increase MRI and NMR sensitivity by several orders of magnitude.¹ A growing number of hyperpolarisation methods have been developed, and some of them are now finding use in clinical diagnosis. In the York facility we use parahydrogen as the source of polarization through processes called parahydrogen induced polarization (PHIP). In its original form, Weitekamp created molecular probes that incorporated atoms previously located in parahydrogen to increase MR sensitivity. More recently, one of several variants of this approach has been termed, signal amplification by reversible exchange (SABRE).² It reflects a route to hyperpolarise materials without changing their chemical identity and achieves its effect by creating a molecular catalyst to move the latent polarization of parahydrogen into a temporarily bound target molecule via its J-coupling network. The PHIP approach has therefore developed substantially from the early starting point of Weitekamp^{3 4}, Eisenberg⁵ and Bargon.⁶

This talk will focus on illustrating how parahydrogen can be used to improve the detection of metal dihydride and dihydrogen species.⁷ Subsequently, it will reveal how NMR can be used to establish a role for normally invisible species in hydrogenation⁸ and hydroformylation catalysis. It will then explore in more detail how the transient binding of parahydrogen to a metal complex containing a weakly bound ligand can lead to its efficient NMR sensitisation in solution.² Finally, more general studies of reactivity will be explored through parahydrogen sensitised NMR such that reaction intermediates can be seen in reactions that occur without hydrogen.⁹

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PL002 - Distance distribution information for ensemble structural biology

Professor Gunnar Jeschke¹

¹ETH Zürich, Department of Chemistry and Applied Biosciences, Zurich, Switzerland

Plenary 1 & 2, Lomond, July 10, 2023, 08:45 - 10:15

Introduction

Six years passed between the first publication of four-pulse double electron electron resonance (DEER) data in 1998 and the publication of the first distance distributions between spin labels for two membrane proteins. It took us two more years to publish the first structural models. Arguably, we then spent a decade on finding out the unique contribution of DEER to structural biology.

Aims

By using distance distribution information, we want to understand how nature uses a distribution of conformations for protein function. Often, this is related to state changes of proteins, for instance, between the apo and holo form, between a protein and its complex with RNA, or between a dispersed protein and the same protein in a biomolecular condensate.

Methods

Ideally, we want to have high-quality distance distributions up to the dimension of the protein chain at physiologically relevant concentration in the micromolar range. This required developments in instrumentation, measurement protocols, sample preparation, and data analysis, all based on understanding of spin dynamics including coherence loss mechanisms. In modeling, we needed fast prediction of spin-label conformations, virtually complete sampling of a large space of system conformations, and integration of the sparse distance distribution restraints with restraints from other experimental techniques.

Results

For RNA-binding proteins and their complexes with RNA, we found a two-state structure (RsmE/RsmZ complex), a structure with two states that each exhibit substantial conformation distribution (PTBP1/EMCD-IRES DtoF), disorder-to-less-disorder transitions (SRSF1 upon binding to RNA), a disordered domain collapsed onto a folded domain (dispersed) hnRNP A1, and random-coil behavior (FUS in the dispersed and condensed state).

Conclusions

Our findings suggest that membrane proteins as well as RNA-binding proteins populate an order-disorder continuum and tend to undergo incomplete disorder-to-order transitions upon binding events.

PL003 - Resolution and selectivity: new methods in small molecule NMR

Professor Gareth Morris¹

¹University Of Manchester, Manchester, United Kingdom

Plenary 3, Lomond, July 10, 2023, 18:15 - 19:00

No matter how expensive the spectrometer, we seem never to have enough resolution. Even 'small' molecules can pose problems, especially in mixtures. Multiplet structure is a mixed blessing: it gives us valuable structural information, but all too often it leads to a thicket of overlapping signals. Pure shift NMR, in which we temporarily sacrifice set aside the information contained in spin-spin couplings, suppresses the effects of homonuclear couplings to leave just one peak per chemical shift – hence the name. For proton NMR this can improve resolution by up to an order of magnitude, and a wide range of pure shift methods are already in use. Some recent developments, published¹⁻³ and unpublished, will be presented, including pure shift methods involving nuclei other than ¹H.

Selective excitation methods offer another route to improve resolution, but once again multiplet structure poses problems: ideally we would like to excite a single chemical shift, not a single frequency. The GEMSTONE variation on chemical shift selective filtration (CSSF)⁴ allows us to do this particularly efficiently, opening up a range of ultrasensitive 1D analogues of 2D experiments.⁵⁻⁷

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⁷Taylor et al., Chem. Commun. 59, 6734-6737 (2023).

PL004 - Ultra-High Field MRI: From Big Magnets to Small Coils

Dr Luisa Ciobanu¹, Nicolas Boulant¹, Alexis Amadon¹, Alexandre Vignaud¹

¹NeuroSpin, CEA-Saclay, Paris-Saclay University, Gif-sur-Yvette, France

Plenary 4 & 5, Lomond, July 11, 2023, 08:45 - 10:15

Magnetic Resonance Imaging (MRI) at ultra-high magnetic fields presents unparalleled opportunities for revolutionizing medical imaging. This plenary lecture aims to provide a fundamental overview of MRI at ultra-high magnetic fields and present the latest advancements in the field, encompassing both human and animal imaging. Ultra-high fields offer significant advantages in terms of sensitivity, contrast, and the acquisition of valuable biological information. However, harnessing these benefits comes with specific challenges, including increased susceptibility artifacts, radiofrequency (RF) field inhomogeneity, and limitations in gradient performance. This lecture will explore the strategies employed to overcome these challenges and achieve the anticipated improvements, focusing on enhanced hardware performance, including shims and radio-frequency detectors, as well as novel and improved imaging methods.

Today, human brain imaging reaches a magnetic field strength of 11.7T, while extreme field strengths ranging from 15-22T open new frontiers for small animal imaging and microscopic investigations. Drawing from recent technological advancements and breakthroughs achieved at NeuroSpin, we showcase the latest results obtained with ultra-high field MRI systems, shedding light on the immense potential this technology holds.

By attending this lecture, participants will gain insights into the capabilities of ultra-high field MRI, its challenges, and the latest developments in hardware and imaging techniques. Prepare to be inspired by the extraordinary world of ultra-high field magnetic resonance imaging.

PL005 - Zero- to ultralow-field NMR: developments and prospects

Professor Dmitry Budker^{1,2}

¹Helmholtz Institute, Johannes Gutenberg University Mainz, Gutenberg, Germany, ²Department of Physics, University of California, Berkley, United States

Plenary 4 & 5, Lomond, July 11, 2023, 08:45 - 10:15

In zero- to ultralow-field (ZULF) NMR, one does not need magnets in some or all of the three stages of an experiment: polarization, encoding, and detection. This unusual NMR modality has witnessed rapid development since the advent of compact and sensitive noninductive sensors, especially, atomic magnetometers that are now available commercially. We will discuss several recent ZULF NMR experiments carried out by our group and collaborators, demonstrating applications in areas as diverse as searches for beyond-the-standard-model particles and interactions, monitoring chemical reaction dynamics in highly inhomogeneous samples within metal catalytic reactors, and detection of breaking down of membranes of biological cells as a result of chemotherapy. ZULF NMR may be combined with hyperpolarization and radioactive detection overcoming the sensitivity limitations. Remarkably, a wide variety of hyperpolarization techniques (including dynamical nuclear polarization, photochemically and parahydrogen induced polarization) are being used in conjunction with ZULF NMR.

PL006 - A Molecular View of Carbon Capture on Surfaces

Professor Jeff Reimer¹

¹UC Berkeley, Berkeley, United States

Plenary 6, Lomond, July 11, 2023, 18:15 - 19:00

Introduction:

Carbon capture by adsorption onto surfaces represents one of the more promising platforms to mitigate anthropogenic emissions of carbon into the atmosphere. The design and analysis of porous adsorbents is informed by a molecular understanding of how CO₂ interacts with these materials, particularly in the presence of water at temperatures and pressures associated with the panoply of gas conditions. This is particularly true in the context of direct air capture with ~420ppm of CO₂.

Aims:

Herein I highlight solid-state NMR results that reveal the reaction of CO₂ in porous materials, with particular attention to designing experiments that inform pilot scale studies of carbon capture at modest scale.

Methods:

Solids NMR (MAS, HETCOR) methods are deployed following controlled dosing of adsorbents with ¹³CO₂ and H₂O. Several other methods, such as adsorption breakthrough curves and isotherms, support the NMR measurements.

Results:

There is a dynamic interplay between adsorbed ammonium carbonate, carbamic acid, and various carbonates that depend strongly on water co-adsorption.

Conclusions:

Two “pre-NMR” strategies portend carbon capture materials: utilizing cooperative adsorption mechanisms and employing computational screening in the context of carbon dioxide sources and sinks. These two approaches directed our solid-state NMR and DFT studies and allowed us to understand the role of molecular interactions and functional groups within porous materials, essential for advancing our knowledge of carbon capture.

* The author acknowledges the USorb-DAC and PrISMa teams led by Susana Garcia (Heriot-Watt University), in partnership with Berend Smit (EPFL), André Bardow (ETH), Georges Mouchaham (CNRS Paris) and Christian Serre (ENS/CNRS Paris). Some of these studies were informed by collaboration with Yi Cui (Stanford University) and Jeff Long (UC Berkeley). Co-researchers performing this work are called out individually during the talk.

PL007 - NMR Chemical Shifts Beyond Numbers

Professor Christophe Copéret¹

¹Department of Chemistry and Applied Biosciences, Eth Zurich, Zurich, Switzerland

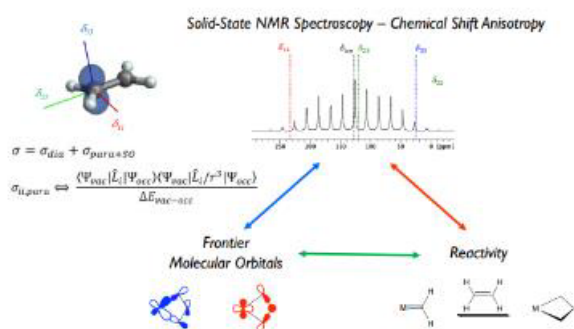
Plenary 7 & 8, Lomond, July 12, 2023, 08:45 - 10:15

Chemical shift has been successfully used since the beginning of NMR to identify the signature of molecules (and materials) making NMR an invaluable tool of characterizations. Because of its power to elucidate molecular structure, NMR interpretation is taught at early stage, often in laboratory courses, even before one understands the fundamentals of spectroscopy and their selection rules. We all remember solving organic and inorganic puzzles based on 1D and 2D NMR spectra during our undergraduate (and graduate...) times.¹

This lecture, targeted for all aficionados of NMR (and those who want to become one), will concentrate on developing a detailed understanding of the origin of NMR chemical shift, and how it can be used to reconstruct the electronic structure of molecules, in particular organometallic intermediates. This lecture will also aim to show that the angular momentum operator has an “ideal” symmetry, that makes NMR a privilege spectroscopic descriptor of reactivity.

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PL008 - Single-molecule magnetic resonance spectroscopy: methods to applications

Professor Jiangfeng Du¹

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Plenary 7 & 8, Lomond, July 12, 2023, 08:45 - 10:15

Magnetic resonance (MR) is one of the most important techniques for characterizing compositions, structure and dynamics of molecules. However, current methods need billions of uniform units on centimeter-scale to accumulate large enough signal-to-noise ratio. High sensitivity MR techniques are urgently needed for new applications on single molecule. A quantum sensor to accomplish single molecule detection is the nitrogen-vacancy (NV) defect center in diamond. By combining the quantum controls and long coherence time of NV, we have experimentally realized single molecule scale nuclear MR and electron spin resonance. This talk introduces the major works we achieved along this line. (I) Single molecule MR spectroscopy. We obtained the first single-protein spin resonance spectroscopy under ambient conditions [Science 347, 1135 (2015)], the electron spin resonance spectroscopy of single molecules under physiological conditions [Nature Methods 15, 697 (2018)], and developed the zero-field electron spin resonance spectroscopy on nanoscale [Nature Communications 9, 1563 (2018); Science Advances 6, eaaz8244 (2020)]. (II) Microscale MR imaging. Using NV sensor, we realized nanoscale MRI of ferritins in a single cell [Science Advances 5, eaau8038 (2019)], and the Immunomagnetic microscopy of tumor tissues with micrometer-resolution [PNAS 119, e2118876119 (2022)]. These results, together with the relation works in the field, open a door to nanoscale/single molecule MR and will be potentially used as a new tool on a broad range of scientific areas from life science to physics and chemistry.

PL009 - Hyperpolarized ^{13}C NMR metabolomics at natural abundance

Professor Patrick Giraudeau¹

¹Nantes Université, CNRS, Nantes, France

Plenary 9, Lomond, July 12, 2023, 18:15 - 19:00

NMR spectroscopy is a central method for metabolomics. However, it mostly relies on 1D ^1H spectroscopy, which provides an acceptable sensitivity but suffers from ubiquitous overlap between complex metabolite patterns. NMR offers a wide range of multi-nuclear and multi-dimensional techniques for analyzing complex samples, which have the potential to profoundly change the way metabolomics studies are conducted. In the past few years, we showed how fast 2D NMR methods could be systematically incorporated into metabolomics workflows, providing improved sample classification and/or biomarker identification. These methods –mainly homonuclear 2D experiments for sensitivity reasons– provide a first stage of dispersion improvement. ^{13}C NMR spectroscopy would be even more advantageous as it provides narrow singlets spread over a broad spectral range. In fact, ^{13}C NMR would be ideal for metabolomics, were it not for the fact that its low sensitivity is not compatible with the detection of low-concentrated analytes at natural abundance.

In this context, we recently showed that Dissolution Dynamic Nuclear Polarization (d-DNP) could provide a unique way to detect ^{13}C NMR metabolomics spectral signatures with a sensitivity enhanced by several orders of magnitude. After reaching excellent repeatability with a prototype d-DNP equipment, we showed that ^{13}C NMR at natural abundance could be applied to plant extracts and incorporated in a full metabolomics workflow. We then systematically optimized the parameters involved in our d-DNP setting, leading to major sensitivity and resolution improvements. Thanks to this optimization, we recently reported the first d-DNP-enhanced ^{13}C NMR analysis of a biofluid - urine- at natural abundance, offering unprecedented resolution and sensitivity for this challenging type of sample. We also showed that accurate quantitative information on multiple targeted metabolites could be retrieved through a standard addition procedure.

These results open many perspectives for ^{13}C NMR-based metabolomics at natural abundance, but also raise a number of analytical challenges in terms of metabolite identification, cost and throughput. We will discuss the potential of this new approach, as well as undergoing methodological developments based on multiple receivers or ultrafast 2D NMR that could further improve its performance.

PL10 - EPR spectroscopy at the interface to NMR: high field ENDOR and DNP

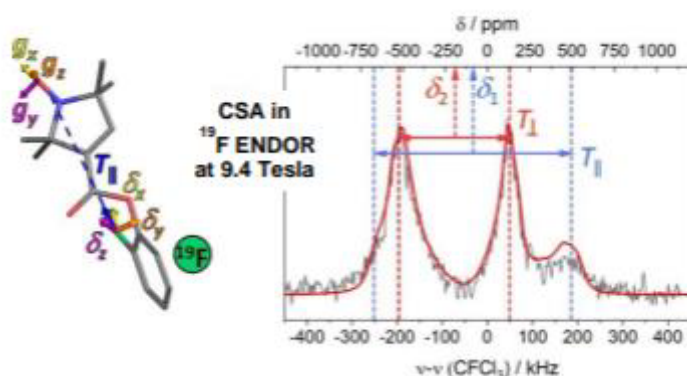
Professor Marina Bennati^{1,2}

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Plenary 10 & 11, Lomond, July 13, 2023, 08:45 - 10:15

Latest developments in magnetic resonance spectroscopy are aimed at increasing sensitivity and resolution for nuclear spin detection, which is limited by the small energy splitting at the available polarizing magnetic fields. A powerful approach is taking advantage of the larger magnetic moment of unpaired electron spins and hyperfine couplings to transfer polarization to nuclear spins. It provides the basis for polarization transfer experiments at the interface between EPR and NMR, which can be cross fertilized. The talk will illustrate recent progress in hyperfine spectroscopy, particularly electron-nuclear double resonance (ENDOR), as well as dynamic nuclear polarization at shared magnetic fields, in the solid and liquid state. In ENDOR, we can detect nuclear spins up to the second ligation sphere ($\lesssim 2$ nm) of a paramagnetic center. We have recently demonstrated that at magnetic fields comparable to NMR (9.4 Tesla), ENDOR can resolve ^{19}F chemical shift tensors, ^{17}O quadrupolar nuclei and, in conjunction with ^{19}F labelling, it can be employed for distance measurements in the angstrom to nanometer range. For DNP-enhanced NMR detection in liquids, we have recently implemented a new instrument at 263 GHz/9.4 Tesla with optimized NMR performance, for which the availability of 263 GHz ESR is mandatory. Progress and strategies for ENDOR and DNP applications will be discussed.

Figure 1: 263 GHz/9.4 Tesla ^{19}F -ENDOR spectrum of a nitroxide radical coupled to a ^{19}F nucleus at a distance of 6.6 Å. Asymmetry in the spectrum reveals chemical shift anisotropy, whereas the peak splitting arises from electron-nuclear dipolar coupling.



PL011 - Chasing the invisible: protein dynamics by MAS- and solution NMR

Prof Paul Schanda¹

¹Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria

Plenary 10 & 11, Lomond, July 13, 2023, 08:45 - 10:15

Visualizing the internal motions of proteins is often instrumental for the understanding the link between a 3D structure and the protein's function, but represents also a significant challenge for any experimental technique. In NMR, often the most interesting parts of proteins seem to become invisible. What makes those states invisible and how can we gain insight into functional dynamics? This presentation highlights the use of NMR, both in solution and in the solid state, and specific isotope labelling to probe dynamics at different time and length scales, ranging from local side chain rotations to entire domains unfolding.

We will show the use of specific isotope labelling schemes to zoom into motions of side chains at great detail. The introduction of isolated ¹H-¹³C pairs in different aromatic rings has allowed us to address questions like: how does the packing of proteins in crystals, or within an amyloid fiber, restrain side chain motions?

We will then show the somewhat unintuitive finding that the formation of a disulfide bond induces extensive μ s dynamics of a large part of a peroxiredoxin enzyme complex of >200 kDa. Lastly, on even longer length scales, we reveal an intriguing mechanism of a protein, which has evolved to stop itself from premature assembly, by co-existing with a state that is largely unfolded.

PL012 - Strong Gradients in MRI: Applications, Caveats, and Future Directions

Assistant Prof Chantal Tax^{1,2}

¹Cardiff University Brain Research Imaging Centre (CUBRIC), School of Physics and Astronomy, Cardiff, United Kingdom, ²University Medical Center Utrecht, Utrecht, Netherlands

Plenary 12 & 13, Lomond, July 13, 2023, 17:00 - 18:30

The launch of the Human Connectome Project in 2010 sparked a resurgence of interest in leveraging stronger gradients for MRI, particularly for quantifying tissue microstructural changes. In diffusion-weighted MRI, stronger gradients offer advantages such as shorter echo times, higher signal-to-noise ratios, and shorter diffusion times for a given b-value. This talk will explore the applications where strong gradients have demonstrated benefits in fundamental and clinical research. The caveats associated with strong gradients will be discussed, and the future of strong gradients in NMR and MRI will be considered, highlighting the potential for further advancements and their impact on increasing our fundamental knowledge and improved patient care.

PL013 - Investigating structure, dynamics, and intramolecular interactions in a peptide catalysed reaction by modern NMR methods

Jens Nowag¹, Matthias Brauser¹, Raffael C. Wende², Peter R. Schreiner², Professor Christina Thiele¹

¹Clemens Schöpf Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, Darmstadt, Germany, ²Institut für Organische Chemie, Justus-Liebig-Universität Gießen, Gießen, Germany

Plenary 12 & 13, Lomond, July 13, 2023, 17:00 - 18:30

We present our study on a peptide-based organocatalyst that enables the enantioselective monoacylation of racemic trans-cycloalkane-1,2-diols.¹ A dynamic binding pocket formed by the acylated catalyst intermediate was proposed by molecular mechanics as well as by DFT computations. Herein, we describe a structural study using RDCs and NOEs yielding a conformer ensemble that allows a detailed analysis of the catalyst's mode of action.²

Furthermore, we present an NMR titration strategy³ of the peptide catalyst alone and in mixtures with the two diol enantiomers to quantify intramolecular interactions by several complementary NMR methods. The values obtained are well in-line with the synthetic results and the observed selectivity. The results indicate that aggregation plays a key role in this system.⁴

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Tutorials Abstracts

TUT001: Beating the odds on Journal Acceptance

Prof Warren Warren

¹Duke University, Durham, United States

Tutorials Session, Lomond, July 11, 2023, 15:45 - 17:45

The scientific journal landscape is changing rapidly, particularly with the handful of highly selective journals which try to cater to broad science audiences. As an example, Science Advances (the open-access member of the Science family) receives about 20,000 submissions per year and ends up rejecting about 90% of them, mostly without review. Understanding the selection process (and thus avoiding some of the mistakes which make it harder for an editor or reviewer to see broad significance and impact) gives your work the best chance to be appreciated. I also expect to discuss the issues associated with open access and publication charges, the process by which manuscript „flow down“ on rejection, and the process for selecting reviewers and associate editors.

TUT001: Best Practice in Scientific Publishing

Professor Tatyana Polenova¹

¹University of Delaware, Department of Chemistry and Biochemistry, Newark, United States

Tutorials Session, Lomond, July 11, 2023, 15:45 - 17:45

In this tutorial intended for early-career researchers, I will discuss best practices in scientific publishing. I will give a broad overview of Journal of Magnetic Resonance and Journal of Structural Biology. I will then outline the publishing process, including a choice of a journal, considerations for how to prepare a strong paper, a general structure of a manuscript, the submission process, and authors' responsibilities. I will also discuss the peer review process, and touch upon general guidelines on how to become a good reviewer.

TUT002: Artificial intelligence for a busy NMR spectroscopist

Professor Ilya Kuprov¹, Prof Vladislav Orekhov²

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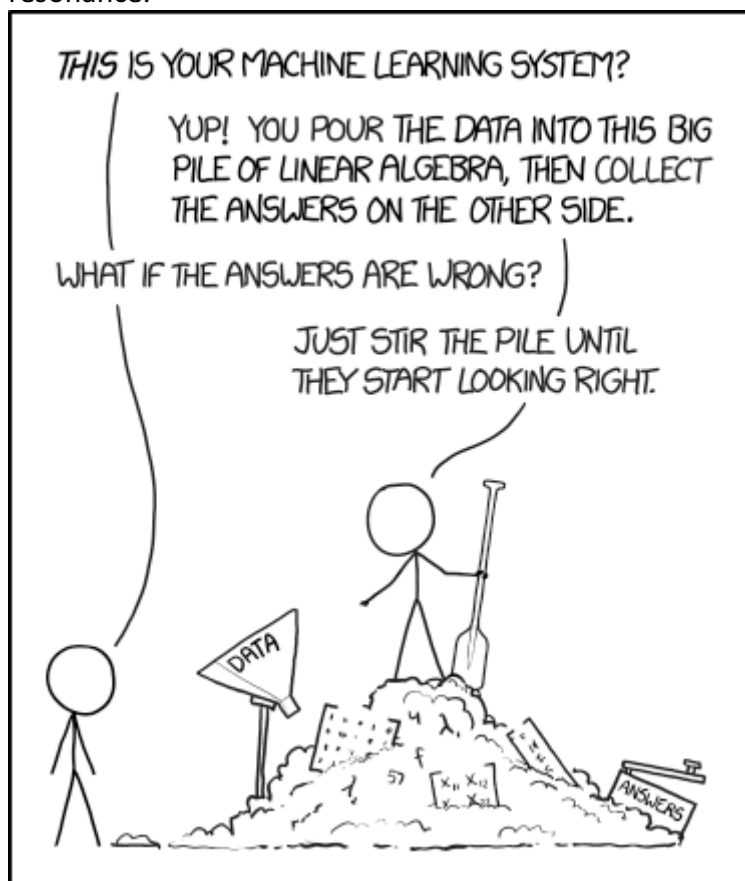
Tutorials Session, Lomond, July 11, 2023, 15:45 - 17:45

Computer scientists and technology start-ups would put on airs of sophistication and have you believe that AI is some ultra-modern wizardry. It is not: neural networks are just matrix-vector product sequences with simple functions in between, machine learning used to be called regression and statistics, and backpropagation is just carefully implemented chain rule. XKCD has a good summary in the figure on the right.

What did vastly improve in the last ten years is data availability and computing power. A threshold had been crossed – mostly by NVidia on the hardware side and by the Internet on the data volume – whereupon variations of existing techniques started yielding dramatic results.

Basic mathematical methods used in artificial intelligence and machine learning go back to 1805 (least squares fitting), 1874 (singular value decomposition), 1933 (probability calculus), 1943 (perceptron), and 1951 (stochastic gradient descent). In this tutorial lecture, we will start with that history and connect the latest newspaper drama to simple algebra – mostly familiar things from matrix quantum mechanics.

This tutorial lecture will come in two parts: first, a fog-lifting tour that will put the AI/ML diagrams and jargon next to the straightforward mathematical operations that are actually being performed; next, an overview of AI/ML data processing and experiment design in the context of magnetic resonance.



Invited Speakers Abstracts

INV001 - Time-resolved solid state NMR of protein folding, amyloid peptide aggregation, and other processes

C. Blake Wilson¹, Jaekyun Jeon^{1,2}, Kent R. Thurber¹, Wai-Ming Yau¹, Dr Robert Tycko¹

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Parallel 1: BioNMR, Lomond, July 10, 2023, 10:45 - 12:45

Introduction and Methods: We have developed experimental methods for probing time-dependent structural conversion processes in biomolecular systems on the millisecond time scale. We call these methods "time-resolved solid state NMR". In our experiments, a process of interest is initiated by rapid mixing of two solutions or by a rapid "inverse temperature jump", both of which can be accomplished in one millisecond or less. After a variable evolution period for structural evolution, the solution is frozen in less than one millisecond to trap transient intermediate states. Low-temperature, DNP-enhanced solid state NMR is then used to extract molecular structural information.

Aims and Results: Previous studies used time-resolved solid state NMR to characterize tetramerization process of melittin, a 26-residue peptide from bee venom, following either a rapid pH jump (from 3.0 to 7.0, see Jeon et al., Proc. Natl. Acad. Sci. 2019) or a rapid temperature drop (from 95° C to 30° C, see Wilson and Tycko, J. Am. Chem. Soc. 2022), and to characterize the process by which calmodulin forms complexes with a target peptide in the presence of Ca²⁺ (see Jeon et al., J. Am. Chem. Soc. 2020; Schmidt et al., Proc. Natl. Acad. Sci. 2022). This lecture will focus on more recent studies.

In one recent study, we used a combination of time-resolved solid state NMR and time resolved light scattering to examine oligomerization by the 40-residue amyloid- β peptide (A β 40), initiated by a rapid pH drop from 12.0 to 7.4 (see Jeon et al., Nature Commun. 2023). The combined data reveal that A β 40 molecules undergo a large change in conformational preferences, from random-coil to β -strand, in less than one millisecond, even before a significant population of dimers appears. Subsequent conformational changes are minor for many minutes, even as oligomers grow to sizes exceeding 100 molecules.

In another recent study, we applied time-resolved solid state NMR with rapid inverse temperature-jump methods to the folding process of HP35, a well-studied model system for protein folding that has been shown previously to fold within 10 μ s. While the NMR data are consistent with prior studies, they also reveal that the microsecond-timescale main folding event is followed by a millisecond-timescale annealing process in which the conformations and packing of amino acid sidechains in the HP35 core is optimized (Wilson et al., manuscript under review).

Conclusions: Results for melittin tetramerization, calmodulin complex formation, A β 40 oligomerization, and HP35 folding amply demonstrate the value of time-resolved solid state NMR methods in studies of the molecular mechanisms of process that have genuine biological importance and biophysical interest. Future studies will apply similar techniques to other classes of processes. For example, preliminary results from studies of liquid-liquid phase separation by low-complexity protein domains and studies of DNA hybridization may be presented, if time permits.

INV002 - Visualizing the multi-step folding trajectory of an intrinsically disordered scaffold protein upon binding to the small GTPase Rac1

Lenette F. École Normale Supérieure¹, Francesco S. Ielasi², Luiza Mamigonian Bessa¹, Laura Mariño Pérez¹, Agneta Kiss¹, Elisabetta Boeri Erba¹, Dr Guillaume Bouvignies³, Andrés Palencia², Dr Malene Jensen¹

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Parallel 1: BioNMR, Lomond, July 10, 2023, 10:45 - 12:45

Introduction

Activation of the pro-apoptotic c-Jun N-terminal kinase (JNK) cell signalling pathway is initiated by binding of the small GTPase Rac1 to the scaffold protein POSH (Plenty Of SH3s). Rac1 is known to recognize so-called CRIB motifs, however, POSH contains only a partial CRIB motif within its 180-amino acid intrinsically disordered region. The molecular details of how Rac1 recognizes POSH therefore remain elusive.

Aims

The aim of the study is to map the precise binding site of Rac1 on POSH, to reveal the structural basis for their interaction and to visualize the structural, dynamic and kinetic details of the folding-upon-binding mechanism of POSH.

Methods

The interaction between Rac1 and POSH is studied by NMR titrations in combination with chemical exchange saturation transfer (CEST) experiments. The thermodynamic profile of the interaction is elucidated by isothermal titration calorimetry, while the structural details are determined using X-ray crystallography.

Results

We identify a novel recognition mode for Rac1-binding to a non-canonical CRIB motif in the intrinsically disordered region of POSH. We demonstrate that the interaction involves two molecular recognition elements (MRE1 and MRE2) covering an impressive 55 amino acids of POSH, and we obtain the crystal structure of the POSH-Rac1 complex at 1.2 Å resolution showing complete folding of both MREs upon binding to Rac1. Using an extensive set of CEST experiments, we map the kinetic details of the folding trajectory of POSH upon binding to Rac1. We show that the interaction initially proceeds through binding and instantaneous folding of MRE1 followed by a reversible folding event of MRE2 on the second time scale on the surface of the GTPase.

Conclusions

Our work provides insight into the complexity of binding mechanisms employed by intrinsically disordered proteins and offers novel structural insight into effector recruitment by Rac1.

INV003 - The use of EPR spectroscopy to study metal transcription factors in-vitro and in-cell

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Parallel 2: EPR/ESR, Alsh, July 10, 2023, 10:45 - 12:45

Bacteria are skilled survivors in high-metal ion environments. Some metals are essential but can be detrimental above a certain concentration in the cytosol. Thus, bacteria have evolved complex processes to regulate intracellular metal homeostasis. Metal transcription factors play a role in this regulation. They respond to metal ions and control gene transcription to protect bacteria from metal-induced stress. Upon metal binding, the metalloregulator protein induces conformational changes in the DNA promoter sequence, leading to transcription initiation and subsequent transcription of genes that mediate adaptive responses to the toxicity of the targeted metal. X-ray crystallography and cryo-EM tools have provided insights into the structures of these transcription factors bound and unbound to the DNA and metal ions. However, intermediate conformations, which offer a better understanding on their mechanism of action, have not been targeted by these methods. EPR spectroscopy can help bridge these gaps. Our lab focuses on studying three copper-sensitive transcription factors from gram-positive and gram-negative bacteria: *E. coli* CueR, *M. tuberculosis* CsoR, and *S. pneumoniae* CopY. Using variety of spin-labelling techniques and EPR measurements on both protein and DNA, both in vitro and in-cell, we demonstrate how metal ions initiate conformational changes that regulate gene transcription. In this presentation, I will discuss the behaviour and control of gene transcription by different transcription factors.

INV004 - In situ and operando film-electrochemical EPR: a new tool that enables insights into surface-bound molecular electrocatalysts

Dr Maxie Roessler¹

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Parallel 2: EPR/ESR, Alsh, July 10, 2023, 10:45 - 12:45

Unpaired electrons play an important role in a wide range of redox-driven catalytic processes in both chemistry ¹ and biology ². Controlling their location and exploiting the interactions with their environment can provide key mechanistic information into these catalytic reactions. In this talk I will discuss how we have used and developed EPR-based techniques to gain mechanistic insights into electrocatalysis.

I will introduce film-electrochemical EPR spectroscopy (FEEPR) and show that it is a powerful tool to investigate surface-bound molecular catalysts, which are increasingly of interest in sustainable chemistry. With FE-EPR we have direct and accurate control over the redox state, even of 'buried' redox centres in proteins.³ We can further monitor the evolution of radicals during redox reactions, including catalysis, in real time under flow conditions, at room temperature and in aqueous solution.⁴ Such in situ and operando FE-EPR provides detailed insight into the mechanism of nitroxide-catalysed alcohol oxidation. FE-EPR gives access to substrate binding affinities, catalytic rate constants and reduction potentials during catalysis, and provides a new means of benchmarking electrocatalysts and their reactions. Lastly, I will provide an outlook for the application of FE-EPR to biocatalytic reactions.

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INV005 - MR meets microelectronics: Recent advances in chip-based MR detection

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Parallel 3: Hardware, Boisdale, July 10, 2023, 10:45 AM - 12:45 PM

Recently, the use of integrated circuit (IC) technology has found significant interest in the magnetic resonance (MR) research community for the integration of entire MR spectrometers into application-specific integrated circuits (ASICs) with millimeter-size footprints. Such chip integrated MR spectrometers open up the road for portable MR detection with wide application ranges from material science to personalized medicine [1] as well as truly broadband MR detection [2]. In addition to miniaturized conventional architectures, the chip-integrated spectrometers frequently use voltage-controlled oscillators (VCOs) [3-6], whose operating principle introduces interesting new possibilities, including the dead time-free detection transient sample magnetization [5] and operation up to very high frequencies beyond 100 GHz [6].

In this invited talk, we will provide an overview of the latest advances in chip-assisted MR detection, covering both NMR and EPR from very low frequencies around 10 MHz up to THz frequencies, including miniaturized conventional architectures and dead time-free VCO based detection.

Literature:

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INV006 - Recent advances in EPR cryoprobes

Dr Vidmantas Kalendra¹, Justinas Turcak¹, Prof. Juras Banys¹, Prof John Morton^{2,3}, Dr Mantas Šimenas¹

¹Faculty of Physics, Vilnius University, Vilnius, Lithuania, ²London Centre for Nanotechnology, University College London, London, United Kingdom, ³Dept. of Electronic & Electrical Engineering, University College London, London, United Kingdom

Parallel 3: Hardware, Boisdale, July 10, 2023, 10:45 - 12:45

Inspired by the success of NMR cryoprobes, we recently reported a leap in EPR sensitivity by equipping ordinary EPR probeheads with cryogenic low-noise microwave amplifiers [1,2]. Here, we discuss recent advances in the field concentrating on the Q-band version of the cryoprobe. Our probehead is equipped with a cryogenic ultra-low-noise amplifier and its protection circuit that are placed close to the sample in the same cryostat. Our cryoprobe maintains the same functionality and compatibility as the commercial instrument allowing high-power pulsed EPR experiments of typical samples. The sensitivity improvement provided by our setup is benchmarked using pulsed and continuous-wave EPR, as well as pulsed ENDOR experiments revealing a significant reduction of the EPR measurement time by a factor of about 15x.

The reported research is supported by funding from the European Union HORIZON-MSCA-2021-PF-01 Marie Skłodowska-Curie Fellowship (Project ID: 101064200; SPECTR)

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INV008 - Elucidating Structural Complexities and Ion Mobility Mechanisms in Li_2OHCl

Dr Karen Johnston¹, Mr George Rudman¹, Dr Theodosios Famprikis², Dr Tavleen Attari¹, Dr Abby Haworth¹, Dr Emma McCabe³, Dr James Dawson⁴

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Parallel 4: Solid State NMR, Alsh, July 10, 2023, 15:45 - 17:45

All-solid-state Li-ion batteries are attracting considerable attention as feasible alternatives to conventional liquid electrolyte-based devices, as they present a viable opportunity for increased energy density and safety. In recent years, numerous candidate materials have been explored as possible solid electrolytes, including Li-stuffed garnets, argyrodites, thio-LISICONs and complex spinels. Li-rich anti-perovskites (LiRAPs), including Li_3OCl and Li_2OHCl , have also generated significant interest, based on their reported ionic conductivities.[1] However, the precise mechanism(s) for ion transport within such systems is still the subject of considerable debate, along with the room temperature structure of Li_2OHCl . Our current research efforts have focused on the synthesis and structural characterisation of Li_2OHCl and the solid-solution $\text{Li}_{3-x}\text{OH}_x\text{Cl}$ to identify the correct room temperature structure of Li_2OHCl and establish the structural motifs responsible for ion conduction within LiRAPs. Using a combination of techniques, including neutron powder diffraction, multinuclear solid-state NMR spectroscopy, muon spin relaxation spectroscopy and ab initio molecular dynamics, we have successfully identified the phases of Li_2OHCl that can exist at room temperature and the conduction pathways available. We will demonstrate that, within Li_2OHCl and $\text{Li}_{3-x}\text{OH}_x\text{Cl}$, Li-ion transport is highly correlated with both the proton and Li-ion vacancy concentrations and temperature. We will also show that the Li ions are free to move through the structure, whilst the protons are restricted to solely a rotation of the OH^- groups. Based on these findings, and the strong correlation between long-range Li-ion transport and 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.[2]

References

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INV009 - To high dimensions and back again

Prof Vladislav Orekhov¹, Mr Amir Jahangiri¹, Xiao Han², Adnane Achour², Dmitry Lesovoy³, Panagiota Georgoulia¹, Dr Tammo Diercks⁴, Dr Björn Burmann¹, Irena Matečko-Burmann⁵, Wolfgang Bermel⁶, Tatiana Agback⁷, Peter Agback⁷

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Parallel 5: Theory and Computation, Boisdale, July 10, 2023, 15:45 - 17:45

NMR spectroscopy provides atomic-level information about molecular structure, dynamics, and interactions. High-dimensional NMR spectroscopy offers the highest resolution and information content by correlating multiple spins. However, processing such experiments requires advanced nonlinear algorithms, and the analysis is usually tedious. In this presentation, we will discuss new developments in spectra processing using artificial neural networks (NN) and deep learning.

An alternative approach to achieving resolution and information similar to that of a high-dimensional spectrum is through Focused Spectroscopy (FOSY). FOSY utilizes frequency selective polarization steps in a short and easily interpretable 2D experiment for a given spin system, such as a protein amino acid residue. This approach achieves higher sensitivity and resolution equivalent to a 4-7D experiment.

We will illustrate this new methodology using several protein systems, including the globular 44 kDa Malt1 and intrinsically disordered Tau441 proteins.

INV010 - Towards exact first-principles predictions of spin-lattice relaxation in magnetic molecules and solid-state defects

Dr Alessandro Lunghi¹

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Parallel 5: Theory and Computation, Boisdale, July 10, 2023, 15:45 - 17:45

Spin-lattice interaction is one of the main limitations to the spin lifetime in semiconductors and the control of such interaction is key for the development of technologies based on spin. Although spin-lattice relaxation has a central role in the physics of magnetism and magnetic resonance, its theoretical formulation is mostly based on a phenomenological approach and a quantitative understanding of its microscopic origin is often lacking.

In this contribution I will show the progresses in developing a computational approach able to tackle the challenge of predicting spin-lattice (T_1) relaxation from first principles. The formalism is based on a spin Hamiltonian description of magnetism and exploits the theory of open quantum systems in order to describe the dissipative effect of phonons on the spin degrees of freedom. This formalism is then mapped onto electronic structure theory, such as Density Functional Theory and Complete Active Space SCF, in order to determine all the parameters of the model in a full first-principles fashion[1].

Such computational strategy is shown to be able to quantitatively predict spin-phonon relaxation time in solid state compounds[1]. Results for both molecular crystals of Kramers ions and solid-state defects/impurities will be presented. The list of materials includes prototypical molecular qubits, such as $S=1/2$ V(IV) complexes[1], single-ion magnets, such as $S=3/2$ Co(II) and $J=15/2$ Dy(III) compounds[1-3], and negative vacancies in diamond and hexagonal boron-nitride[4].

Ab initio spin dynamics is able to explain the dependence of spin-lattice relaxation time with respect to temperature for all the investigated compounds, making it possible to individuate the origin of slow relaxation. Moreover, we successfully disentangled all the spin and molecular interactions leading to spin-phonon coupling, making it possible to discuss future directions for this field and possible strategies for the enhancement of spin lifetime in solid-state spin systems.

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INV011 - Pure Shift NMR of Aqueous Biofluids: Towards Quantitative Metabolic Profiling

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Parallel 6: Solution NMR Methods, Lomond, July 10, 2023, 15:45 - 17:45

Lymphoma refers to the group of blood cancers developing from lymphocytes, which are key infection-fighting cells of our immune system. Diffuse Large B-Cell Lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma. Although DLBCL treatment outcome has significantly improved, 40% of patients will be refractory or relapse and die.

Over the last years, metabolism has become a major topic in cancer research, unveiling new approaches to decipher the mechanism of the disease and develop new treatments.[1] In this context, we are carrying out a collaboration with Dr. V. Baud (INSERM, Univ. Paris Cité) and Prof. C. Thieblemont (Hospital Saint-Louis, Paris) to describe the basal metabolism of Lymphoma cell lines and understand how strategies based on the combined use of metabolic inhibitors can overcome drug resistance in DLBCL.

We will present the methodological developments carried out in our group to acquire Pure Shift ¹H NMR spectra [2-3] with efficient suppression of the water signal on biofluids. We will show how this approach can be used to detect in an hour all the metabolites at concentration as low as 10 μ M in extra-cellular media, yielding a very good separation of their NMR signals. We will describe how the statistical analysis of the metabolic profiles determined from these ultrahigh resolution data allows for getting a unique insight into the metabolic pathways that are key to Lymphoma cells and understand the mechanism of action of antimetabolic drugs.[4] We will also present for the first time a workflow for quantifying metabolites in several biofluids based on Pure Shift data.[5]

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INV012 - NMR Discrimination of Enantiomers at Submicromolar Concentration via Parahydrogen-Induced Hyperpolarization

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Parallel 6: Solution NMR Methods, Lomond, July 10, 2023, 15:45 - 17:45

Reversible association of small molecules and parahydrogen to an iridium catalyst forms the basis of SABRE techniques for nuclear spin hyperpolarization in solution [1]. Under suitable conditions, hyperpolarization of the transient complex resulting from this reversible association can be exploited to detect specific analytes at concentrations much lower than routinely observed in thermal NMR measurements. In this respect, the iridium catalyst employed in SABRE can act as an NMR chemosensor, allowing the selective detection of dilute analytes in complex mixtures, with no interference from the signal background due to other species in solution [2,3].

Here, recent work concerning the NMR detection and discrimination of enantiomers in complex mixtures by non-hydrogenative Parahydrogen Induced Polarization (nhPHIP) will be presented. These results demonstrate that it is possible to quantitatively discriminate enantiomers at sub-micromolar concentrations in biofluids or natural extracts without any prior functionalization or separation.

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INV013 - Solid-State NMR of Pharmaceuticals and Plant Cell Walls

Professor Steven Brown¹

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Parallel 7: Materials, Lomond, July 11, 2023, 10:45 - 12:45

Solid-State magic-angle spinning (MAS) NMR is a valuable tool in the characterisation and study of active pharmaceutical ingredients (APIs) [1-5]. Heteronuclear ^1H - ^{13}C correlation experiments are invaluable for assignment, while homonuclear ^1H - ^1H double-quantum (DQ) single-quantum (SQ) experiments reveal proximities (typically under 3.5 Angstroms) among pairs of hydrogen atoms. In addition, ^{14}N - ^1H spectra show one-bond NH connectivities or additionally longer-range NH proximities depending on the recoupling time employed. In the emerging NMR crystallography concept (recognized as a sub area by the International Union for Crystallography and, in the UK, by funding for a collaborative computational program for NMR crystallography, www.ccpnc.ac.uk), experimental solid-state NMR is complemented by first-principles calculations of NMR parameters using the GIPAW (gauge-including projector augmented wave) density-functional theory planewave approach that is particularly suited to periodic solids. In addition, the talk presents the application of ^{13}C - ^{13}C refocused INADEQUATE DQ-SQ to plant cell walls incorporating ^{13}C labelling [6,7].

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INV014 - Solid-State NMR Studies of Materials for Energy Conversion and Storage

Professor Arno Kentgens¹, Hongtao Qu¹, Ernst R.H. van Eck¹, Miss Shrestha Banerjee¹, Paul Tinnemans¹, Helen Grüniger¹, Jop Wolffs¹, Dr Jennifer S. Gomez¹, Dr. Gilles de Wijs¹

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Parallel 7: Materials, Lomond, July 11, 2023, 10:45 - 12:45

In our quest to reduce our carbon footprint, ultimately to net zero emissions we face many technological and fundamental challenges in chemistry, physics, and materials science. Therefore, a multitude of research is taking place on new materials for energy conversion and storage. As the function of these materials relates to their structure and dynamics, NMR has a relevant role to play in this research. During the past decades, solid-state NMR has emerged as a powerful tool to aid in understanding the working and failing mechanisms of energy conversion [1,2] and storage [3] materials and devices.

All-solid-state lithium batteries have gained recognition as the future of (mobile) energy storage, offering a secure and extended lifespan while significantly surpassing current technology in terms of energy density. We investigated Al-incorporation in β -Li₃PS₄, using NMR spectra to provide insights in the effect of Al-doping on the structure and dynamics in Li-Al-P-S systems [4,5]. ²⁷Al NMR studies of aluminum nuclei coordinated by sulfur are very scarce, however. Therefore, we undertook a ²⁷Al solid-state NMR study of various polymorphs of aluminum sulfide (Al₂S₃) and aluminum thio-ortho-phosphate (AlPS₄), which has shown promise as electrode material in Li- and/or Al-ion batteries. In combination with powder X-ray diffraction and density functional theory (DFT) calculations, we clarify the assignments of ²⁷Al NMR signals to specific Al-S coordinations and report their NMR parameters including chemical shift anisotropy and quadrupolar interaction parameters.

Over the past decade, hybrid halide perovskite materials have captured the attention of many researchers with their exceptional photoluminescent and photovoltaic properties, sparking intense interest and exploration for potential applications in areas such as photovoltaics, light-emitting diodes (LEDs), photodetectors, and beyond. Their unique ABX₃ structure means that perovskite-based materials properties are highly tunable, by changing the size and number of A/B/X species, where X is a halide. The ¹²⁷I quadrupolar interaction proves to be very sensitive to small changes in its surroundings. As reviewed in [2] ¹²⁷I NQR spectra of MAPbI₃ have been used to monitor phase transitions and its line width correlates to the powder quality as well as to the short-range dynamics. It will be shown that ¹²⁷I NQR spectra can be used to study compositional variations in halide as well as cation mixing.

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INV015 - *In vivo* MRI enhanced by dynamic nuclear polarization at ultra-low field for future proteolysis detection

Dr Elodie Parzy¹

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Parallel 8: Benchtop/Low Field MR, Alsh, July 11, 2023, 10:45 - 12:45

Introduction: Molecular imaging is a promising tool for a more personalized medicine. Magnetic Resonance Imaging (MRI) offers a great potential, provided selectivity and sensitivity issues are solved. Our team has implemented an Overhauser-enhanced imaging method (OMRI) at 0.2 Tesla to detect and localize proteolysis *in vivo* in mice with enzyme-targeted smart biocompatible nitroxide radicals. MRI contrast is brought by electron-to-proton polarization transfer in a double resonance experiment (5.4 GHz for EPR, 8.25 MHz for NMR). Such an approach would be invaluable to diagnose inflammatory diseases where upregulated massive proteolysis occurs but the current approach is limited by low penetration depth of the microwaves.

Aims: The objective is to transfer our method to a very low magnetic field which allows proteolysis detection on larger animals. Therefore a MRI system operating at 206 μ T was built in order to carry out OMRI in living rats.

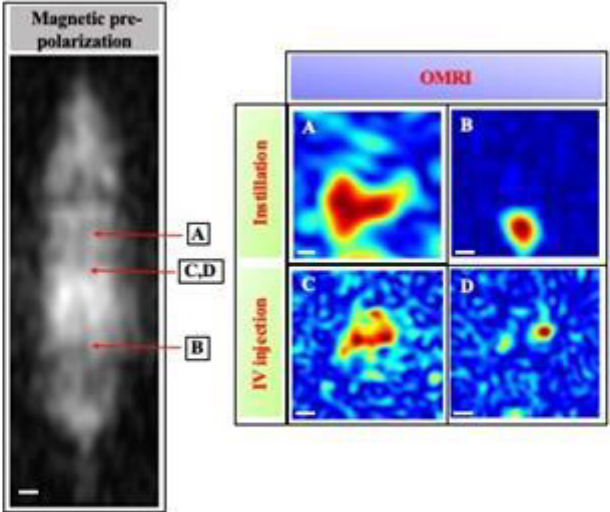
Methods: The hardware is composed of a 3-axis B0 cage, a gradient set, a pre-polarization coil (20 mT), a transmit-receive 1H RF-coil (8.7 kHz), a transmit-only EPR coil (72 MHz), a pre-polarization driver, independent transmitter channels for proton and electron, a proton receiver channel, and a system control unit. The NMR signal was recorded at 206 μ T both in pre-polarized and in OMRI experiments.

During an exam, a stable nitroxide (3CarboxyProxyl, 100mM, 100 μ l) was instilled in rat lungs prior to the *in vivo* OMRI experiment. 3D MR images were also acquired after the pre-polarization step for OMRI signal localization.

Results: Results show that nitroxides are visualized in 3D within a few minutes post-administration in the lungs, the kidneys and the bladder.

Conclusion: As a proof-of-concept the system allows *in vivo* OMRI in rats and shall be used for molecular imaging of inflammation using protease-specific nitroxide probes.

Funding: EC H2020/FETOPEN programme GA No 863099.



INV016 - Overhauser DNP for Online NMR Spectroscopy

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Parallel 8: Benchtop/Low Field MR, Alsh, July 11, 2023, 10:45 - 12:45

NMR spectroscopy is an attractive analytical technique for reaction and process monitoring. The robust benchtop NMR spectrometers that have become available recently have extended the applicability of the method to industrial processes. Process monitoring is often carried out on-line: the mixture that is to be analysed is pumped through the analytic instrument, which is operated in flow mode. In these setups, the volume of the line between process and analysis should be small and flow rates should be high to enable a fast transport to the analytic instrument. In NMR spectroscopy, this is in conflict with the time needed for sufficient polarization build-up, which is particularly problematic for benchtop NMR spectrometers because of their compact design. However, hyperpolarization methods like Overhauser Dynamic Nuclear Polarization (ODNP) are well suited to overcome this problem because hyperpolarization build-up happens on very short timescales and can be performed under continuous flow [1]. We demonstrate continuous-flow ODNP enhanced ¹H NMR measurements with a 1T benchtop spectrometer with immobilized TEMPO radicals [2] in pure solvents and in binary solvent mixtures and show that the range of accessible flow rates can be greatly expanded by the application of ODNP.

It is desirable to use also ODNP enhanced ¹³C NMR spectroscopy for online monitoring application because of its superior chemical resolution which enables the analysis of complex mixtures. For the hyperpolarisation of ¹³C via ODNP under continuous flow we compare different strategies: direct polarisation of ¹³C via dipolar and/or scalar hyperfine interaction to the radical or cross-polarisation from ODNP-polarized ¹H nuclei. We demonstrate that ODNP enables also continuous-flow ¹³C NMR spectroscopy with a 1T benchtop spectrometer.

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INV017 - Pulse(d) dynamic nuclear polarization

Dr Guinevere Mathies¹

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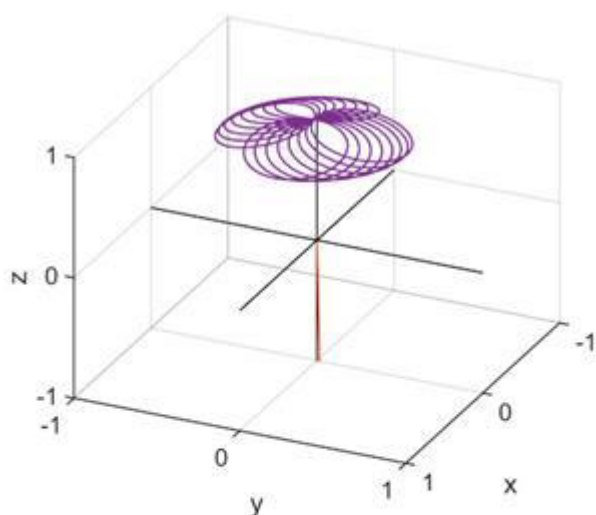
Parallel 9: Hyperpolarization, Boisdale, July 11, 2023, 10:45 - 12:45

In pulsed dynamic nuclear polarization (DNP), electron polarization is transferred to nuclei by means of a microwave pulse sequence. With the help of spin dynamics and optimal control, the polarization transfer can be fully optimized. The eventual goal is to improve the sensitivity of high-resolution magic-angle spinning (MAS) NMR beyond what is currently possible with continuous-wave DNP methods like the solid-effect and the cross-effect.

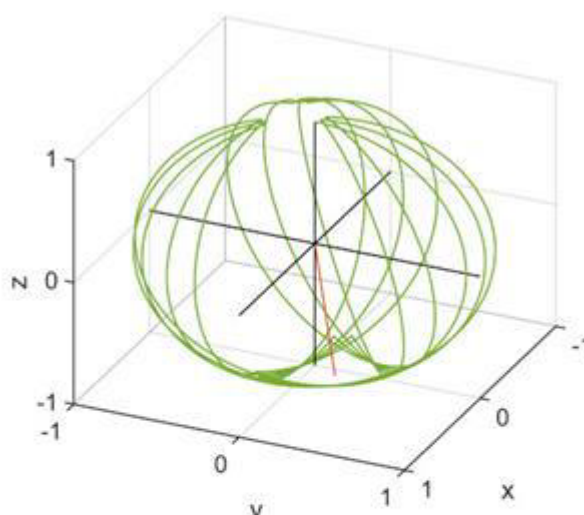
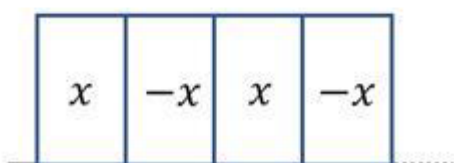
For the time being, pulsed DNP experiments are only possible at low magnetic fields. The basic reason is that suitable microwave sources are not available above roughly 95 GHz. Nevertheless, in recent years considerable progress has been made in the development of DNP pulse sequences. Beyond the original NOVEL sequence,[1] there now exists a family of DNP sequences, for which matching can be accomplished at conditions compatible with high-field MAS DNP.[2-5]

The theoretical description of the transfer of polarization by periodic DNP pulse sequences in a dipolar-coupled electron-nuclear spin system is under control (Figure 1). Numerical simulations of this process are a useful tool to investigate the efficiency of pulsed DNP conditions, but, in the current form, form do not always correctly predict the experimental results. Moreover, the ultimate DNP pulse sequence has not yet been found. In my talk I will update you on the latest developments and insights.

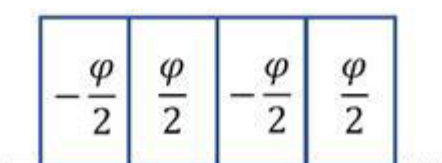
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XiX DNP



TPPM DNP



INV018 - DNP on a GPCR – enhancement strategies and frozen motions

Dr Johanna Becker-Baldus¹, Dr. Alexei Yeliseev², Dr. Thomas T Joseph³, Prof. Snorri Th. Sigurdsson⁴, Prof. Clemens Glaubitz¹

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Parallel 9: Hyperpolarization, Boisdale, July 11, 2023, 10:45 - 12:45

Introduction

Tremendous progress has been made in the structural characterization of G-protein coupled receptors (GPCRs) in the last decade. Structural information alone could so far not provide a detailed understanding of signalling specificity, and the dynamic nature of GPCRs probably plays an important role in their function. There are not many methods to characterize protein dynamics. NMR is such a method but not all time-scales are easily accessible. An alternative approach is to stop the motions by freezing the protein resulting in heterogeneously broadened spectra, representing the conformational space available to the protein at ambient temperature regardless of the timescale of the motion.

Low measurement temperatures are also required for DNP-enhanced MAS NMR methods and can be ideally combined with studies of GPCRs at low temperatures to boost the sensitivity and enabling experiments which otherwise would be impossible to do. Best signal-enhancement depends on the type and concentration of the polarizing agent applied and the composition of the matrix and have to be tailored to the type of sample.

Aims

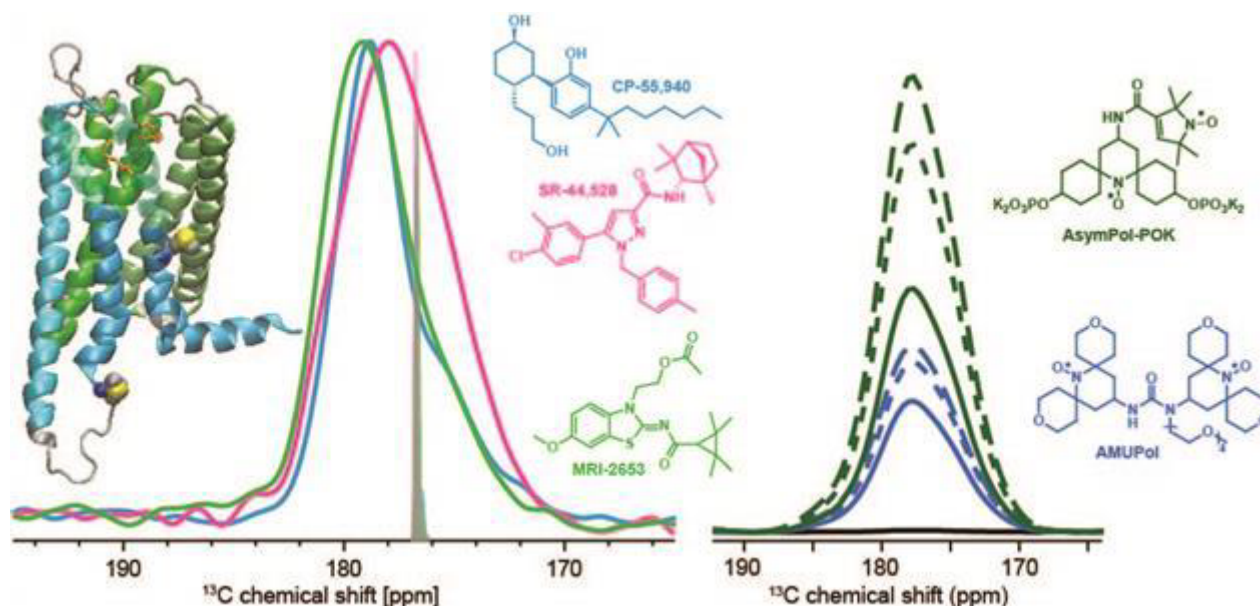
First, we aim to find optimal sample preparation conditions for DNP-enhanced MAS NMR on detergent micelles of membrane proteins. Secondly, we want to study the conformational space of the GPCR cannabinoid receptor 2 (CB2) in the presence of different ligands using the unique pair approach.

Methods

DNP-enhanced MAS NMR spectroscopy, Unique-pair labelling

Results and Conclusion

We could show that the amount of glycerol can be reduced in DNP sample preparations and that AsymPol-POK is very suitable for the study of membrane protein detergent micelles. Two sites in CB2 could be studied with three different ligands, showing large heterogeneous broadening as expected from a mobile protein. We could identify differences in mobility of the two sites and in one of the sites the mobility depended on the type of ligand.



INV019 - Optically generated spin polarization in diamond and chromophore-radical systems

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Parallel 10: Single Molecule/NV, Alsh, July 12, 2023, 10:45 - 12:45

The negatively charged nitrogen vacancy (NV⁻) center in diamond exhibits a triplet ground state that can be spin polarized and read out optically. As a result, the NV⁻ defect can operate as a sensitive magnetometer as well as a spin polarizing source for increasing the sensitivity of NMR. Many schemes involving magnetic field shuttling, pulsed and/or continuous wave microwave irradiation have successfully led to large ¹³C nuclear spin polarization in the bulk diamond. However, none of these methods so far have been used to effectively polarize a bulk sample external to the diamond. In this presentation I discuss some of the challenges faced when polarizing nuclei external to the NV diamond lattice, some possible strategies as well as distinct chromophore-radical (C-R) systems that could also serve as spin polarizing sources. Unlike NV⁻ centers, carefully designed C-R systems could have the advantage of being compatible with generalizable dynamic nuclear polarization methods. Given the vast chemical space that is possible in these systems, computational studies are vital to aid in the rational design of C-R molecules with desired electronic and spin properties. I present some recent work where we calculate vertical excitation energies and spin-correlation parameters of several pentacene-radical systems using existing computational methods. We use the obtained results to rationalize some of our past and recent transient electron paramagnetic resonance and transient absorption results on pentacene-radical and pentacene-biradical systems.

INV020 - Nuclear magnetic resonance at nanoscale enabled by diamond spin qubits

Prof Fedor Jelezko

¹Institute of Quantum Optics, Ulm University , Ulm, Germany

Parallel 10: Single Molecule/NV, Alsh, July 12, 2023, 10:45 - 12:45

Nuclear magnetic resonance is a key technique for unravelling molecular structure and dynamics. The major drawback of this method is its limited sensitivity related to noisy inductive detection and low polarization of nuclear spins under ambient conditions. We will report the new technique based on diamond spin qubits allowing to improve the sensitivity of NMR and improve spatial resolution and sensitivity of the NMR spectroscopy to nanoscale. Furthermore, we will discuss the use of optically polarizable diamond spin qubits as a source of polarization of nuclear spins. We will also discuss the limits of spectral resolution of liquid state nanoscale NMR methods to overcome limitations related to diffusion related broadening.

INV021 - A two-part NMR metabolomics story: optimizing stem cell osteogenic differentiation and searching for markers of therapy resistance in breast cancer

Professor Ana Gil¹

¹Department of Chemistry and CICECO-Aveiro Institute of Materials, University Of Aveiro, Aveiro, Portugal

Parallel 11: Metabolomics, Lomond, July 12, 2023, 10:45 - 12:45

The first part of this talk will focus on stem cell (SC) differentiation into bone tissue for biomedical bone repair protocols. As bone tissue growth does not always achieve identical characteristics of native bone, one way to optimize the process is to use osteoinductive metabolites, to stimulate differentiation while ensuring minimal amounts of co-differentiation (mainly adipogenesis) occurs. An NMR metabolomics characterization will be presented of the endo- and exometabolome time-course adaptations of human adipose mesenchymal SC (hAMSC) to osteogenesis, in 2D cultures. Results unveil candidate osteoinductive metabolites (both polar and lipidic) that may stimulate differentiation into a pure osteogenic lineage. The effects of different donors (a poorly understood and largely unpredictable issue, often hindering efficient differentiation) and underlying cell proliferation will be considered, to help elect a specific and donor-independent metabolic signature specific of SC osteogenesis.

In the second part of the talk, the issues of therapy resistance in estrogen receptor-positive (ER+) and in triple-negative breast cancer (BC) will be addressed making use of the medroxyprogesterone acetate (MPA) mice tumors and cell metabolomics, respectively. In the former case, the transition between responding and resistant ER+ tumors will be described through untargeted metabolomics and particular pathways highlighted as potential new therapeutic targets. Regarding triple-negative BC, a new platinum-resistant cell line will be compared with a sensitive cell line, as to their metabolic traits as a function of time. The identified distinguishing metabolic features will be advanced as candidates for targeted therapies in resistant cases of triple-negative BC.

Funding

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INV022 - Investigating the Metabolic Landscape of p53 Mutations

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Parallel 11: Metabolomics, Lomond, July 12, 2023, 10:45 - 12:45

Introduction. TP53 is the most mutated gene in cancer, with about 50% of cancers harboring a single missense mutation. p53 is a DNA-binding transcription factor frequently dubbed “guardian of the genome” that regulates multiple cellular functions and acts as a tumor suppressor. Over the last decade, it has also been identified as a key regulator of cell bioenergetics metabolism. In addition to the loss of tumor suppression function, many mutations of p53 result in the stabilization of mutant forms of the protein exerting a wide range of gain-of-function effects.

Aims. We aim at gaining specific insights into the metabolic landscape of TP53 mutations, in order to decipher the complex interplay between genetic factors, cell metabolism and alterations in epigenetic landscape underlying carcinogenesis.

Results. We present a comprehensive NMR metabolomics investigation of TP53 mutations across model cancer cell lines, primary human fibroblasts with constitutive p53 mutations, and mouse models. An integrative, multi-omics, multivariate analysis of the obtained molecular fingerprints will be presented.

INV023 - Deuterium Magnetic Resonance Imaging and Spectroscopy in Human Subjects

Professor Richard Bowtell¹, Daniel Cocking¹, Robin Damion¹, Matt Brook², Dorothee Auer³

¹Sir Peter Mansfield Imaging Centre, Schools of Physics and Astronomy, University of Nottingham, Nottingham, United Kingdom, ²School of Life Sciences, University of Nottingham, Nottingham, United Kingdom, ³School of Medicine, University of Nottingham, Nottingham, United Kingdom

Parallel 12: MRI & In vivo, Boisdale, July 12, 2023, 10:45 - 12:45

The low natural abundance ($\sim 0.015\%$) and small gyromagnetic ratio (6.54 MHz/T) of deuterium (^2H) reduce the available NMR signal compared to ^1H . However, the quadrupolar moment of ^2H leads to shorter longitudinal relaxation times that allow faster signal averaging, partially compensating for the reduction in intrinsic signal-to-noise ratio. The minimal equipment modifications required for implementing ^2H imaging and the simplicity of the required pulse sequences, mean that ^2H imaging has significant potential for clinical application. This has led to an increasing interest in the use of deuterium magnetic resonance in conjunction with injection or ingestion of ^2H -labelled compounds, particularly labelled glucose or heavy water, as a means of monitoring cellular metabolism (1,2). Residual quadrupolar splitting of the ^2H signal from water also potentially provides a way of probing tissue microstructure in vivo (3).

Using a Philips 7T scanner equipped with a dual-tuned $^2\text{H}/^1\text{H}$ head coil we have monitored glucose brain metabolism in 10 healthy subjects following ingestion of D_2 - or D_7 -labelled glucose (0.75g/kg body weight). Signals from the glucose (Glc) and its metabolic products (glutamate/glutamine=Glx and water=HOD) are readily detected, with significantly increased signal strength ($\sim \times 3.5$ Glc, $\sim \times 1.5$ Glx and $\sim \times 4.5$ HOD) for D_7 -labelled glucose. The same system has been used to characterise the HOD signals from different brain tissues in healthy participants who increased their deuterated water content to $\sim 1.5\%$ by drinking heavy water (4). We also measured the time-course of ^2H signal changes in the brain immediately following ingestion of heavy water. At 3T, we have investigated the orientation dependent quadrupolar splitting of the ^2H signal from HOD in muscle (3) and have demonstrated double quantum filtered chemical shift imaging in vivo. We are currently evaluating the level of incorporation of ^2H in fat in subjects loading with heavy water over a 4-week period.

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INV024 - Field-cycling NMR relaxometry for the development of paramagnetic large protein assemblies as high relaxivity MRI contrast agents

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Parallel 12: MRI & *In vivo*, Boisdale, July 12, 2023, 10:45 - 12:45

Introduction

The strategy to reduce the risks associated with the administration of MRI gadolinium(III) contrast agents can pass through the use of complexes with higher efficiency, so that the injected dose can be sizably reduced. Molecular reorientation times in the nanosecond timescale are needed to achieve the highest efficiency at the fields of MRI scanners. This can be achieved by functionalizing low molecular weight gadolinium(III) complexes to bind noncovalently to macromolecules, by confining them within nanosized matrices, like nanogels, or by exploiting nanosized gadolinium(III)-based compounds. To this aim, gadolinium(III)-complexes have been conjugated to multimeric proteins and their relaxation properties have been investigated as a function of the magnetic field using the fast field cycling relaxometry technique.

Results

The relaxivity profiles of Gd-labeled large protein assemblies, as the tetrameric protein asparaginase¹ or the protein cages AaLS-13 and OP,² show a remarkably high relaxivity at MRI fields, ca. five times higher than that of clinically used contrast agents. The analysis of the relaxivity profiles sheds light on the origin of the observed relaxivity enhancement. The main contribution arises from a correlation time modulating the proton-electron dipole-dipole interaction amounting to few nanoseconds, i.e., only one order of magnitude longer than that of the unbound paramagnetic complex, but optimal for high field MRI.

Conclusions

The large relaxivity of protein assemblies opens up the possibility of their use as protein-based theranostic agents. Asparaginase, for instance, is currently in clinical use against acute lymphoblastic leukaemia. Furthermore, since each of the four protein subunits contains 22 lysine residues, which can be largely functionalized, a huge amount of paramagnetic chelates can be conjugated, so that asparaginase represents an attractive carrier for the delivery of a high payload of paramagnetic ions.

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INV025 - Tracking Degradation in Commercial Li Batteries with High Chemical and Temporal Resolution

Professor Lauren Marbella¹

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Parallel 13: Materials, Alsh, July 12, 2023, 15:45 - 17:45

Anode-free batteries offer the highest specific energy density for Li-based batteries, but practical application is plagued by the growth of high surface area Li deposits. The presence of these Li filaments is strongly correlated with the formation of dead (electrochemically inactive) Li that leads to low Coulombic efficiency and serious safety concerns. Yet, electrifying large-scale modes of transportation will rely on energy dense technologies. Commercial batteries present unique challenges because the way that electrodes are stacked inside of a multilayer cell impacts Li deposition due to differences in pressure in the system. In this talk, I will discuss our efforts to use operando NMR spectroscopy to probe buried interfaces in these systems and quantitatively detect Li growth, dead Li, and electrolyte decomposition. We find that electrolyte formulation and cathode degradation dominates battery performance for anode-free configurations. Once these parameters are optimized, electrode stacking geometry can be tuned to further increase Coulombic efficiency.

INV026 - An integrative view on bioinspired silica-lysozyme composites

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⁴Florence Data Science, Università degli Studi di Firenze, Sesto Fiorentino, Italy

Parallel 13: Materials, Alsh, July 12, 2023, 15:45 - 17:45

Hen egg-white lysozyme promotes the formation of condensed silica microparticles from silicic acid solutions under milder conditions than the standard sol-gel synthesis (1). A similar chemistry appears to be applicable to titania and other IV-oxidation state oxides. However, little is known about the mechanism of the reaction, and even less so about the fate of the protein after the reaction is completed.

Over the past years, we applied a wide range of structural biology methodologies - including MAS-NMR and spin-labelling EPR - for understanding the interaction between the protein and the precursor (2), and for studying the protein-silica interface (3-5). Our results indicate that the nature of the protein-precursor interaction is chiefly electrostatic. After the reaction, the main contribution to the confinement of the protein within the composite is steric, even though electrostatics provide some orientational preference.

From the NMR standpoint these composite represent a significant challenge because of the dilution that the matrix imposes on the protein component and vice versa. To overcome this limitation, we have applied DNP (3) but also developed alternative processing methods for increasing the SNR a posteriori (6,7).

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INV027 - SHARPER NMR Spectroscopy

Professor Dusan Uhrin¹

¹University of Edinburgh, Edinburgh, United Kingdom

Parallel 14: Solution NMR Methods, Lomond, July 12, 2023, 15:45 - 17:45

Introduction. ^1H and ^{19}F NMR spectra typically contain signals split by ^1H - ^1H , ^1H - ^{19}F or ^{19}F - ^{19}F coupling constants. Removing these splittings can substantially enhance the sensitivity of NMR detection. However, the general use “pure shift” NMR methods typically degrade the sensitivity. When the task is to remove such splittings from a single resonance, SHARPER^{1,2} (Sensitive, Homogeneous And Resolved PEaks in Real time) is a superior technique, as it eliminates magnetic field inhomogeneity and produces sharp signals that approach their natural linewidths.^{3,4}

Aims. To further improve the sensitivity of SHARPER spectra and optionally to remove chemical shift dispersion of signals by using a CPMG acquisition mode.

Results. We will demonstrate that, compared to the original use of selective pulses for the removal of homonuclear splittings, the use of short spin-echo intervals (< 1.0 ms) in combination with non-selective pulses represents a more efficient approach. We also show that the use of 90° spin-echo pulses is advantageous in many instances and demonstrate a feasibility of removing the chemical shift modulation of signals spanning thousands of Hz. By applying these concepts to the measurement of translational diffusion of molecules, we have developed a SHARPER-DOSY protocol⁵ that boosts the sensitivity of DOSY experiments by up to two orders of magnitude, enabling fast and reliable determination of diffusion coefficients at low concentration of pure compounds.

Conclusions. The concept of collapsing multiplets, parts or complete spectra, opens new possibilities for characterisation of molecules and their mixtures, and is expected to find numerous applications in liquid-state NMR spectroscopy.

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INV028 - Fast 2D NMR spectroscopy for online reaction monitoring

Dr Jean-nicolas Dumez

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Parallel 14: Solution NMR Methods, Lomond, July 12, 2023, 15:45 - 17:45

Two-dimensional (2D) NMR spectra provide a wealth of information on mixtures of small molecules. 2D NMR can notably address overlap and compound identification issues met with 1D NMR. Classic 2D methods typically have durations of 10 minutes or more. Fast 2D NMR methods can reduce durations by one order or magnitude or more by, e.g., spatial parallelisation of the indirect dimension. This is particularly relevant for the real monitoring of chemical reactions.

Online monitoring by flow NMR has emerged as a powerful approach to study chemical reactions in diverse and realistic conditions, with applications in fields such as catalysis and polymer science [1]. The sample flow makes it necessary to revisit some of the core concepts of NMR pulse sequences. Interferences are for example expected between the flow and the effect of magnetic field gradients, and this can prevent the use of fast 2D NMR methods.

Here we describe the development of fast 2D NMR that are applicable in flow, and for the real-time monitoring of chemical reactions. First, we show that ultrafast (UF) 2D NMR methods based on spatial parallelisation can be adapted to yield COSY spectra in one or a few scans [2]. Second, we show that flow-compatible, fast diffusion ordered NMR spectroscopy (DOSY) methods can be designed to collect 2D diffusion data in about one minute [3]. These developments are illustrated with the monitoring of several organic reactions, as well as in-line detection for a photochemical flow synthesis [4].

These developments open many perspectives to extract detailed information from ongoing reactions, with applications that include mechanistic studies of batch reactions and the design of autonomous flow reactors.

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INV029 - Improving SABRE Hyperpolarization Using Non-Intuitive Fields and Sequences

Prof Warren Warren¹, S. L. Eriksson¹, X. Li¹, M. W. Mammen¹, J. R. Lindale¹

¹Duke University, Durham, United States

Parallel 15: Theory and Computation, Boisdale, July 12, 2023, 15:45 - 17:45

Introduction: SABRE variants can produce significant polarization levels on clinically interesting molecular targets. However, current advantages of SABRE (low cost, rapid polarization, broad ligand suitability) are counterbalanced by generally lower polarization levels than d-DNP.

Aims: SABRE operates in an unusual regime for magnetic resonance (in the heteronuclear case, the optimal experimental continuous field is $\approx 0.5 \mu\text{T}$), where Zeeman splittings, scalar couplings, and exchange rates are all comparable. As a result, most of the assumptions that make NMR tractable are invalid. A practical consequence is that, while low fields offer the unique opportunity to fully manipulate fields in three dimensions, very little exploration of parameter space has been done. This presentation will develop a comprehensive theoretical picture of SABRE evolution dynamics, validated by experiment.

Results: Sequences of rectangular or shaped z-fields which never approach a matching condition can improve polarization. We also report two approaches with multiaxial pulse sequences that facilitate direct measurement of the initial coherent dynamics in this complex system. The first approach, analogous to many high-field experiments, used decoupling along a perpendicular axis to preserve hydride singlet order. The second approach gets away from assuming a sequence structure, and used an evolutionary strategy to optimize polarization under an arbitrary multiaxial field.

Conclusions: Excitation field optimization (Multi-Axis Computer-aided HETeronuclear Transfer Enhancement approach, or MACHETE SABRE) improves polarization more than 7-fold over continuous field SABRE SHEATH. An average Hamiltonian approach turns out to be insightful to understand this. These waveforms, compatible with any 3-channel AWG and a simple multiaxial coil array, present a new strategy for understanding polarization transfer and optimizing population transfer in both magnetized and singlet states.

INV030 - Non-Fourier dimensions in NMR spectroscopy – signal acquisition, processing, and applications.

Professor Krzysztof Kazimierczuk¹

¹University of Warsaw, Warsaw, Poland

Parallel 15: Theory and Computation, Boisdale, July 12, 2023, 15:45 - 17:45

Multidimensional NMR experiments are particularly precious tools for chemical analysis. Typically, recording a multidimensional signal amounts to measuring a series of one-dimensional FIDs at different delay settings in a pulse sequence. Such data are processed using a multidimensional Fourier transform, often supported by extra procedures, such as, e.g., non-uniform sampling reconstruction.

But are we limited to "Fourier" dimensions? Are time intervals the only parameters we can change when generating a multidimensional signal? Numerous examples contradict this limitation. My presentation will summarize the non-Fourier multidimensional experiments my group proposed in recent years and the appropriate signal processing procedures.

Specifically, I will focus on the experiments where sampling of an extra dimension corresponds to varying external conditions, such as temperature, concentration, or pH. I will describe the data collection and dedicated processing methods involving compressed sensing and Radon transform, as well as their related variants.

The results show that series of NMR spectra can be effectively processed using the Radon transform, providing sensitivity boosts and facilitating the analysis. Moreover, many serial 2D+ experiments can be effectively accelerated by a non-stationary NMR approach, where the experimental conditions are varied between indirect time-domain points.

In summary, I will show that it is often worth considering a series of N-dimensional NMR spectra as an (N+1)-dimensional data object and apply alternative sampling and processing methods to the extra, non-Fourier dimension.

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INV031 - Probing the conformational landscape of biomolecules in situ using NMR spectroscopy

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Parallel 16: BioNMR, Lomond, July 13, 2023, 10:45 - 12:45

Increasing evidence suggests that a full understanding of biomolecular function and disease requires in-situ approaches that probe molecular structural and dynamics in a native setting. NMR is a non-invasive method that has made significant progress to study biomolecular systems in a native-like environment including bacterial, fungal or human cells. In our contribution, we have introduced novel solid-state NMR (ssNMR) techniques to study complex biomolecular systems in a bacterial^{1,2}, fungal^{3,4} and human cell⁵ setting. These methods maximize spectral resolution and sensitivity and are geared towards elucidating complex molecular systems including microtubular protein complexes⁵ as well as deciphering the dynamic landscape of proteins inside cells and cell organelles. Examples of such applications will be shown.

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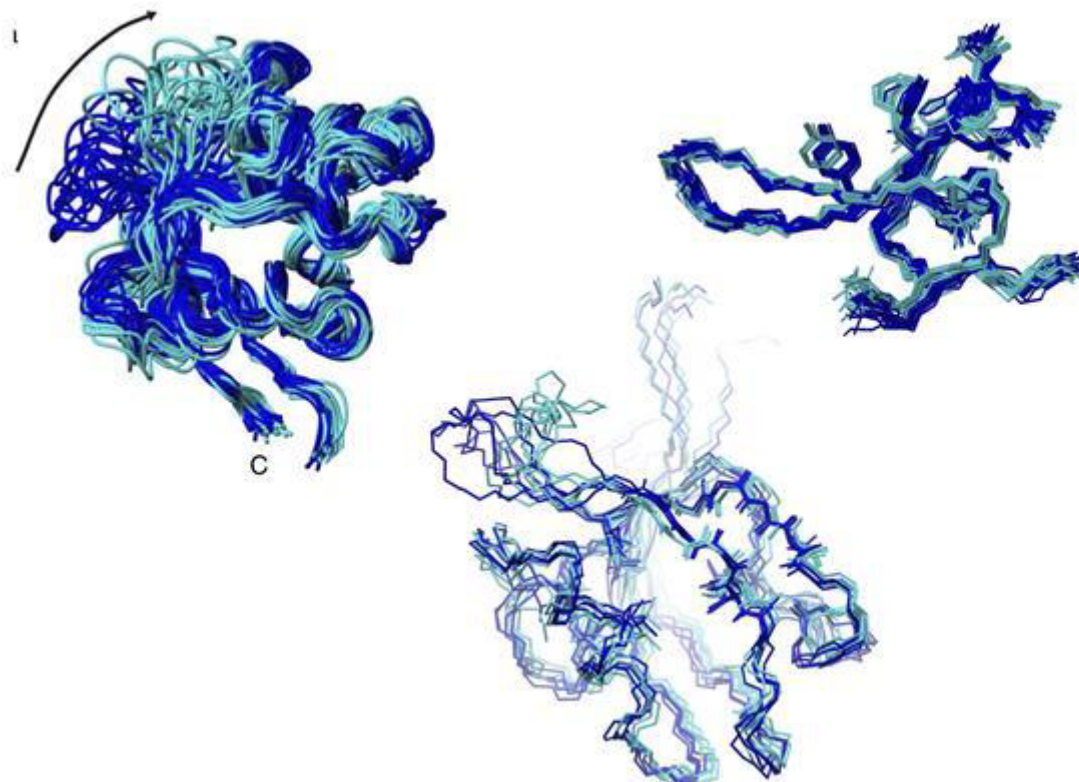
INV032 - Multi-state protein structures *in vitro* and in cells

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Parallel 16: BioNMR, Lomond, July 13, 2023, 10:45 - 12:45

Despite its essential role in the activity of proteins, the structural landscape with its multiple states and the dynamic exchange between them is not well-studied partially attributed to a lack of experimental approaches. With the advent of introducing exact Nuclear Overhauser Enhancement (eNOEs) multi-state protein structures of a handful of proteins such as cyclophilin, WW domain (Strotz et al., Angew. Chemie, 2020), PDZ (Ashkinadze et al., Nat. Comm. 2022) and GB3 is established elucidating the role of concerted motion in enzymatic function and protein allostery. However, the established multi-state protein structure determination protocol requires the collection of many precise distance restraints restricting the analyses to well-behaved systems and NMR experts only. Theoretical and experimental findings to be presented indicate that even in standard NMR structures, of which more than 10'000 have been deposited in the PDB, multiple states are embedded in a significant portion of the ensembles unlike one would expect. This opens an avenue to a simple identification of correlated motions that are relevant for enzyme activity and protein allostery. Given the large data set of known NMR structures, the general properties of protein allostery and correlated motions can be elucidated and translated into a predictive tool for their signatures in proteins for which first steps will be presented. In addition, recent in cell NMR investigations show not only that protein allostery is also present in cells, but that proteins comprise several distinct structural states in cells in part dependent on their localization (Kadavath et al. Angew. Chemie, 2022).



INV034 - MAS NMR analysis of proteins in trehalose matrix

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Parallel 17: Hardware, Alsh, July 13, 2023, 10:45 - 12:45

As previously shown in EPR experiments [1], the sugar trehalose can provide a solid matrix for proteins that can be studied with MAS NMR studies [2].

This method also allows to increase the concentration of the protein - compared to an aqueous buffer solution - to trap reactive intermediates and to shield the protein from oxygen. In addition, MAS NMR experiments are possible at room temperature, eliminating the cost of refrigeration.

In our group, we have studied a whole range of proteins in trehalose: photoreceptors as phytochromes and cyanobacteriochromes [3], plastic-degrading enzymes [4] and flavoproteins [5].

Data from photo-CIDNP MAS NMR experiments suggest that the lifetimes of the light-induced states are slightly altered, apparently because the electrical polarity of the protein environment is lower in sugar matrix than in frozen buffer.

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INV035 - Solid-state NMR of Paramagnetic Materials: many good reasons for faster Magic-Angle Spinning

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Parallel 18: "ParaNMR", Boisdale, July 13, 2023, 10:45 - 12:45

NMR studies of paramagnetic solids have been traditionally plagued with low resolution and sensitivity due to strong hyperfine couplings and relaxation enhancements, resulting in significant isotropic shift dispersions, large anisotropies, and broadening or total absence of signals by relaxation. This often results in the failure to detect signals by conventional NMR methods.

The recently attainable magic-angle spinning (MAS) rates >100 kHz and development of new broadband RF pulse schemes has led to considerable improvements in resolution and excitation bandwidth, permitting the acquisition of spectra spanning >1 MHz with a single offset. We will take the moves from a critical analysis of recent literature data and discuss the expected impact of fast MAS on resolution and sensitivity of different NMR experiments on different classes of diamagnetic and paramagnetic samples.

Furthermore, combining these experimental methods and state-of-the-art quantum chemical calculations results in a powerful tool for determining local and electronic structures in paramagnetic solids. We will demonstrate the power of this technique on iron-based catalysts and olivine-type mixed-phase Li-ion battery materials.

INV036 - Synergy of paramagnetic NMR and computational chemistry

Dr Elizaveta Suturina¹

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Parallel 18: "ParaNMR", Boisdale, July 13, 2023, 10:45 - 12:45

Paramagnetic NMR (pNMR) can be a highly informative characterisation technique for small metal complexes in solution. However, it is rarely employed by synthetic chemists due to difficulties in analysis of pNMR spectra. Here, we show several case studies of lanthanide and transition metal complexes where computational chemistry helps to assign the peaks of solution ¹H, ¹³C pNMR spectra. And more importantly we show how paramagnetic shift and paramagnetic relaxation enhancement (PRE) data can be used to get accurate estimations of the anisotropy of magnetic properties including g-tensor and D-tensor as well as electron relaxation time.

The case studies include the shift and relaxation data analysis of (i) series of lanthanide complexes relevant to PARASHIFT MRI probes [1]; (ii) an intermediate spin tBu(PNP)Fe–H complex, with a record shifted hydride signal at –3560 ppm at 295 K and (iii) binuclear radical-bridged Co and Ni complexes with extremely large exchange coupling.

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INV037 - Solid-state NMR insights into how tissue mechanics influence cell behaviour

Professor Melinda Duer¹

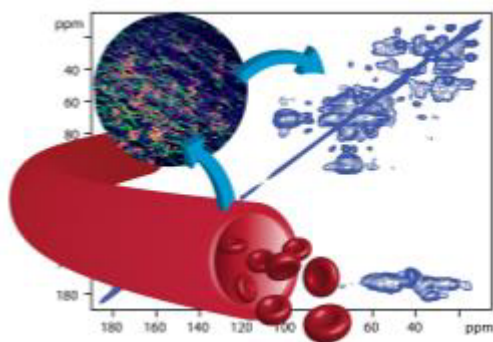
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Parallel 19: Solid State NMR and AI, Lomond, July 13, 2023, 1:45 PM - 3:45 PM

The extracellular matrix (ECM) forms the bulk of our structural tissues and provides them with their mechanical properties. Equally importantly, cell fate and function is determined by the molecular structures of the ECM and their interface with cells. Thus, the ECM manages very different roles on different lengthscales, all ultimately underlined by the ECM molecular structures, dynamics and organization. Ultimately, if we can understand how the extracellular matrix molecular structure and dynamics dictates the behaviour of cells and the tissue mechanical properties, then we can develop ways to combat the effects of ageing, reprogram cancer cells and effectively treat many degenerative diseases. Arguably, solid-state NMR spectroscopy is one of the few techniques that can deliver molecular structural and dynamics information in such heterogenous materials as the ECM. In this talk, I will describe the NMR approaches that my group has taken and is continuing to develop to tackle these complex questions.

Using multidimensional solid-state correlation NMR spectra as fingerprints of the underlying molecular structures in isotope-labelled native tissues has allowed us to develop laboratory-grown tissues that have demonstrably highly similar molecular structures to native tissues, and thus represent demonstrably good models of native tissue.^[1-3] Detailed SSNMR investigations of these in vitro and in vivo models are giving us new insight into how extracellular matrix mechanics influence cell binding and function. In this talk, I will discuss two studies that exemplify our approaches.

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INV038 - Using deep learning to unleash the full potential of NMR spectroscopy

Professor D Flemming Hansen¹

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Parallel 19: Solid State NMR and AI, Lomond, July 13, 2023, 13:45 - 15:45

Introduction

Artificial intelligence (AI) and deep learning are some of the most important technologies of our time. In magnetic resonance imaging (MRI), AI methods are deployed extensively, whereas the uptake of AI in nuclear magnetic resonance (NMR) spectroscopy has been slower - but this picture is now swiftly changing.

Aims

The aim of this work is to integrate deep learning with NMR spectroscopy to allow for robust autonomous analyses of complex NMR data and for new NMR-based methods.

Methods

We have shown that deep neural networks (DNNs) for analysis and transformations of NMR data can be trained on fully synthetic data. Thus, tailored network architectures were trained with synthetic data, whereas cross-validations were performed with both synthetic and experimental data.

Results

¹³C-detected protein NMR methods can be advantageous because they offer superior resolution, however, homonuclear scalar couplings often reduce the sensitivity and resolution. Our results show that DNNs can be trained for virtual homonuclear decoupling of ¹³C-detected spectra, where decoupling of the spectra can be achieved by passing a single spectrum through the trained DNN to yield a singlet spectrum of high quality.

Many tools have been developed to characterise dynamic and exchanging systems; however, analyses of the resulting NMR data often hinge on complex least squares fitting procedures and human intuition. Deep neural networks were developed for the analysis of ¹H chemical exchange saturation transfer (CEST) data, where the DNN not only accurately predicts the chemical shifts of the nuclei in the exchanging species, but it also determines the uncertainties associated with these predictions.

Conclusions

NMR spectroscopy is a vital tool for many fields of science, however, analysis of NMR spectra and development of new NMR methods still hinge on human intuition. Our research promises to propel NMR into the future, where integration of deep learning with NMR allows for autonomous analyses and new scopes.

INV039 - ^{17}O Hyperfine Spectroscopy in Surface Chemistry and Catalysis

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¹Department of Chemistry, University of Torino, Torino, Italy

Parallel 20: EPR/ESR, Alsh, July 13, 2023, 13:45 - 15:45

Oxygen is a ubiquitous element and oxide-based materials are of key technological importance in different areas including advanced functional materials, solid state chemistry and catalysis. Many of the key questions concerning these areas involve the understanding of the chemical bond, with oxygen being in the first or subsequent coordinating shells. The spectroscopic study of oxygen is therefore of fundamental importance to elucidate the complex interfacial coordination chemistry that underlies the development of materials featuring supported metal atoms on oxide surfaces.

“Adsorbed, chemisorbed, embedded, anchored, grafted” are all different words used to describe the variety of bonding interactions of an atom with the surface of a support, which can range from weak dispersive interactions to covalent or ionic bonds depending on the degree of orbital overlap and energy difference of the interacting orbitals. In the case of open-shell species, the key features of the chemical bond can be recovered at once by measuring the hyperfine interaction with isotopically labeled oxide ions.

The hyperfine coupling between the electron and ^{17}O nuclear spins is a unique source of information about the local binding environment around open-shell metal centers that allows to rationalize structure–property relationships in the most diverse systems. In this talk I will offer a perspective on ^{17}O surface enrichment of oxide materials and the use of hyperfine spectroscopies to investigate the interaction of paramagnetic metal atoms or ions with different oxidic supports featuring different chemical properties such as basicity, ionicity and reducibility. Emphasis will be given to the breadth of information provided by ^{17}O hyperfine interactions on the redistribution of the electron spin density over the support and to the assessment of the local geometric structure in polycrystalline materials.

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INV040 - Light-induced pulsed dipolar EPR spectroscopy based on electron spin hyperpolarization

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Parallel 20: EPR/ESR, Alsh, July 13, 2023, 13:45 - 15:45

Electron Paramagnetic Resonance (EPR) pulsed dipolar spectroscopy is a well-established technique to determine precise distance distributions between paramagnetic centers with distances ranging from around 1.6 nm up to 16 nm. In combination with site-directed spin labeling, it is ideally suited for structural characterizations of macromolecules and complexes, and has emerged as valuable tool in structural biology.

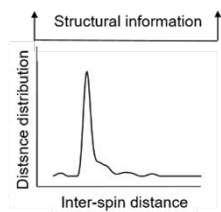
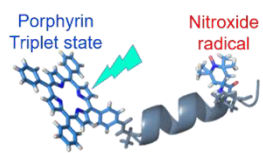
In recent years, porphyrins have been introduced in the selection of spin labels for dipolar spectroscopy applications. In their ground state, these chromophores are diamagnetic and thus EPR-silent, but, upon laser photoexcitation, their triplet state can be populated via intersystem crossing from the lowest excited singlet state, generating in this way the paramagnetic center.

Here we present various light-induced techniques, which exploit the distinctive properties of the porphyrin triplet state including the electron spin polarization. In combination with nitroxide spin labels or using two porphyrin probes, they enable both the distance and angular distributions between the two paramagnetic moieties to be determined. Pulsed dipolar spectroscopy has been applied on peptide-based spectroscopic rulers in order to test the accuracy, sensitivity and distance limits and it has been extended to paradigmatic proteins, containing an endogenous porphyrin probe.¹⁻⁴ Different chromophores with high triplet yield have also been introduced and the hyperpolarization of the nitroxide radical has been considered for a further increase in sensitivity.⁵

The methodology has a high potential for measuring nanometer distances in more complex biological systems and for future in-cell applications.

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INV041 - Breaking the limits in understanding glycan recognition in NMR

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Parallel 21: Small Molecule/Drug Discovery, Boisdale, July 13, 2023, 13:45 - 15:45

Introduction

Molecular recognition by specific targets is at the heart of the life processes. 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.

Aims

Thus, unravelling the structural and conformational factors and the physicochemical features that rule the interactions of these molecules is of paramount interest.

Methods

Solution NMR is unique in providing stereochemical and conformational information. Given the inherent flexibility and dynamic properties of sugars, we use NMR as key tool for deducing at atomic resolution molecular recognition processes in which glycans are involved, also assisted by a variety of synthetic, molecular biology, computational and biophysical techniques. This presentation is focused on the application of state-of-the-art NMR methods both from the ligand and receptor's perspective to study molecular recognition processes between receptors of biomedical interest and glycans.

Results & Conclusions

As recent examples, key details of glycan recognition by these receptors will be shown, with special emphasis in the application of novel ¹³C-based and paramagnetic-NMR methods, including the interactions of the spike protein of SARS CoV-2 with human immune lectins and cell glycans.¹⁻⁸

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Promoted Talks

Abstracts

PT001 - New insights on the intrinsically disordered amyloid-beta peptide through singlet-state, multi-quantum and high-pressure NMR and molecular simulation

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Parallel 1: BioNMR, Lomond, July 10, 2023, 10:45 - 12:45

Amyloid-beta (A β) aggregation is widely regarded as a key event in pathogenesis of Alzheimer's disease (AD). A vast body of knowledge on the structure, dynamics and aggregation of the wild-type and mutated A β has been accumulated over the past two decades. A full characterization of the conformational ensemble of the intrinsically disordered A β however requires extending our experimental access to the whole range of the multiscale structural dynamics of A β and integrating the complementary experimental and computational techniques. Recently, we have developed a glycine-based singlet-state NMR method, which allows accessing glycine residues of proteins in a site-specific manner (1). Using this method, we demonstrate that the ratio between the singlet-state and spin-lattice relaxation times (T_s/T_1) is a sensitive probe of dynamics at intermediate timescales and reveals the distinctive temperature-dependent rigidification of residue G33 in A β in contrast with the mobilization of its other five glycines. Integrating data from glycine-based singlet-state and methyl cross-correlated relaxation methods, we propose that the G33-L34-M35 segment plays a crucial role in the early steps of A β aggregation (2). On another line, we employ high-pressure NMR and the recently developed multi-quantum CEST methods (3), and provide, to our knowledge, the first experimental evidence of a salt bridge involving R5 sidechain in the N-terminal region of A β (4). Through combining with MD simulation and quantum chemical calculations we further characterize the R5-based salt bridges in A β and the potential effect of AD-related S8 phosphorylation on them. Finally, we employ high-pressure NMR and MD simulation and demonstrate that the AD-related mutations of A β alter stability of A β fibrils in distinct ways and potentially contribute to the remarkable phenotypical heterogeneity of familial AD (5).

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PT002 - A multidisciplinary approach to study the structure and the maturation of protein liquid droplets

Dr Leonidas Emmanouilidis¹, Ettore Bartalucci², Dr Yelena Kan³, Dr Mahdiye Ijavi⁴, Maria Escura Pérez¹, Dr Pavel Afanasyev⁵, Dr Daniel Böhringer⁵, Johannes Zehnder⁶, Prof. Sapun H. Parekh^{3,7}, Prof. Mischa Bonn⁷, Prof. Eric Dufresne⁴, Prof. Thomas C.T. Michaels¹, Prof. Dr. Thomas Wiegand^{2,8}, Prof. Dr. Frédéric Allain¹

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Parallel 1: BioNMR, Lomond, July 10, 2023, 10:45 - 12:45

Cells and most subcellular organelles are defined from the environment with lipid membranes. While membranes are a very efficient way to define distinct compartments, recently it was shown that the cells are fundamentally organized in much smaller and numerous functional membraneless entities. Currently, growing evidence show that these phase-separated membraneless organelles (MO) are ubiquitous and essential in all kingdoms of life. Essential functions for the cellular homeostasis and reproduction involving RNA synthesis, processing, storage and translation are linked with the MOs, indicative examples are the nucleolus, paraspeckles, Cajal bodies, P bodies and stress granules. Recently we have established a method to study phase separated liquid droplets in biphasic samples by NMR, EPR and RAMAN spectroscopies. The stabilization of the droplets inside an agarose gel allows not only to study simultaneously the structure of the molecules in the two phases but also explore the mechanism of droplet rigidification over time or maturation. Even though droplet maturation has been reported on many systems there is no reported mechanism. We report here the mechanism of how the droplets of the RNA-binding protein Fused in Sarcoma (FUS) transition from a disordered liquid to a fibrillary solid state. Both solution and solid state NMR confirm the presence of an invisible species that is converted into amyloid. Moreover the rate of amyloid conversion followed by NMR in the biphasic sample was found about two times faster than in the pure condensed phase. Combining the data with other biophysical techniques we propose the first model to explain how and why protein droplets transition from liquid into solid state. This model has great implications on how we understand phase separation as it implies inhomogeneity inside the liquid droplets and NMR should be further developed on this basis.

PT003 - Quantitative analysis of sterol modulated monomer-dimer equilibrium of β 1-adrenergic receptor by DEER spectroscopy

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Parallel 2: EPR/ESR, Alsh, July 10, 2023, 10:45 - 12:45

G protein-coupled receptors (GPCR) play a vital role in intracellular signaling pathways and control various physiological processes in eukaryotes. The oligomerization properties of GPCRs, and hence their cellular functions, may be modulated by various components within the cell membrane (such as the presence of cholesterol). Modulation may occur directly via specific interaction with the GPCR or indirectly by affecting the physical properties of the membrane. Despite extensive investigations on structure-function relationships of membrane G protein-coupled receptors (GPCR), the effects of membrane components, such as cholesterol and its derivatives, on oligomerization preferences remains to be clarified.

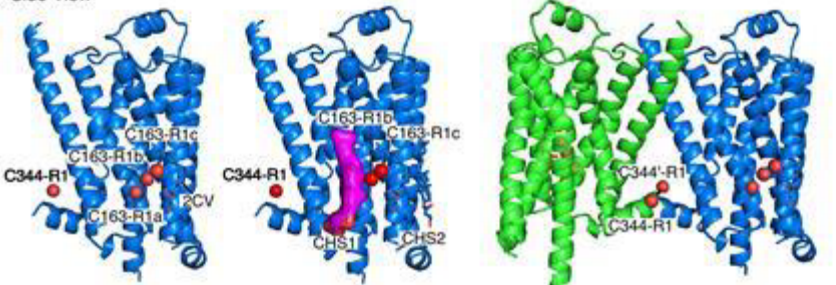
Using double electron electron resonance (DEER) spectroscopy, we demonstrate different effects of soluble cholesterol analog cholesteryl hemisuccinate (CHS) and cholesterol derivative bile salt sodium cholate on the oligomerization propensities of β 1-adrenergic receptor (β 1-AR) in DDM micelles. β 1-AR is mostly expressed in cardiac tissue, where failure of cholesterol regulation can develop into various heart diseases. Global fitting of DEER echo curves for spin-labeled β 1-AR upon titration with sodium cholate and CHS demonstrates that saturation of micelles with the former induces receptor dimerization, while specific binding of the latter to β 1AR inhibits dimerization and stabilizes the monomeric form.

The more novel technique for mimicking the natural membrane conditions are nanodiscs. Nanodiscs are self-assembled phospholipid bilayer enclosed in two helical membrane scaffold proteins. To investigate the dimerisation process we incorporated two β 1-adrenergic receptor molecules into a single nanodisc. It appeared that unlike micelles, which restrict GPCR motion, the native-like environment of nanodiscs provides more degrees of freedom, which is important for recognition by different binding partners.

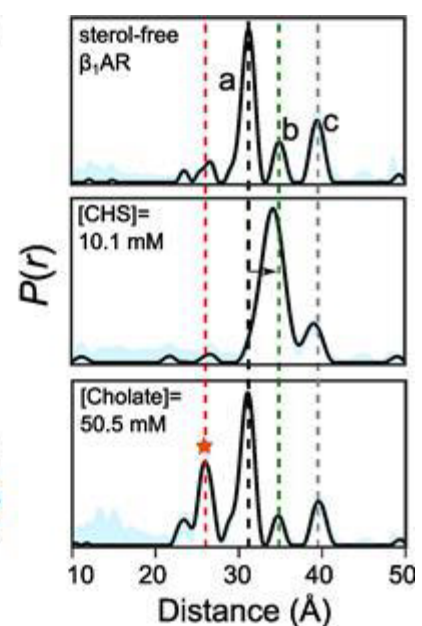
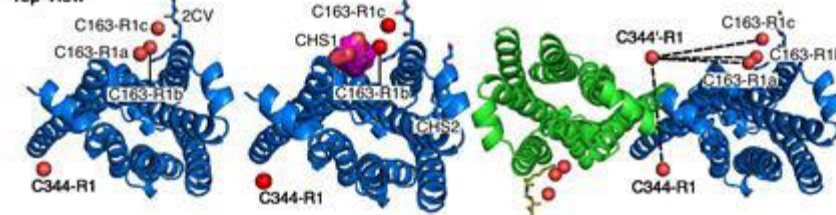
Knowledge about the role of membrane composition in modulating the GPCR oligomerization process has far-reaching pharmaceutical application. Our results illustrate how quantitative analysis of DEER data can contribute to studies of GPCR oligomerization.

A: sterol-free β 1AR B: CHS bound β 1AR C: Cholate induced dimeric β 1AR

Side View



Top View



PT004 - Investigating native metal ion binding sites in mammalian histidine-rich glycoprotein

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Parallel 2: EPR/ESR, Alsh, July 10, 2023, 10:45 - 12:45

Histidine-rich glycoprotein (HRG) is a ~70 kDa mammalian blood plasma protein involved in many essential regulatory biological processes, such as blood coagulation, cell migration and adhesion, and angiogenesis. Surprisingly little is known about the structure of HRG. It exhibits a multi-domain arrangement (see Figure) including two N-terminal (N1, N2) and one C-terminal (C) domains, and a central histidine-rich region (HRR) flanked by two proline-rich regions (PRR1, PRR2). An experimental high-resolution structure is only available for the N2 domain, while the PRR1-HRR-PRR2 stretch is predicted to be intrinsically disordered.

Metal ion binding to HRG, particularly of zinc(II), plays a crucial role in regulating protein function. HRG has multiple metal ion binding sites, however, since mammalian plasma is the main source of pure HRG protein, systematic investigation of metal ion binding has been limited.

Here, we use a suite of EPR methods in combination with ITC and protein structure prediction to assemble a holistic picture of metal ion binding to native mammalian HRG. We first demonstrate that copper(II) can be used as a proxy for zinc(II). Results show that HRG has 10-12 metal ion binding sites of equal and high affinity, involving two or more histidine residues for coordination per site, and a much larger number of lower affinity sites not involving histidine residues.

In summary, data suggest a variable, adapting fold of the predicted intrinsically disordered PRR1-HRR-PRR2 stretch.¹ Transient structural features of HRG induced by changes in metal ion loading could be key for enabling tight regulation of HRG affinities for interaction partners and thus, HRG function.

[1] K. Ackermann, S. Khazaipoul, J. L. Wort, A. I. S. Sobczak, H. EL Mkami, A. J. Stewart, B. E. Bode J. Am. Chem. Soc., accepted/in press.

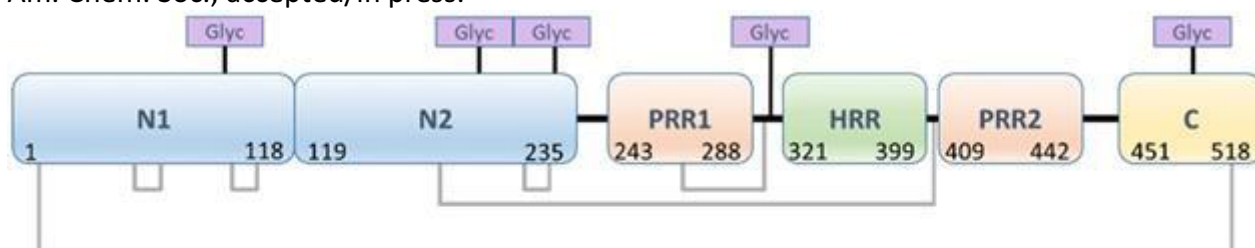


Figure 1. Domain structure of rabbit HRG. Disulfide bridges are indicated by grey lines.

PT005 - Stationary Phase EPR Spectroscopy Reveals Functional Conformational Ensembles of Adenosine A2A Receptor

Dr Joerg Reichenwallner¹, MSc Boyuan Liu¹, Dr Anling Kuo¹, Dr Ned van Eps¹, Dr. Adnan Sljoka^{2,3}, Prof. Dr. R. S. Prosser^{1,4,5}, Prof. Dr. O. P. Ernst^{1,6}

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Parallel 2: EPR/ESR, Alsh, July 10, 2023, 10:45 - 12:45

Stationary phase electron paramagnetic resonance (EPR) spectroscopy, or on-resin EPR, has the potential to considerably simplify purification procedures and functional analysis of G protein-coupled receptors (GPCRs). GPCRs play essential roles in a plethora of biological functions linking extracellular stimuli to intracellular signaling pathways. Accordingly, these proteins represent a major target for pharmacological treatment and play a crucial role in drug development and discovery. Adenosine A2A receptor (A2AR) is a prototypical family A GPCR which adopts complex conformational ensembles, depending on ligand and G protein binding. A stationary phase EPR spectroscopic platform was developed for characterizing the conformational ensembles of A2AR, while bound to an immobilized metal ion affinity chromatography (IMAC) resin. This on-resin platform provides sufficiently high local A2AR concentrations for conducting experiments ranging from continuous wave (CW) to pulsed EPR without the risk of protein aggregation. Furthermore, this approach yields comparable results to traditional in-solution measurements. Specifically, from double electron-electron resonance (DEER), we found that transmembrane helix 6 (TM6) of A2AR adopts four separable conformational states showing a distinct outward displacement upon receptor activation. The relative occupation of the inactive, intermediate and active states in the ensemble depend on the type of ligand and G protein binding as expected for an allosteric network regulating GPCR activation. The stationary phase EPR platform proves to be highly efficient and suitable for further understanding receptor activation. It could serve as a tool in drug discovery to investigate signal transduction pathways.

PT006 - Coherent DNP with Chirped Pulses

Professor Robert Griffin¹

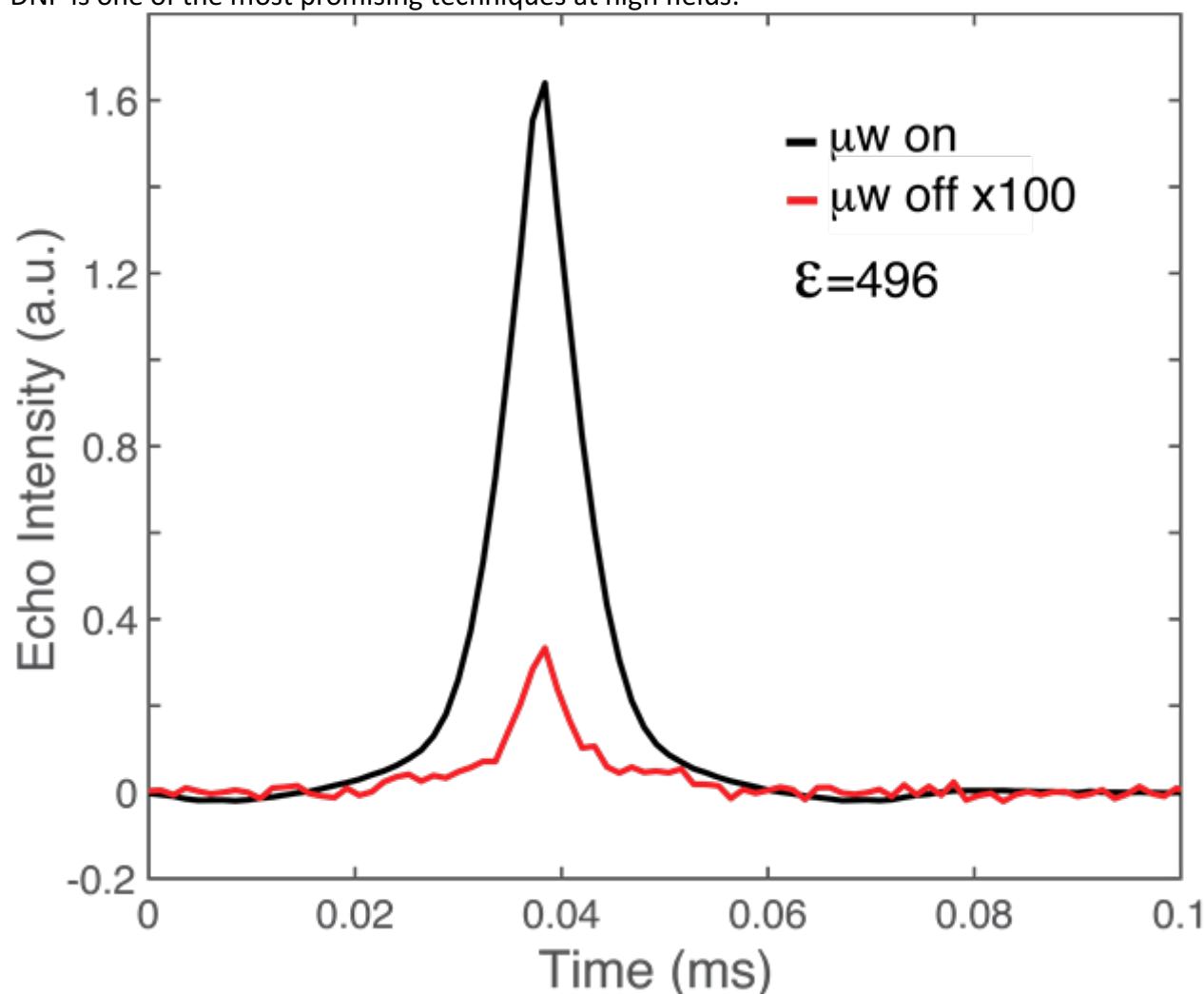
¹Massachusetts Institute of Technology, Cambridge, United States

Parallel 3: Hardware, Boisdale, July 10, 2023, 10:45 - 12:45

Recently, a theoretical and experimental study was presented that explains many aspects of the spin dynamics associated with the three microwave chirped pulse dynamic nuclear polarization (DNP) experiments [1]. With an improved understanding of the spin dynamics of chirped pulsed DNP, we performed experiments using the 94 GHz HiPER (High Power quasi-optical EPR) spectrometer located at the National

High Magnetic Field Laboratory. Using chirped pulses, the polarization transfer efficiency can be optimized and an enhancement $\epsilon = 496$ was observed using 10mM trityl-OX063 as the polarizing agent in a standard d8-glycerol:D2O:H2O : 6:3:1 glassing matrix at 70 K. The frequency swept pulses enhance the nuclear magnetic resonance (NMR) signal, and also reduce the recycle delay, accelerating the NMR signal acquisition.

Coherent pulsed DNP is still mostly limited at X-band and Q-band. We believe that our experimental results at W-band provide strong support that coherent pulsed DNP methods should be further developed at higher magnetic fields, where the NMR resolution can be optimized. Chirped pulsed DNP is one of the most promising techniques at high fields.



PT007 - Instrumentation for dual DNP-EPR MAS detection

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¹Eth Zürich, Zürich, Switzerland

Parallel 3: Hardware, Boisdale, July 10, 2023, 10:45 - 12:45

Introduction

We present improvements to solid-state nuclear magnetic resonance (ssNMR) methods using versatile instrumentation comprised of a magic-angle spinning (MAS) dynamic nuclear polarization (DNP) spectrometer with a high-power frequency-agile gyrotron as a microwave source, a Martin-Puplett interferometer (MPI), and an electron paramagnetic resonance (EPR) detection circuit.

Aims

The implementation of the EPR detection will greatly improve the sensitivity of ssNMR as nuclear coherences can be detected through the more highly polarized electron spins. Moreover, detection of the gyrotron output will enable precise tuning of the “electron control channel” for experiments utilizing the microwave frequency-agility, such as DNP with electron decoupling.

Methods

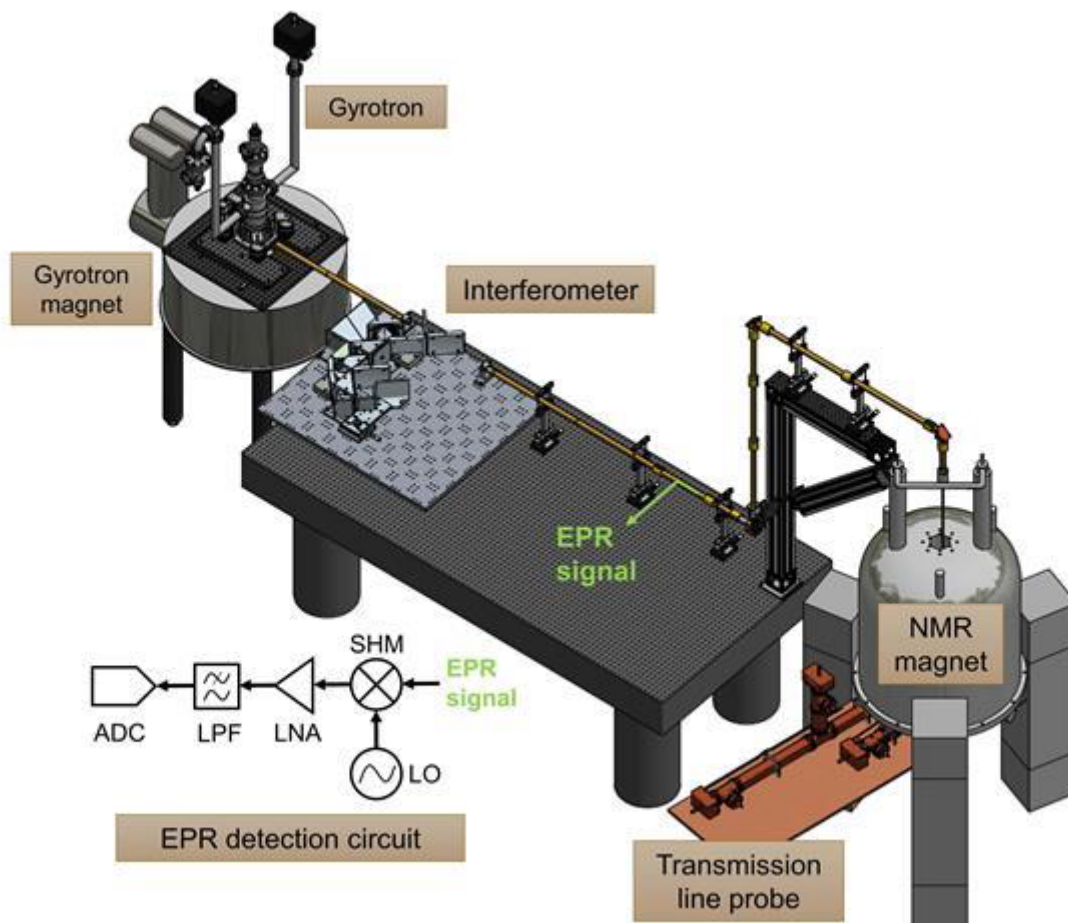
This assembly allows for complete control of microwave properties, e.g. frequency, power, and polarization providing optimal conditions for DNP experiments with EPR detection. The gyrotron is able to generate frequency-chirped microwaves over a bandwidth of 600 MHz with a power of approximately 30 W. The MPI allows adjustments of the microwave power, and polarization from linear to circular by changing the path length in the interferometer. While circular polarization is desirable for DNP experiments, linear polarization will allow for induction-mode EPR detection.

Results

As the interaction with electron spins in DNP and EPR experiments is polarization sensitive, it is important to understand the generated microwave polarization. In general, gyrotrons emit linearly polarized radiation, yet in this case elliptically polarized microwaves were observed from the gyrotron window as was revealed through the use of the MPI. The effect of the microwave polarization on the DNP enhanced signal was investigated and an intensity increase of 34% for circular versus linear polarization was observed. The EPR detection circuit has already been used for characterizing the gyrotron output, constituting an important step towards electron spin detection.

Conclusion

With the control over the microwave properties, the basic framework for dual MAS DNP-EPR was established.



PT008 - Sensitivity-Enhanced Experiments for Protein Solid-State NMR using Optimal-Control-Based Transverse Mixing Pulses

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Parallel 4: Solid State NMR, Alsh, July 10, 2023, 15:45 - 17:45

Proton-detected solid-state MAS NMR is a promising and versatile method for structure and function determination of insoluble proteins such as amyloid fibrils, protein complexes, and membrane domains. Multidimensional sequences (>3D)[1] are proposed to resolve spectral overlap challenges and to yield connectivity between atoms. However, sensitivity remains the major obstacle to broader applicability due to the integrated loss of sensitivity through multiple magnetisation transfer in high-dimensional pulse schemes.

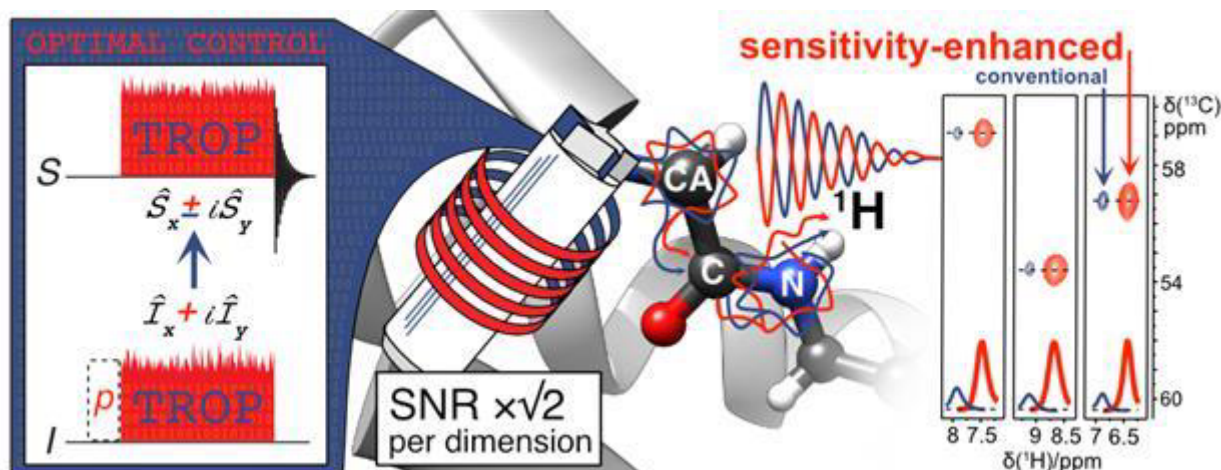
The sensitivity of the multidimensional experiments can be systematically improved when both transversal components of the magnetisation are transferred simultaneously after an evolution period, allowing the preservation of equivalent pathways (PEP), known in solution-state NMR.

Here, we present solid-state homo- and heteronuclear TRAnsverse mixing based on Optimal-control Pulses (TROP) in multidimensional pulse schemes that allow one to systematically increase sensitivity of the experiments by a factor of $\sqrt{2}$ per each indirect dimension[2] as we have demonstrated in collaboration with several partner NMR facilities. Additional gain is obtained by improving the robustness of optimal-control-based pulses against rf-field inhomogeneities.[3] Sensitivity-enhanced 2D hNH and 3D hCONH, hCOcNH, hCANH, and hCAcNH experiments with TROP were tested using standard samples of fMLF and SH3, as well as the human lambda-III immunoglobulin light chain. In this challenging sample, the 300% sensitivity gain enables the detection of minor fibril polymorph that has escaped detection so far.

We anticipate that the systematic use of the PEP strategy in nD spectroscopy of solids presents a game-changing factor turning the demanding 5D HNcoCANH experiments (about one month of acquisition for tryptophan synthase)[1] into a routine tool that provides protein backbone assignment in couple of days (a factor of 16 in time saving).

References.

- [1] Klein, A. et al. PNAS. 119, e2114690119 (2022).
- [2] J. Blahut et al. JACS 144, 17336–17340 (2022).
- [3] Z. Tošner et al. Sci. Adv. 7, 1–11 (2021).



PT008 - Self-Diffusion in Solid Lithium Metal Revisited Using ^7Li Field-Cycling NMR Relaxometry

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Parallel 4: Solid State NMR, Alsh, July 10, 2023, 15:45 - 17:45

Field-cycling (FC) nuclear magnetic resonance (NMR) was employed for the first time to measure the spin-lattice relaxation rate T_1^{-1} of ^7Li in solid lithium metal. A wide Larmor frequency range of 1 kHz – 10 MHz was covered as well as a temperature (T) range of 232 K – 397 K. A detailed discussion and comparison with literature highlight the benefits of FC NMR and its advantages in the investigation of diffusion mechanisms in solids. Choosing Li metal as model system enables us to test and compare theoretical models using the FC NMR data. We revisited Li metal in terms of Korringa constant ($T_1 \cdot T$), self-diffusion coefficient and jump correlation. It is again shown that a jump mechanism via monovacancies takes place in metallic lithium. This does not exclude the possible influence of divacancies at higher temperatures near the melting point (454 K) which were not investigated here. Understanding mechanisms and effects like correlation and dimensionality of diffusion in solids can be a wide field to be investigated by FC relaxometry. This could supplement and enhance other solid-state NMR methods.

PT009 - Optimizing chemistry at the surface of prodrug-loaded cellulose nanofibrils with MAS-DNP

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Parallel 4: Solid State NMR, Alsh, July 10, 2023, 15:45 - 17:45

Cellulose nanofibrils (CNF) are renewable, biodegradable and biocompatible, which makes them ideal candidates for numerous applications, including carriers for drug delivery. However, in-depth chemical and structural characterization of the CNF surface chemistry is mandatory to make further progress. Thanks to the development of Dynamic Nuclear Polarization (DNP), and in particular the design of improved polarizing agents,[1,2] we and others, are continuously pushing back sensitivity limits. Importantly, the latest generation of polarizing agents (cAsymPol-POK, PyrroTriPol, etc.) has improved performance at high field, fast MAS, and still performs well on protonated systems, which significantly broadens their application range.[3,4]

As a result, we recently showed that such on-going advances can be used to unravel the surface chemistry of drug-functionalized CNFs (using heterogeneous aqueous chemistry). Using ¹³C and ¹⁵N DNP-enhanced solid-state NMR, one can locate the position of functionalization and quantify adsorption versus covalent grafting (~1 wt% of modified metronidazole in our case).[5] Such results cannot be achieved with other characterization techniques (FT-IR, elemental analysis) which also missed the presence of residual coupling agents at the CNF surface. The latter information provides a unique opportunity to rationalize the effect of various coupling agents (DMTMM vs EDC/NHS). This approach was then applied to a large prodrug of ciprofloxacin designed for controlled release. Besides quantifying the drug grafting, we also evidenced the challenge to control concurrent adsorption and to optimize efficient washing procedure. Finally, we discovered an unexpected but highly relevant prodrug cleavage mechanism triggered by the presence of carboxylates at the CNF surface.[6]

[1] Mentink-Vigier et al., JACS 2018 140, 35 [2] Halbritter et al., Chemical Science 2023, 10.1039/D2SC05880D [3] Harrabi et al., Angewandte 2022, 10.1002/anie.202114103, [4] Smith et al., SSNMR 2023, 123, 101850 [5] Kumar et al., Chemical Science, 2020, 11, 3868, [6] Kumar et al., Communications Chemistry (accepted).

PT010 - Structure-based protein chemical shift assignment from minimal NMR data with the hybrid deep learning approach ARTINA

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¹ETH Zürich, Zürich, Switzerland, ²Goethe University, Frankfurt am Main, Germany, ³Tokyo Metropolitan University, Hachioji, Japan

Parallel 5: Theory and Computation, Boisdale, July 10, 2023, 15:45 - 17:45

Chemical shift assignments are required for most protein NMR studies and often demand most of the measurement and analysis time. Here, we present a hybrid automated approach for protein chemical shift assignment that allows to reduce the number of spectra to be measured and the time to analyze them. It is based on machine learning for visual spectra analysis with ARTINA (1,2), structure prediction with AlphaFold2 (3), chemical shift prediction with UCBShift (4), and automated assignment with FLYA (5). Results from more than 10'000 assignment calculations with 100 proteins show that a small number of spectra suffices to establish the backbone and side-chain assignments. In conjunction with AlphaFold2 structures, the five 3D spectra 15N-NOESY, 13C-NOESY, CBCAcoNH, HCCH-TOCSY, and CCH-TOCSY yield on average better assignments than if ARTINA is run with all available spectra but without AlphaFold2 structures. NOESY spectra are particularly valuable for automated assignment. To be beneficial, structures should have an accuracy better than 2 Å.

This new version of ARTINA, which is available for use at the open NMRtist web server (2), offers users the general possibility to load, in addition to NMR spectra, 3D structures, manually or otherwise prepared peak lists, chemical shifts, distance restraints and torsion angle restraints as additional input for the ARTINA shift assignment and ARTINA structure determination applications. The new version of NMRtist also provides quality scores for peak picking, shift assignments, and structure calculations that are computed purely on the basis of the input data without recourse to manually obtained reference results.

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3. Jumper, J. et al. Nature 596, 583–589 (2021).
4. Li, J. et al. Chem. Sci. 11, 3180–3191 (2020).
5. Schmidt, E. et al. Am. Chem. Soc. 134, 12817–12829 (2012).

PT011 - A Bayesian approach to semi-automated assignment of NMR spectra of molecular organic solids

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Parallel 5: Theory and Computation, Boisdale, July 10, 2023, 15:45 - 17:45

Introduction: Robust structural assignment is essential whenever solid-state NMR spectra are being used as more than simple fingerprints of solid materials. This is particularly relevant for ‘NMR crystallography’[1], where the agreement between the experimental chemical shifts and chemical shifts calculated from a proposed structure is often used to validate the structural model. Moreover, the widespread use of DFT-predicted shifts has highlighted several examples where ¹³C spectra of molecular solids have been incorrectly assigned. If NMR crystallographic approaches are to be used more widely, more robust methods of assignment are required.

Methods: The methodology builds on the principles of Goodman’s DP4 parameter[2], which provides a relative probability that alternative structures match experimental ¹³C and ¹H shifts. The very limited resolution of ¹H NMR in the solid state, and frequent spectral overlap even in ¹³C NMR means, however, that the shift-only approach of DP4 is not practical; the number of potential assignments rapidly becomes impossible to consider. By adding data from additional experiments within a Bayesian framework, assignments can be refined until a sufficient level of confidence and completeness is achieved.

Discussion: Tested on a variety of systems, this approach reproduced “manual” assignments in simple cases, but crucially allowed more difficult systems to be tackled in a semi-automated fashion. By providing a consistent “scoring” of different assignments, the robustness of a given assignment can be easily independently reviewed. Although evaluated using non-interactive Python scripting, this approach is well suited to implementation in an interactive graphical user interface, with the goal of improved overall robustness and reproducibility of NMR crystallographic studies.

[1] P. Hodgkinson, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2020, 118–119, 10.

[2] S. G. Smith and J. M. Goodman, *J. Am. Chem. Soc.*, 2010, 132, 12946.

PT012 - Xe NMR modelling in porous liquids

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¹NMR Research Unit, Faculty of Science, University of Oulu, Oulu, Finland

Parallel 5: Theory and Computation, Boisdale, July 10, 2023, 15:45 - 17:45

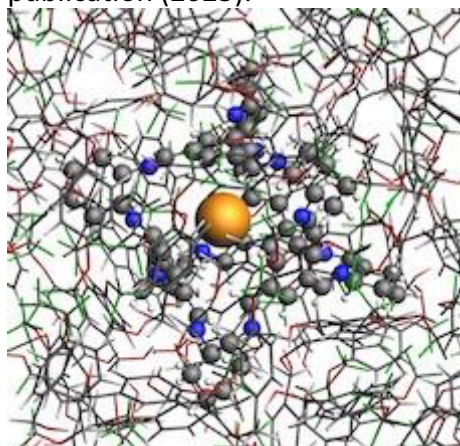
Xe NMR spectroscopy in molecular cavities and solvents provides detailed local information about the microscopic structure of the host molecule and surrounding solvent. While Xe NMR spectral features are very sensitive to physical conditions-dependent dynamical processes affecting both the host and the guest, to connect them to the processes at the microscopic level requires theoretical first principles modelling.

Here we study recently developed porous liquids (PLs) with permanent intrinsic cavities formed by porous organic cages (POCs) dissolved in size-excluded solvents. Our aim is to increase the amount of information obtained from Xe NMR spectra by first principles modelling that both confirms and helps to explain spectral features.

We will describe the approach, in which reliable Xe NMR parameters are obtained by carrying out state-of-the-art relativistic density functional theory (DFT) calculations of the Xe NMR shielding tensors for snapshots extracted from semi-empirical GFN2-xTB molecular dynamics (MD) simulations. We will discuss a few examples of PLs made from scrambled POC mixture of CC3-R and CC13 precursor cages in different solvents. In TBA solvent (see Figure), we reach quantitative agreement with experimental Xe NMR chemical shifts [1]. This confirmed the validity of the two-site exchange model used in the experimental analysis and allowed quantification of e.g. cage occupancy and cage-solvent exchange rate.

The developed computational approach for NMR parameters modelling including full dynamics of the system in real physical conditions is shown to be necessary in detailed Xe NMR studies of neat and porous solvents. Universality makes the approach widely applicable in NMR modelling of atoms and molecules in different environments.

[1] Sarah E. Mailhot, Petri Peuravaara, Rachel J. Kearsey, Benjamin D. Egleston, Sanna Komulainen, Rebecca L. Greenaway, Andrew I. Cooper, Juha Vaara, Perttu Lantto, Ville-Veikko Telkki: Cage Occupancy and Exchange Dynamics in Porous Liquids Elucidated by ¹²⁹Xe NMR, submitted for publication (2023).



PT013 - Steady-state generation of

long-lived nuclear spin states using cascades of pulses

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Parallel 6: Solution NMR Methods, Lomond, July 10, 2023, 15:45 - 17:45

With the advent of long-lived states, nuclear magnetic resonance spectroscopy has expanded storage of spin polarization far from equilibrium for periods much longer than the longitudinal relaxation time constant^{1–3}. This is due to the cancellation of relaxation mechanisms imposed by quantum mechanical laws, e.g. the immunity of the two-spin singlet-state to the intrapair dipolar interaction⁴. Still, there are many other relaxation mechanisms shortening the lifetime of long-lived states as well as hampering the efficiency of the pulse sequences used to excite them. Converting equilibrium magnetization into singlet state usually requires accessing transient states with various spin and coherence orders via pulses and synchronized delays, imposing a time constraint for efficient excitation. On this timescale, relaxation mechanisms like dipolar (DD) or chemical shift anisotropy (CSA) interactions can significantly shorten the lifetime of transient states, thus reducing the singlet state yield.

Here, we propose methods that do not require system-specific delays of free evolution to reach a steady-state singlet-state population. This can be achieved through two different pathways: coherently by cascades of frequency-selective inversion pulses⁵, or incoherently by exploiting the intrapair DD/CSA cross-correlation mechanism⁶ during a loop of hard inversion pulses. These two methods allow for a parameter-free excitation that overcomes the time constraints of other pulse sequences, as well as sustaining non-equilibrium spin correlation indefinitely during the preparation stage.

PT014 - Field cycling Singlet-Assisted Diffusion NMR: a new tool for probing translational dynamics in space and time heterogeneous systems

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¹University of Southampton, Southampton, United Kingdom

Parallel 6: Solution NMR Methods, Lomond, July 10, 2023, 15:45 - 17:45

Translational dynamics is perhaps one subject where NMR best reveals its advantages over other techniques. Diffusion NMR methodologies, for instance, include diffusion ordered spectroscopy, a successful tool used by communities within and outside NMR. But, diffusion NMR is not only a solution state tool; it is exploited to produce diffusion weighted images (DWI) and diffusion tensor imaging (DTI) in MRI and to characterize porosity and other structural parameters in porous media applications. However, all conventional diffusion NMR methods are limited by the duration of spin memory, i.e., by how long spin order can store positional information for (typically of the order of seconds, at best).

In my laboratory, we have taken the challenge to extend the capabilities of diffusion NMR using long-lived spin order methods, an NMR topic we have long and deeply specialized in. Long-lived spin order allows information to be stored for long time, typically of the order of several minutes. An extended spin memory translates into extended diffusion NMR capabilities allowing, for example, to measure smaller diffusion coefficients or slower flows, to characterize structures with larger pores or to measure tortuosity in porous media. We are particularly interested in measuring diffusion tensors and tortuosity in tissue cultured on 3D-printed scaffoldings, a heterogeneous system in space and time with pores too large (300-500 μm) for conventional diffusion NMR and where magnetic susceptibility inhomogeneities varying as the cells infiltrate the scaffoldings complicate the approach.

Here we discuss a methodology that merges field-cycling, long-lived spin order and pulsed field gradients that we have developed for these studies. We also discuss a simulation approach that combines μ -Computed Tomography data, Monte Carlo methods and spin dynamics theory to predict spin relaxation due to spins diffusing in the presence of magnetic susceptibility inhomogeneities in an actual sample of arbitrary complexity.

PT015 - Oxide Ion Diffusion Mechanism in Melilite from ^{17}O and ^{71}Ga Variable Temperature MAS NMR

Miss Lucia Corti^{1,2}, Professor Matthew J. Rosseinsky^{1,2}, Professor Frédéric Blanc^{1,2,3}

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³Stephenson Institute for Renewable Energy, University of Liverpool, Liverpool, United Kingdom

Parallel 7: Materials, Lomond, July 11, 2023, 10:45 - 12:45

The design of fast oxide-ion conductors exhibiting elevated oxide-ion conductivity between 600°C and 800°C is crucial for the development of efficient solid oxide fuel cells and $\text{La}_{1.54}\text{Sr}_{0.46}\text{Ga}_3\text{O}_{7.27}$ has attracted research interest owing to its remarkably high oxide-ion conductivity in this temperature range[1]. This work aims at elucidating the differences in structure and oxide-ion dynamics between the poorly conductive $\text{LaSrGa}_3\text{O}_7$ and the highly conductive $\text{La}_{1.54}\text{Sr}_{0.46}\text{Ga}_3\text{O}_{7.27}$ phases.

Structural information is provided by multinuclear MAS and multiple-quantum MAS (MQMAS) NMR experiments aided by the computation of NMR parameters using an ensemble-based approach[2] to model site disorder. Powerful insight into local dynamics is gained from a range of multinuclear variable temperature (VT) MAS NMR approaches, namely ^{17}O and ^{71}Ga lineshape analysis, ^{17}O relaxometry and ^{17}O - ^{17}O exchange spectroscopy (EXSY), making use of exciting probe capabilities which enable experiments up to 700°C to be recorded.

The ^{17}O MAS NMR spectrum of $\text{La}_{1.54}\text{Sr}_{0.46}\text{Ga}_3^{17}\text{O}_{7.27}$ displays one strongly deshielded signal not observed for $\text{LaSrGa}_3^{17}\text{O}_7$ and assigned to oxide-ion interstitials formed upon La(III) doping. A five-coordinate Ga site accommodating the interstitial defects is also probed in the ^{71}Ga MAS NMR spectrum of $\text{La}_{1.54}\text{Sr}_{0.46}\text{Ga}_3\text{O}_{7.27}$. Coalescence of all signals in the ^{17}O VT MAS NMR spectra of $\text{La}_{1.54}\text{Sr}_{0.46}\text{Ga}_3^{17}\text{O}_{7.27}$ above 300°C indicates chemical exchange between all oxide ions, in agreement with the spectral changes observed in the ^{71}Ga VT MAS NMR experiments and the appearance of cross peaks between the bridging oxide ions in the EXSY spectrum at 130°C.

The observation of chemical exchange between all framework oxygen sites implies the anticipated participation of all oxide ions in the diffusion mechanism and addresses a profound debate in the literature on this extensively studied family of fast oxide-ion conductors.

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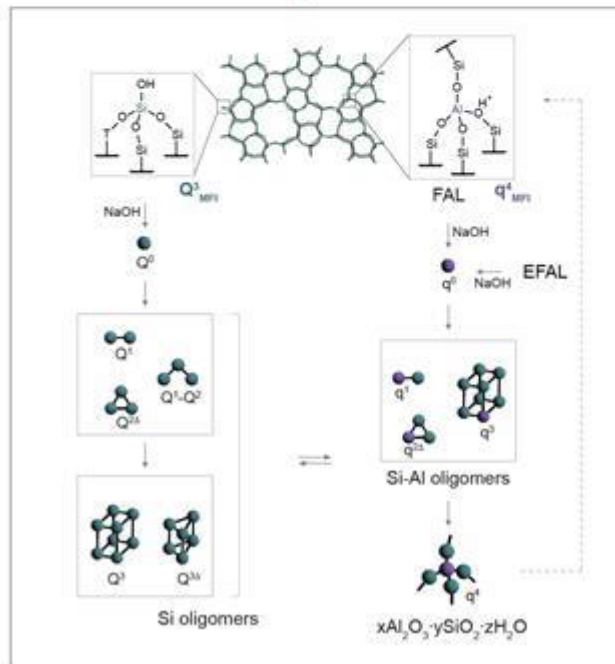
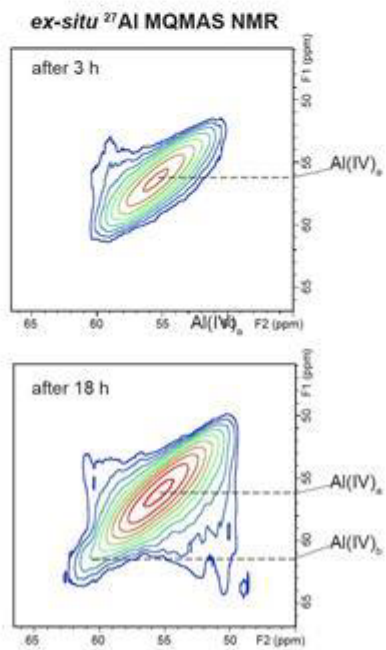
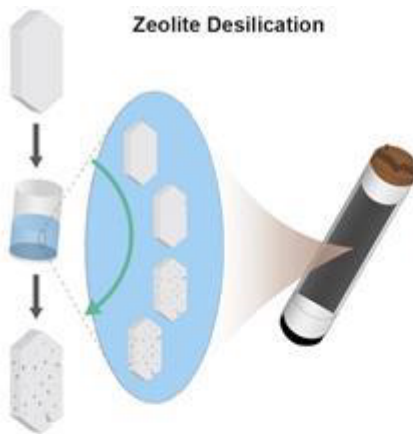
PT016 - In-situ MAS NMR Shed Light on the Formation of Hierarchical Zeolites by Desilication

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Parallel 7: Materials, Lomond, July 11, 2023, 10:45 - 12:45

Desilication of zeolites introduces secondary mesoporosity to classical microporous zeolites, and the resulting hierarchical structure shows improved transport properties. However, the underlying chemistry of zeolite desilication remains unclear since the study of solid-liquid systems by spectroscopic techniques is challenging. Here, we show the structural changes of zeolites and their chemical environment during the desilication process by in-situ MAS NMR spectroscopy. First, the desilication of zeolite (ZSM-5, Si/Al = 40) was studied by in-situ ²⁹Si and ²⁷Al MAS NMR single-pulse experiments. The results show desilication occurs in three steps: (I) the formation of Si and Al monomers indicating the initial dissolution of the zeolite, (II) the formation of Si-Si and Si-Al oligomers during the pore growth, and (III) re-insertion of Al into the zeolite and the formation of the polymeric species. Due to the tetrahedral geometry observed by ²⁷Al SS NMR studies, the re-inserted Al species are commonly considered to be fully incorporated into the zeolite framework. We revisited ex-situ ²⁷Al MAS NMR using z-filtered 3Q MAS pulse at an ultra-high magnetic field (21.1 T) and observed the unique chemical shift of the newly formed species ($\delta^{27}\text{Al}$ 59.9–63.4 ppm). Along with other characterization results, such as in-situ XRD and N₂ physisorption, we provide a comprehensive view of zeolite desilication, as well as crystallization, as both processes occur in an alkaline environment.



PT017 - Investigating Structure and Dynamics of Confined Photoswitches by Solid-State NMR

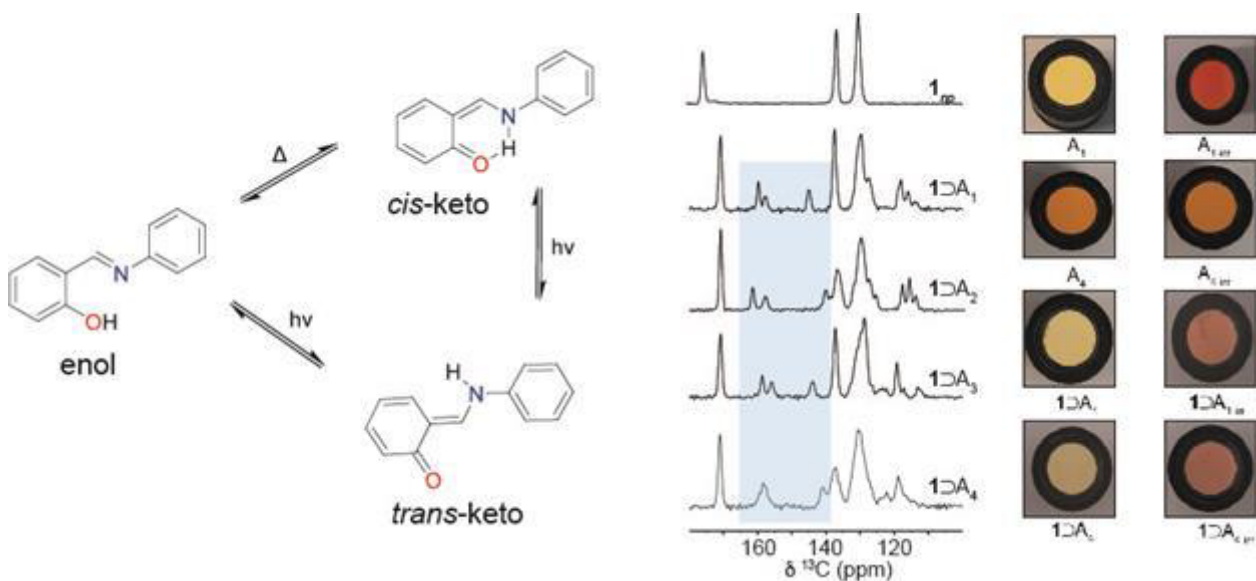
Dr John Griffin¹, Dr Kieran Griffiths¹

¹Lancaster University, Lancaster, United Kingdom

Parallel 7: Materials, Lomond, July 11, 2023, 10:45 - 12:45

Molecular photoswitches are molecules which can alter their isomeric or tautomeric structures in response to light. They have a wide range of potential applications including sensing, data storage and energy conversion. A key requirement for most photoswitches is the availability of sufficient free volume to allow structural changes to take place. In the design of solid-state photoresponsive materials, the lack of free volume in condensed phase can significantly limit or preclude the structural change necessary for photoswitching to occur. We have been investigating molecular photoswitches confined as guest molecules within metal-organic frameworks (MOFs) as a method to immobilise them within a solid architecture while still retaining sufficient free volume within the pore to change structure in response to light.

In this work, we have been studying salicylidene-aniline (anil) derivatives confined within the breathable MOF MIL-53. Some anils exhibit photochromism due to light-induced tautomerism between the ground-state enol and metastable *cis*- and *trans*-keto molecular configurations. However, photochromism is not always observed and the link between structure and photochromic properties is not well understood. For a set of four model anils, comparison of experimental ¹H and ¹³C chemical shifts shows that all are in the ground state enol tautomer in the bulk crystalline state. Upon incorporation within the MOF, two non-photochromic anils become photochromic. Single molecule DFT calculations show that the torsional angle across the C-N bond has a significant influence on the chemical shifts of the imine and quaternary ring carbons. On this basis it is possible to infer differences in the molecular conformation between the bulk crystal structure and the MOF composite that help to explain the onset of photochromism upon confinement. These results provide insight into mechanism of photoswitching in MOF composites, as well as into host-guest interactions in the wider context of breathable MOFs.



PT018 - Benchtop NMR for lab-on-a-chip

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¹Molecular Imaging for Precision Medicine, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain, ²Oxford Instruments, Abingdon, United Kingdom, ³Multiwave Imaging, Marseille, France

Parallel 8: Benchtop/Low Field MR, Alsh, July 11, 2023, 10:45 - 12:45

INTRODUCTION: We have developed a benchtop NMR spectrometer to accommodate microfluidic platforms containing 3D in vitro models and enable the study of their cellular metabolism in real-time, in situ and non-destructively by hyperpolarization-enhanced MRS.

METHODS & RESULTS: A 1.4 T commercial benchtop NMR spectrometer (Oxford Instruments) has been modified to allow for planar microfluidic chips to be inserted into the scanner. A microfluidic chip was fabricated in PDMS with channels interconnecting a cylindrical science chamber for NMR detection and a passive membrane pump for delivery of hyperpolarized solutions (Fig.1A). A Tx/Rx 6-turn cylindrical saddle coil was designed to be embedded in the chip and to maximise SNR and B1 homogeneity in the science chamber. The averaged amplitude in the sample volume is 1.15 mT at 15 MHz for 1W of input power. This efficiency approaches 75% of the standard solenoid probe used in the regular benchtop spectrometer setup. The homogeneity is evaluated in the sample volume at 20% of relative standard deviation. Shimming procedures were tested including a 3D spatial field mapping protocol analogous to gradshim. This protocol could deliver a linewidth of less than 0.1ppm (Fig.1C). Neither the material used for the chip nor the sample caused susceptibility artifacts. A carrier was 3D printed from a photopolymer resin to accommodate the chip outside the scanner prior to the NMR experiment, improving usability (Fig. 1D). Our sample consisted of a 3D cell model containing liver spheroids onto a carboxymethyl cellulose scaffold. To test the system, hyperpolarized [1-13C]pyruvate will be prepared as in ref.3 and injected into the microfluidic system containing the cell-laden scaffold to follow its metabolic conversion in real time.

CONCLUSION: The equipment developed in this project will be an easy-to-use and low-cost microfluidic platform for NMR studies of biomedical tissue engineering, environmental control and chemical industry.

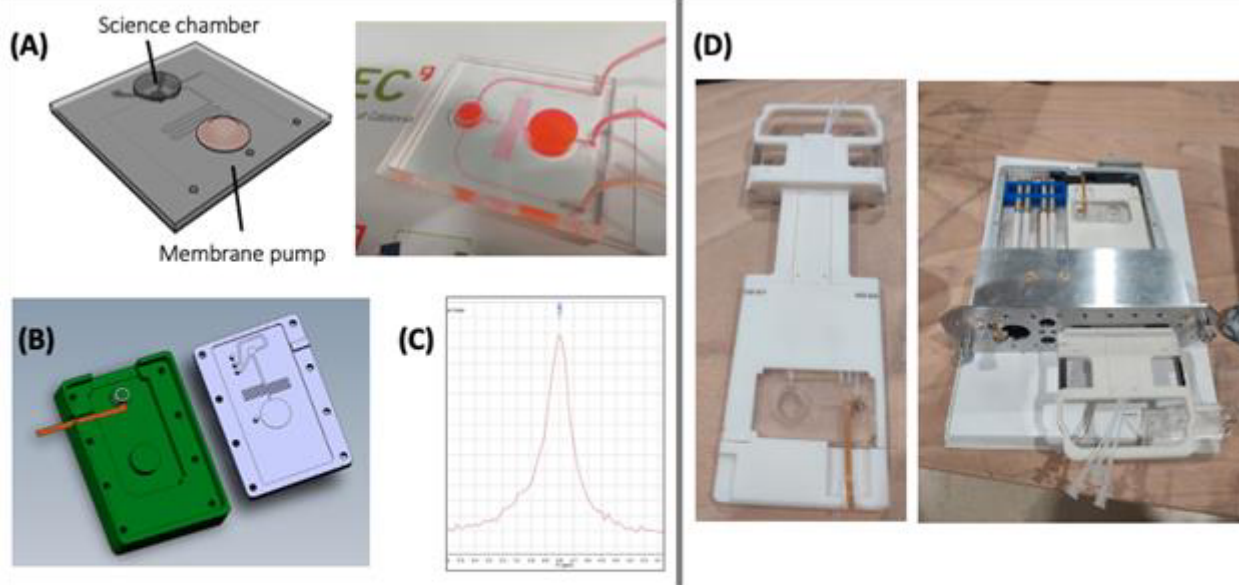


Figure 1. *Left:* Microfluidic chip designed for a fast and controlled delivery of a hyperpolarized solution while retaining the media renewal capabilities of microfluidic chip bioreactors. (A) Design and fabricated chip. (B) Mould designed to facilitate the fabrication of the chip with an embedded RF coil. (C) NMR signal of water doped with CuSO_4 in a chip. The system resolution can be deduced as being better than 0.1ppm as the linewidth is dominated by the intrinsic FWHM of the doped water ($\sim 10\text{Hz}$, 0.17ppm). *Right:* Carrier to ease microfluidic chip insertion into the benchtop NMR scanner probe. (D) 3D printed carrier with microfluidic chip and demonstration of the carrier fit into the spectrometer's probe.

PT019 - ^{27}Al NMR study of the $\text{Al}_{0.5}\text{TiZrPdCuNi}$ in high-entropy alloy and metallic glass forms

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Parallel 8: Benchtop/Low Field MR, Alsh, July 11, 2023, 10:45 - 12:45

High-entropy alloys (HEAs) are crystalline solid solutions, composed of five or more different chemical elements in near-equiatomic ratios. A large number of HEAs contain more than one phase and their multiphase structure frequently results in technologically relevant and tunable physical-mechanical properties like high hardness and strength (due to the precipitation strengthening mechanism), a combination of hardness and ductility (one phase is hard and the other one ductile) and a combination of magnetic softness and zero magnetostriction for supersilent alternating-current applications like a “non-humming” grid transformer [1].

HEAs are characterized by a simultaneous presence of a crystal lattice and an amorphous type chemical disorder. In order to unravel the effect of crystal-glass duality on their physical properties, we have studied a 6-component $\text{Al}_{0.5}\text{TiZrPdCuNi}$ that can be prepared either as a HEA or as a metallic glass (MG) at the same chemical composition. We performed a comparative ^{27}Al NMR study of the distribution of EFG tensors and the local electronic density of states (DOS) $g(\epsilon_F)$ at the Fermi level at the position of ^{27}Al nuclei for both structural modifications [2]. A theoretical $I = 5/2$ quadrupole-perturbed NMR spectrum, pertinent to both cubic HEAs and amorphous MGs, has also been derived. The EFG distribution function of the MG state is about twice broader than that of the HEA state, reflecting the existence of a (distorted) crystal lattice in the latter and its absence in the former. The T^2 -dependence of the Knight shift indicates that the DOS is changing rapidly within the Fermi-level. The local DOS at the ^{27}Al sites of the HEA sample is about 10% larger than that of the MG state, indicating comparable degrees of disorder.

[1] J. Luzar, et al. Adv. Mater. Interfaces 9 (2022) 2201535.

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PT020 - Operando Benchtop NMR Method for Studying Redox Flow Batteries

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¹Radboud University Nijmegen, Nijmegen, Netherlands

Parallel 8: Benchtop/Low Field MR, Alsh, July 11, 2023, 10:45 - 12:45

Introduction

Redox flow batteries (RFB) are very promising energy storage systems for future renewable energy resources. High field NMR-based operando analyses of RFB systems have yielded significant insight into their working mechanisms. Nevertheless, the high cost and large footprint of a high-field NMR system limit its implementation for a wider electrochemistry community.

Aims

In this study, we demonstrate the feasibility of performing an operando NMR study of a RFB system on a low-cost and compact 43 MHz benchtop spectrometer.

Methods

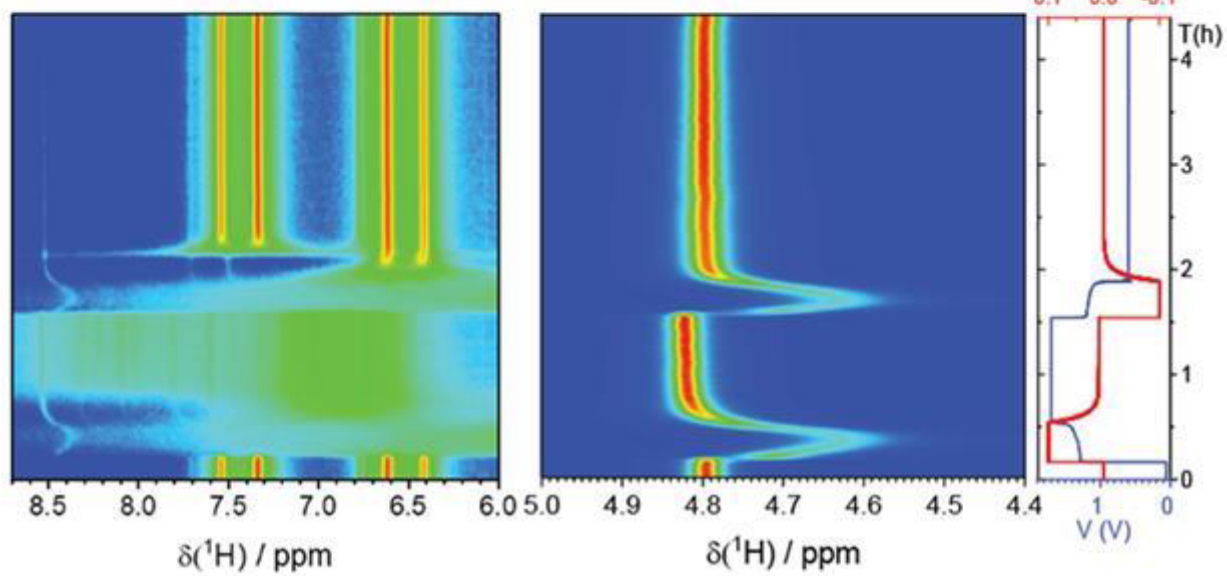
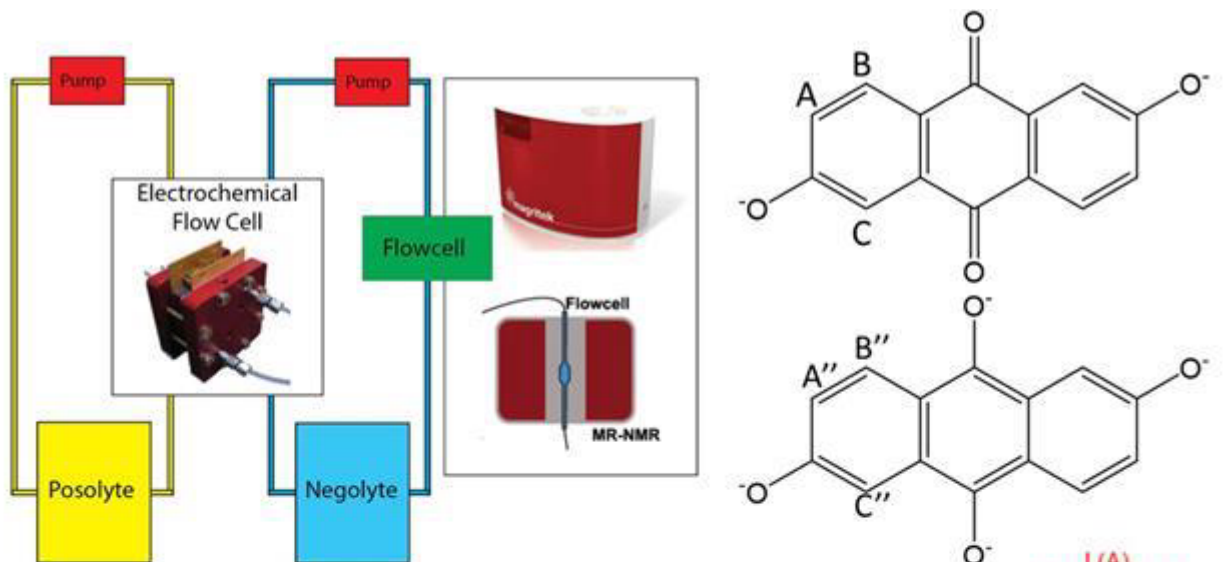
Online NMR analyses of an anthraquinone/ferrocyanide-based RFB using a Magritek benchtop NMR using a flow tube setting.

Results

Evans method was utilized to estimate the concentrations of paramagnetic anthraquinone radical and ferricyanide anions. The degradation of 2,6-dihydroxy-anthraquinone (DHAQ) to 2,6-dihydroxy-anthrone and 2,6-dihydroxy-anthranol has also been quantified. Furthermore, the impurities commonly present in the DHAQ solution were identified to be acetone, methanol and formamide. The crossover of DHAQ and impurity molecules through the Nafion® separation membrane was captured and quantified, and a negative correlation between the molecular size and crossover rate was established.

Conclusions

This study shows that a benchtop NMR system has sufficient spectral and temporal resolution and sensitivity for the operando study of RFBs. A broad range of applications of operando benchtop NMR methods is anticipated for studying flow electrochemistry targeting a range of environmentally relevant processes.



PT021 - SABRE-hyperpolarized [1-¹³C]pyruvate metabolism detected *in vivo*

Professor Thomas Theis¹, Austin Browning, Keilian McCulloch, Professor Eduard Chekmenev, Professor Boyd Goodson, Prof. Matthew Rosen, Dr. David Guarin Bedoya, Dr. Carlos Dedesma, Dr. Patrick TomHon, Prof. Yi-Fen Yen

¹NC State University, Raleigh, United States

Parallel 9: Hyperpolarization, Boisdale, July 11, 2023, 10:45 - 12:45

Introduction:

Signal Amplification By Reversible Exchange (SABRE) is a particularly fast and simple hyperpolarization modality. Since its inception, SABRE has received significant attention in the scientific community because of its relative ease and great promise for delivering hyperpolarized molecular imaging probes. Yet, *in vivo* measurements have not been shown because of challenges with biocompatibility. Here we overcome biocompatibility concerns and present real-time metabolic tracking, and Chemical Shift Imaging (CSI) in various organs, the whole body at multiple sites, and a multiple magnetic field strengths.

Aims:

The aim of this study was to demonstrate the detection and imaging of SABRE-hyperpolarized [1-¹³C]pyruvate and its metabolic conversion *in vivo*. We aimed for demonstrations in multiple organs, whole body, at multiple sites and at multiple magnetic field strengths to illustrate the broad scalability of the approach.

Methods:

[1-¹³C]pyruvate was hyperpolarized by SABRE and processed with phase transfer and catalyst-filtration to obtain biocompatible solutions. The solutions were injected into the tail or jugular veins of healthy Wistar rats. The Measurements were performed on the liver and kidney with a surface coil at 4.7 T at MGH to obtain time resolved spectra as well as spectrally resolved CSI. Also, whole body metabolic measurements were obtained with a volume coil of a 1.5 T cryogen-free MRI system at NC State.

Results:

Metabolic conversion of pyruvate to lactate, alanine, pyruvate-hydrate and bicarbonate was detected. The animals vitals, heart and breathing rate, remained stable, CSI images were obtained, multi-site validation was provided, and both standard-high field MRI and cryogen-free lower-field approaches were found to deliver comparable results.

Conclusions:

In conclusion, we showcased the feasibility and scalability of SABRE hyperpolarized imaging across sites and magnetic fields. Looking ahead, the combination of a simple, and fast SABRE hyperpolarization scheme with affordable, low-cost MRI may lead to broadly scalable molecular imaging.

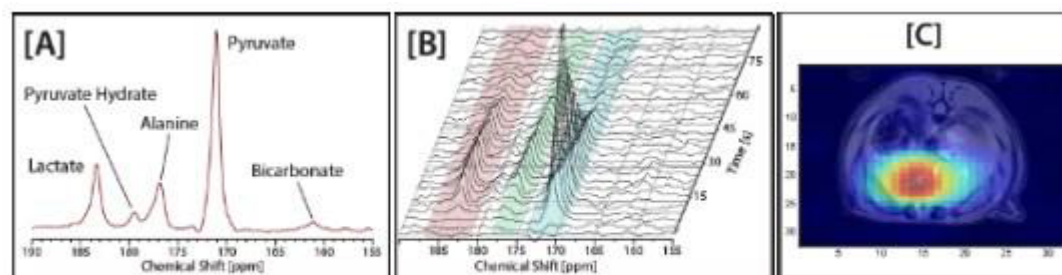


Figure 1. SABRE-hyperpolarized [1-¹³C]pyruvate metabolism and imaging in vivo. A) Metabolic conversion of pyruvate to lactate, alanine, pyruvate-hydrate and bicarbonate. B) Metabolism as a function of time acquired every 2 seconds. C) CSI image of hyperpolarized pyruvate in the liver.

PT022 - Solid-effect dynamic nuclear polarization in viscous liquids using narrow-line polarizing agents at 9.4 T

Dr Andrei Kuzhelev¹, Dr Vasyi Denysenkov¹, Ms Iram Ahmad², Dr Olga Rogozhnikova³, Dr Dmitry Trukhin³, Prof Elena Bagryanskaya³, Dr Victor Tormyshev³, Prof. Snorri Th. Sigurdsson², Prof Thomas Prisner¹

¹Goethe University Frankfurt am Main, Frankfurt Am Main, Germany, ²University of Iceland, Reykjavik, Iceland, ³N. N. Vorozhtsov Institute of Organic Chemistry, Novosibirsk, Russia

Parallel 9: Hyperpolarization, Boisdale, July 11, 2023, 10:45 - 12:45

Dynamic nuclear polarization (DNP) is a hyperpolarization method that is widely used for increasing the sensitivity of nuclear magnetic resonance (NMR) experiments. DNP is efficient in solid-state and liquid-state NMR but its implementation in the intermediate state, namely viscous media, is still less explored. We recently demonstrated that α,γ -bis(diphenyl)- β -phenyllalyl (BDPA) radicals can be used to polarize lipid bilayers in the fluid-phase through the solid effect mechanism at high magnetic fields [1]. Here, we show that ^1H DNP enhancement of over 50 can be obtained in viscous liquids at a magnetic field of 9.4 T and a temperature of 315 K. This was accomplished by using narrow-line polarizing agents in glycerol, both a water-soluble BDPA and triarylmethyl radicals, and a microwave/RF double-resonance probehead. We observed DNP enhancements with a field profile indicative of the solid effect and investigated the influence of microwave power, temperature and concentration on the ^1H NMR results. To demonstrate potential applications of this new DNP approach for chemistry and biology, we show hyperpolarized ^1H NMR spectra of tripeptides in glycerol- d_8 [2]. In addition, we studied direct ^{13}C and indirect $^1\text{H} - ^{13}\text{C}$ DNP using triarylmethyl radicals as polarizing agent in glycerol- $^{13}\text{C}_3$ at a magnetic field of 9.4 T and temperature of 315 K.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, grant 518679424) and FOCUS program funded by Goethe University Frankfurt am Main.

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PT023 - Probing Biomolecular Interactions in Homogeneous Media and Reversed Micelles Using Para-Hydrogen Polarization

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¹Texas A&M University, College Station, United States

Parallel 9: Hyperpolarization, Boisdale, July 11, 2023, 10:45 - 12:45

Introduction

Nuclear spin hyperpolarization provides new opportunities for biomolecular NMR measurements in the physiological concentration regime, at faster time scale and with enhanced selectivity. Para-hydrogen based hyperpolarization methods can supply large signal enhancements at low cost, when they can be made applicable to biological molecules.

Aims

We aim to demonstrate the use of para-hydrogen polarized probes for measuring biological interactions. Reverse micelles provide a nano-scale phase separated medium optimizing parahydrogen delivery in an organic environment and solubilizing proteins in the aqueous phase.

Methods

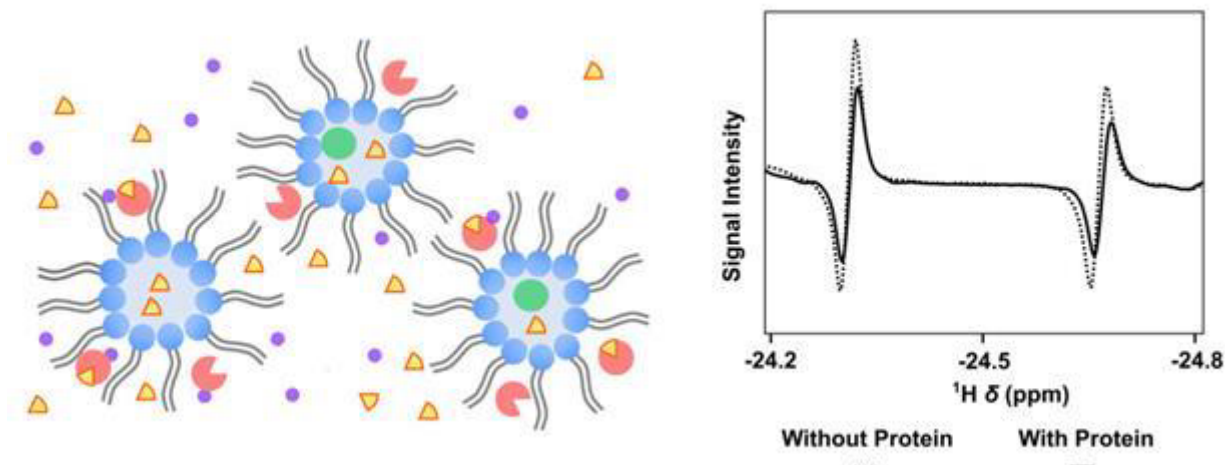
Molecules with nitrogen containing heterocycles were designed to bind to the iridium of a signal amplification by reversible exchange (SABRE) catalyst, while having the ability to interact specifically with a target protein. Para-hydrogen was introduced to the heterogeneous sample containing solubilized protein in the reverse micelles, or was introduced to molecules in homogeneous solution, which were subsequently mixed with protein. ¹H and ¹⁹F NMR signals were measured.

Results

Signal enhancements of several hundred-fold at 400 MHz were obtained for probes targeting the trypsin protein, when hyperpolarized in methanol solution. A change in the R2 relaxation rate was observed after dilution of a small aliquot with the protein solution. The binding affinities of competing ligands were derived from the R2 measurements. Alternatively, the interaction of a small molecule with a protein was detectable in reverse micelles in a single pot reaction (Figure). Thereby, the repartitioning of the small molecule between the aqueous and organic phases was sensed using a change in the strongly enhanced iridium hydride signals.

Conclusions

Para-hydrogen polarized probes provide a sensitive means for detecting biomolecular interactions. Reverse micelles optimize the use of parahydrogen by containing an organic phase. The application of para-hydrogen polarization to study biomolecular problems is broadened, including topics such as protein-ligand and protein-protein interactions or protein folding.



PT024 - Imaging local diffusion in microstructures using NV-based pulsed field gradient NMR

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Sustainable Systems Engineering (INATECH), University of Freiburg, Freiburg, Germany, ⁵Fraunhofer Institute for Applied Solid State Physics, Freiburg, Germany

Parallel 10: Single Molecule/NV, Alsh, July 12, 2023, 10:45 - 12:45

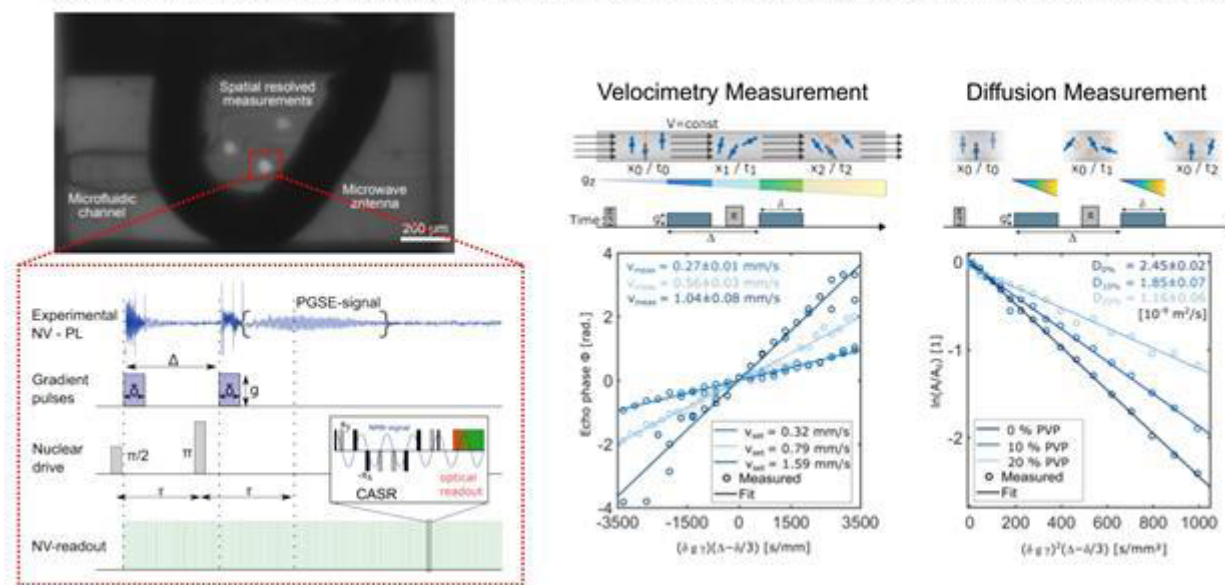
The understanding of diffusion within microstructures is crucial in various scientific fields, such as energy research, cancer research, and neuroscience. Magnetic resonance methods are widely used for quantitative diffusion measurements, but they lack sensitivity in measuring and resolving diffusion within individual microstructures. In this study, we introduce nitrogen-vacancy (NV) center-based nuclear magnetic resonance (NMR) spectroscopy [1] as a novel tool to probe diffusion in individual structures on a microscopic scale. Our experimental scheme combines pulsed gradient spin echo (PGSE) with optically detected NV-NMR, allowing us to quantify molecular diffusion and flow within nano-to-picoliter sample volumes [2]. We demonstrate correlated optical imaging with spatially resolved PGSE NV-NMR experiments to probe anisotropic water diffusion within a model microstructure [3]. This method has the potential to extend the current capabilities of investigating diffusion processes to the microscopic scale, allowing for the probing of tissue microstructures, single cells, or ion mobility in thin film materials for battery applications.

[1] R. D. Allert et al., Chem. Commun., 2022,58, 8165-8181

[2] F. Bruckmaier et al., Imaging local diffusion in microstructures using NV-based pulsed gradient NMR, arXiv:2303.03516v1 [physics.app-ph]

[3] R. D. Allert et al., Lab Chip, 2022,22, 4831-4840

Measuring spatially resolved flow and diffusion in microstructures using PGSE NV-NMR



PT025 - Tunable sub-THz masing of nitrogen-vacancy centers in high magnetic fields

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Parallel 10: Single Molecule/NV, Alsh, July 12, 2023, 10:45 - 12:45

Nitrogen-vacancy (NV) centers already proved their best in the field of metrology, especially as highly sensitive magnetometers. Their application as qubits or spin photon interfaces is also promising based on their long coherence time and their unique electronic structure resulting in the phenomenon of optical spin polarization. In the field of quantum computing this means that just by continuous optical pumping, the ground state triplet can be initialized into the $m=0$ spin state due to intersystem crossings. This effect can be exploited in microwave amplifiers or oscillators because in an external magnetic field higher than ≈ 0.1 T the $m=0$ state is no longer the state with the lowest energy therefore the spin polarization leads to population inversion. Previously, continuous masing was achieved by putting the NV centers in an X-band microwave resonator and applying resonant external magnetic field[1]. Since then, quantum limited performance of NV based microwave amplifiers was also reported[2]. Here, we show that the masing can be scaled up to higher magnetic fields extending the corresponding emission frequency into the sub-THz regime. However, even in high magnetic fields up to 16 T the relative angle between the external magnetic field and the NV axis i.e. the zero field splitting (ZFS) \hat{z} -axis seems to play a crucial role in the spin polarization effect. Surprisingly, the ZFS can not be handled as a small perturbation in high magnetic field. We present temperature dependent high field/ high frequency electron spin resonance (ESR) spectra, light induced ESR and simulate the population of states in the strongly illuminated limit to achieve a better understanding of the results[3].

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- [3] S. Kollarics, et al., Stimulated THz emission from diamond nitrogen-vacancy centers: a route towards tasers (prep.)

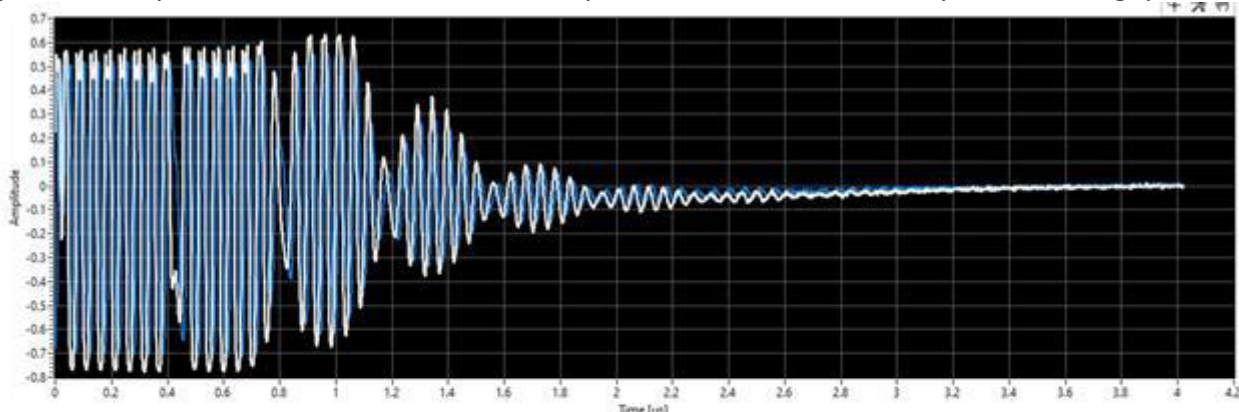
PT026 - Observation of quantum cavity-spin dynamics using diamond NVs at ambient conditions

Professor Aharon Blank¹

¹Technion - Israel Institute of Technology, Haifa, Israel

Parallel 10: Single Molecule/NV, Alsh, July 12, 2023, 10:45 - 12:45

Magnetic resonance (MR) can be used as a testbed for observing, demonstrating and studying fundamental concepts of quantum cavity interaction with a two-level system. Such experiments are mostly carried out at low cryogenic temperatures with spin systems that are highly polarized, embedded in a very high quality (Q-factor) superconducting cavity. The photons in the cavity can excite the spin system, which in turn, can also excite the cavity back, revealing a plethora of non-linear quantum phenomena, such as splitting the resonance frequency of the cavity (strong coupling), multiple echo formation and superradiance. In this work we show that using a unique tailor-made low Q cavity, in conjunction with diamond crystals having large concentration of nitrogen vacancy (NV) centers, such non-linear quantum phenomena can be observed even at ambient conditions. For example, we present measurements and theoretical analysis of multiple echoes appearing after a simple two pulse Hahn sequence. We also discuss the possibility of demonstrating superradiance with such system. Finally, we show that our experimental system can also be used to cool the electromagnetic mode of the resonator to a temperature well below its ambient temperature, with potential implications to microwave-related quantum circuits, such as superconducting qubits.



PT027 - Brain Regions Show Different Metabolic and Protein Arginine Methylation Phenotypes in Frontotemporal Dementias and Alzheimer's Disease

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²Key Laboratory of Gastrointestinal Cancer (Fujian Medical University), Ministry of Education, Fuzhou, China, ³Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology,

London, UK, ⁴Queen Square Brain Bank for Neurological Diseases, UCL Queen Square Institute of Neurology, London, UK, ⁵BioTechMed-Graz, Graz, Austria

Parallel 11: Metabolomics, Lomond, July 12, 2023, 10:45 - 12:45

Introduction

Frontotemporal dementia (FTD) is a heterogeneous neurodegenerative disease with multiple histopathological subtypes. FTD patients exhibit similar symptoms as in Alzheimer's disease (AD), and are often misdiagnosed with AD despite the consensus clinical diagnostic criteria. Therefore, identification of FTD-specific biomarkers is considered a necessity. For this purpose, NMR could serve as a powerful tool allowing high-throughput quantitative analysis of complex biological matrices with high reproducibility.

Aims

We aimed to explore metabolic alterations in FTLD-TDP and identify relevant tissue biomarker candidates. Since protein arginine methylation is connected to pathological processes in neurodegeneration, we investigated the relation between arginine methylation level and metabolic phenotypes in FTD and AD.

Methods

A complex analysis of post-mortem tissue from different brain regions of control, FTLD-TDP subtypes and AD patients was conducted using untargeted and targeted NMR metabolomics. For arginine methylation quantification, we used NMR-based method developed in our lab relying on CPMG and JRES experiments.

Results

Our results indicate that brain subdivisions responsible for different functions show different metabolic patterns. Different FTD subtypes and AD share similar metabolic phenotypes in cerebellum, but AD exhibits distinct metabolic patterns in frontal and occipital regions compared to FTD. We provide NMR-based metabolite panels that might help in systematic subtyping of the diseases. Furthermore, protein arginine methylation levels were revealed to be region-specific and correlate with FTD-/AD-specific metabolic alterations.

Conclusions

NMR spectroscopy was proved to be a suitable technique for detection of metabolic phenotypes in brain tissues. Identified brain region-specific biomarkers might serve as a tool for distinguishing FTD subtypes and AD and provide the first insights into metabolic changes related to neurodegenerative diseases in different brain regions. Our findings highlight the relationship between arginine methylation and metabolic changes in FTD and AD that could be further explored for a deeper understanding of pathogenesis molecular mechanism.

PT028 - Real Time Pulse Chase in-cell NMR, RTPC-NMR, spectroscopy reveals that mRNA in lipid nanoparticles rescues cells from lipid-induced mitochondrial dysfunction.

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¹University at Albany, SUNY, Albany, United States, ²New York University, New York, United States

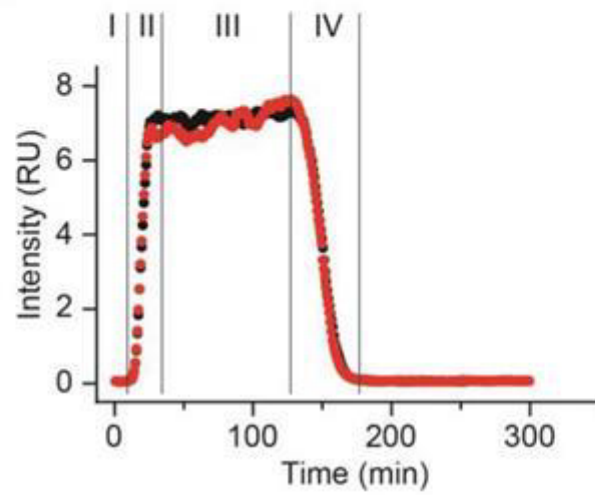
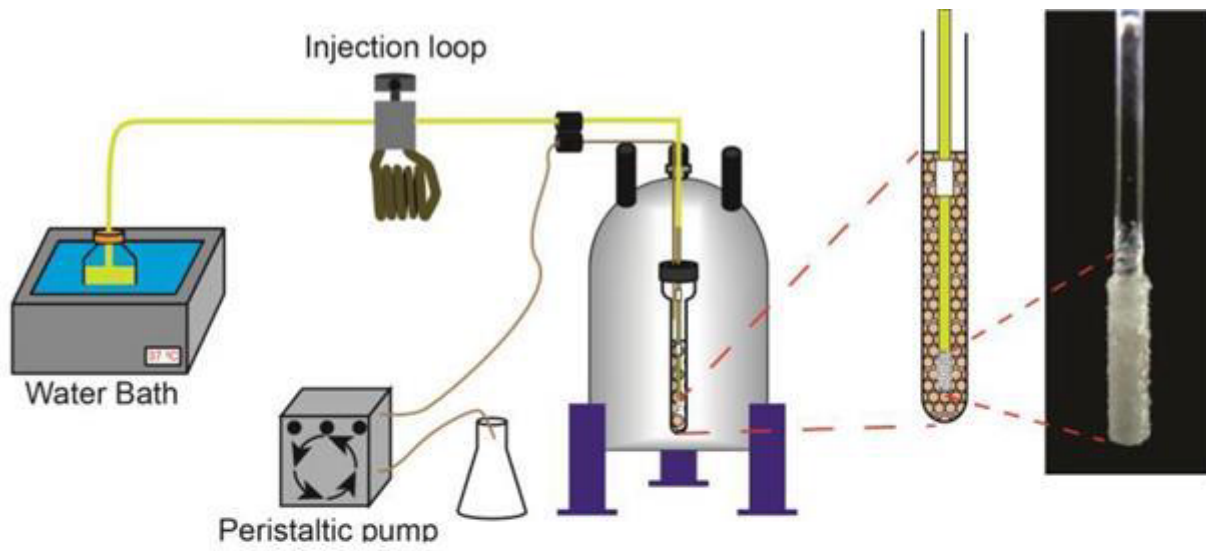
Parallel 11: Metabolomics, Lomond, July 12, 2023, 10:45 - 12:45

Introduction. Analytical tools to study cell physiology are critical for optimizing drug-host interactions. Real time pulse chase in-cell NMR spectroscopy, RTPC-NMR, was introduced to monitor the kinetics of metabolite production in HEK 293T cells treated with COVID-19 vaccine-like lipid nanoparticles, LNPs, with and without pseudouridine-modified mRNA.

Methods. RTPC-NMR, combines the use of ¹³C-glucose and ¹³C-edited proton NMR with an ultrasensitive NMR cryoprobe and the improved design of a bioreactor to increase the time resolution of the experiments to 47 s allowing rapid changes in metabolic fluxes in response to stimuli to be followed. Kinetic flux parameters were resolved for the incorporation of isotopic label into metabolites and clearance of labeled metabolites from the cells.

Results. Changes in the characteristic times for alanine production implicated mitochondrial dysfunction as a consequence of treating the cells with lipid nanoparticles, LNPs. Mitochondrial dysfunction was largely abated by inclusion of mRNA in the LNPs, the presence of which increased the size and uniformity of the LNPs.

Conclusions. The RTPC-NMR methodology is applicable to study metabolic kinetics in all cultured cells.



PT029 - NMR metabolomics multi-tissue study of the chronic low-dose exposure to a cocktail of Persistent Organic Pollutants

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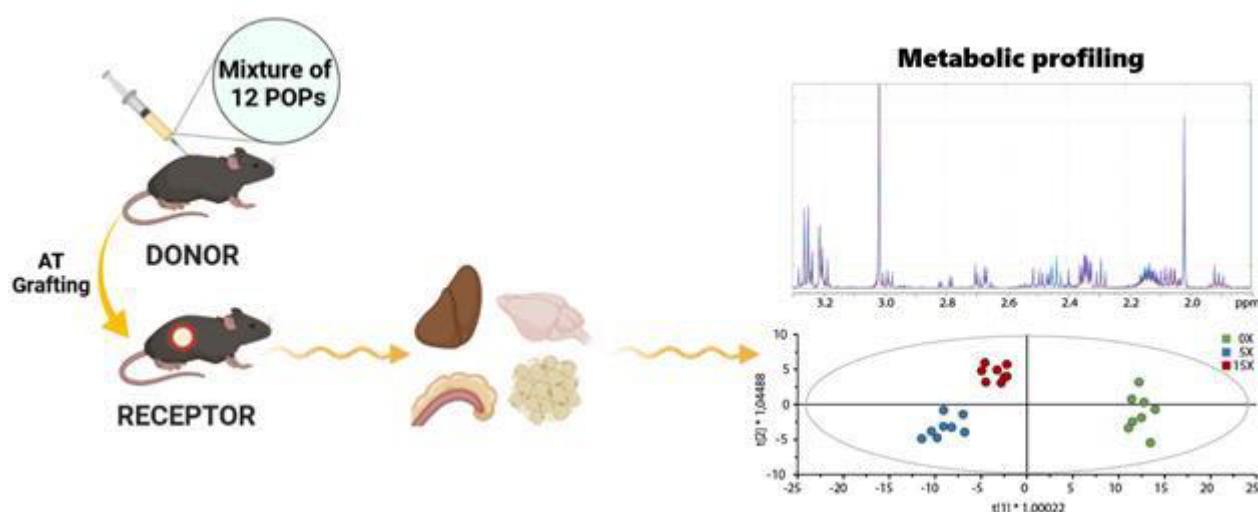
Parallel 11: Metabolomics, Lomond, July 12, 2023, 10:45 - 12:45

Exposure to endocrine disrupting chemicals (EDCs) including persistent organic pollutants (POPs) represents one of the most critical public health threats nowadays. EDCs interfere in the body's endocrine system and have been associated with a diverse array of health issues. POPs deserve a particular attention among them since due to their lipophilic properties and resistance regarding the xenobiotic metabolism, they can bio-accumulate for long periods of time in adipose tissues (AT).¹ This innovative untargeted metabolomics study aims to look into the low-dose and chronic internal exposure to a cocktail of POPs, on multiple tissues known to accumulate these lipophilic compounds. In order to mimic a chronic internal exposure,² a group of donor mice was injected with a cocktail of 12 POPs at different doses (0x, 5x and 15x the LOAEL*). Their adipose tissues were grafted into a group of receptor mice, from which biopsies of liver, brain, epididymal and retroperitoneal AT were obtained after different exposure times (3 and 21 days). Polar metabolites were extracted from the tissues and analysed by NMR.

Interestingly, the metabolic response differs among the selected tissues in mice. In liver, we observed a dynamic effect according to the exposure time and doses of POPs. In brain the presence of POPs gives immediately a saturated effect which is independent of the dose and exposure time studied. In the opposite, for both adipose tissues nearly no effect is observed. This metabolic profiling leads to a holistic and dynamic vision on the main metabolic pathways impacted in lipophilic tissues by a cocktail of POPs, extending our knowledge of what could be reproduced in exposed human population.³

* LOAEL: Lowest Observed Adverse Effect Level

1. Müllerová D. et al, *Physiol Res.* (2007)
2. Joffin N. et al, *Environ Int.* (2018)
3. Lucas-Torres, C. et al, *NMR in biomedicine* (submitted)



PT030 - 3D velocity mapping of slow flow in porous media

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Parallel 12: MRI & In vivo, Boisdale, July 12, 2023, 10:45 - 12:45

Understanding root water uptake is indispensable for the optimization of plant growth and crop yield against the background of climate change. One strategy is to understand how root water uptake functions at the root-soil interface. Whereas direct imaging of fluxes in the above-ground plant stem has been performed by the group of Van As [1], little is known about the 3D flow pattern, dispersion and velocities in the soil-root compartment. This is due to the heterogeneous and hierarchical structure of the root system [2], resulting in small fluid displacements of some tens of micrometer per second based on the transpiration rate of the plant. It has been shown by Spindler et al. [3] that mean flow rates of a homogeneous flow as low as 0.06 mm/s can be measured using 13-interval stimulated echo multi-slice imaging (STEMSI).

Here we report on further developments for the acquisition of water transport around the roots of a life plant system. While a 3D MRI image of the root system with sufficient spatial resolution is necessary it is also important to obtain the 3D information of the water velocity in the plant roots. These requirements need to be balanced against the necessary acquisition time for this 6D data set since the plant is growing and therefore changing its root system over time. To meet this requirement the Stimulated Echo Acquisition Mode (STEAM) [4] have been combined with STEMSI, thus enabling rapid multi-slice acquisition while retaining sufficient signal to noise ratios.

References

- [1] H. Van As et al., in Encyclopedia of Magnetic Resonance. 2012, John Wiley & Sons.
- [2] Y. Liu et al.: Scientific Reports, 8, 2960 (2018).
- [3] N. Spindler et al., Journ. Magn. Reson., 212, 216-223 (2011).
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PT031 - Rapidly Signal-Enhanced 1-¹³C-Pyruvate via Parahydrogen-Induced Polarization for Magnetic Resonance Spectroscopic Imaging of Metabolism *in Vivo*

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Parallel 12: MRI & *In vivo*, Boisdale, July 12, 2023, 10:45 - 12:45

MRI (Magnetic Resonance Imaging) is a tool widely used in clinical diagnostics of various diseases, even though it is limited by a relatively low sensitivity. Parahydrogen-induced polarization (PHIP) is a method to rapidly signal-enhance ¹³C-enriched molecules by over four orders of magnitude, paving the way to investigate potential tracers for MR(S)I (Magnetic Resonance (Spectroscopic) Imaging), and to observe their metabolic conversion *in vivo* and in real-time. Pyruvate is a molecule of special interest, as it is involved in various metabolic pathways such as glycolysis and the TCA cycle. In cancerous tissue, the metabolic conversion of pyruvate to lactate is upregulated (Warburg effect) making it a promising marker for cancer diagnosis and determination of tumor aggressiveness. The aim of the presented work is to demonstrate the application of 1-¹³C-pyruvate-d₃, rapidly hyperpolarized with PHIP in seconds, as a robust method to characterize the metabolism in xenografts of different cancer types.

Human-derived colon and pancreatic cancer cells were injected subcutaneously above the flanks of athymic mice to generate tumors. Following injection of 1-¹³C-pyruvate-d₃ into the tumor-bearing animals, slice-selective NMR spectra were acquired and the data obtained were analyzed to obtain rate constants of metabolic conversion. Pyruvate converted to lactate and alanine could be shown for these two types of cancer.

These data demonstrate the first successful application of 1-¹³C-pyruvate-d₃ for the metabolic analysis of human derived colon and pancreatic tumors and represent a first step towards application of this non-invasive imaging approach to human malignancies. We envision rapidly hyperpolarized MRI to accompany PET and other tools to aid in the early and differential diagnosis of cancer.

PT032 - *In situ* NMR study of a diverse set of electrochemical storage devices using optimized parallel-plate resonator RF probes

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Parallel 12: MRI & *In vivo*, Boisdale, July 12, 2023, 10:45 - 12:45

Introduction: Li-ion batteries (LIBs) with graphite anodes have become ubiquitous in society; however, improved lifetime of LIBs requires increased energy density, achievable partially by using anodes with higher capacities than graphite (372 mAh/g). Silicon (>3500 mAh/g) is an attractive alternative, but huge volume expansion (~300%) upon lithiation and subsequent disintegration hinders its adoption as a viable anode[1]. Beyond mobile devices, a Net-Zero future requires appropriate energy storage for the grid, for which LIBs are impractical. Alternative chemistries are composed of earth-abundant materials and aqueous electrolytes, which are environmentally friendly, sustainable, and cost-effective[2]. NMR is uniquely suited to study local atomic environments in batteries, and the choice of RF probe is crucial to achieving high sensitivity measurements.

Aims: To monitor aging and defect formation in various battery chemistries by *in situ* NMR.

Methods: The parallel-plate resonator (PPR) has recently been proposed as a prime choice due to natural geometric matching to prismatic electrochemical cells and production of uniform B_1 fields, yielding high sensitivity[3,4]. Optimized ^1H and ^7Li PPRs provide sensitive *in situ* and *operando* NMR with fine temporal resolution.

Results: Repeated cycling of silicon-based anodes at moderate/high rates yields accumulation of irreversible lithium metal and concentrated lithium silicides, identifying a key capacity fade mechanism by ^7Li NMR. The response of plated Li-metal signal to increasing charging currents is particularly revealing and is quantified using ssNake[5] with a home-written intuitive sequential fitting tool. Additionally, ^1H NMR identifies the accumulation of Mn(II) in the aqueous electrolyte of a MnO_2 half-cell upon repeated cycling.

Conclusions: New insight into Si anode aging and cathode degradation are achieved with *in situ* and *operando* NMR measurements utilizing optimized RF probes. This opens the door to routine NMR studies of fast-charged electrochemical systems by way of high sensitivity measurements with ample temporal resolution.

[1]-<https://doi.org/10.1021/cr500207g>

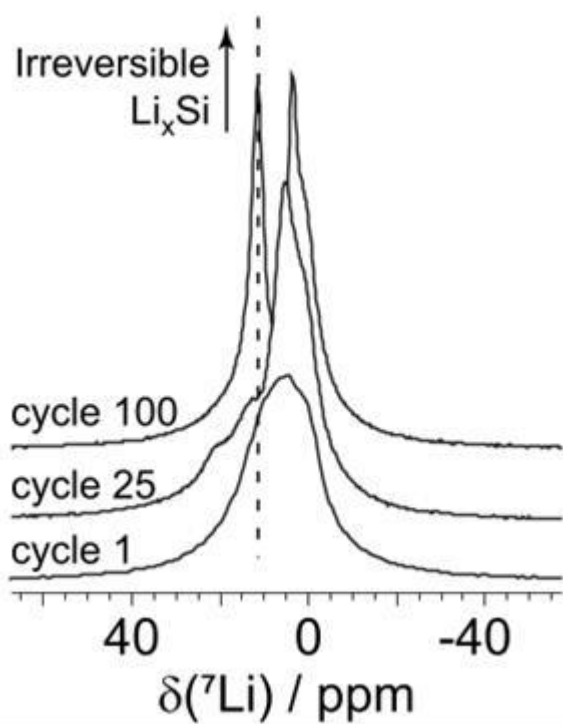
[2]-<https://doi.org/10.1016/j.joule.2020.03.002>

[3]-<https://doi.org/10.1016/j.mrl.2023.01.002>

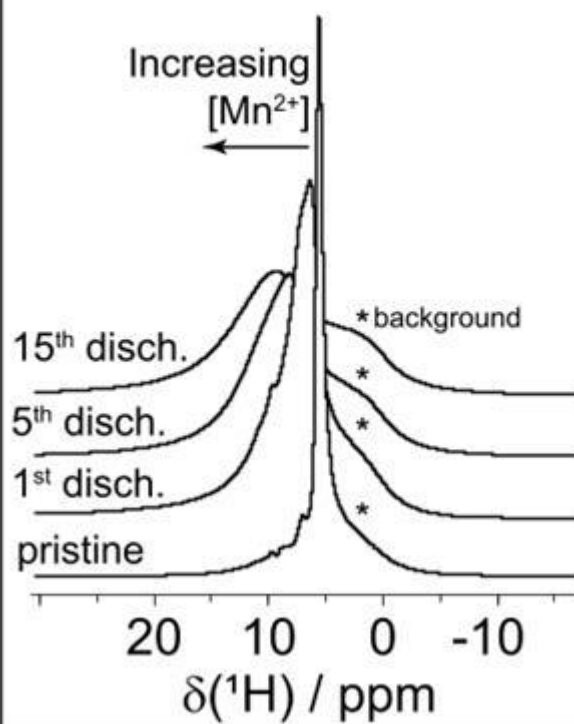
[4]-<https://doi.org/10.1016/j.carbon.2021.12.082>

[5]-<https://doi.org/10.1016/j.jmr.2019.02.006>

Li-ion, Si anode



MnO_2 cathode, aqueous



PT033 - Operando NMR Visualisation of Ion Dynamics in Conducting Polymer on Electrochemical Gating

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Parallel 13: Materials, Alsh, July 12, 2023, 15:45 - 17:45

Introduction

Organic mixed ionic-electronic conductors (OMIECs) are a rising class of soft functional materials with coupled ionic and electronic conductivity which are exploited for neural recording, sensing and energy storage. At the core of OMIEC device operation is the conversion of ionic fluxes from the electrolyte to the electronic charges in the doped polymer modulated by an external potential. While properties in the dry-state are reasonably well understood, the ion-polymer interactions and ion-electron coupling of the polymer in the wet-state and during device operation are less well studied. This is mainly due to challenges in probing the molecular and electronic structure in a heterogeneous polymer matrix and in differentiating between electronic and ionic charge carriers using electrical techniques alone.

Aims and Methods

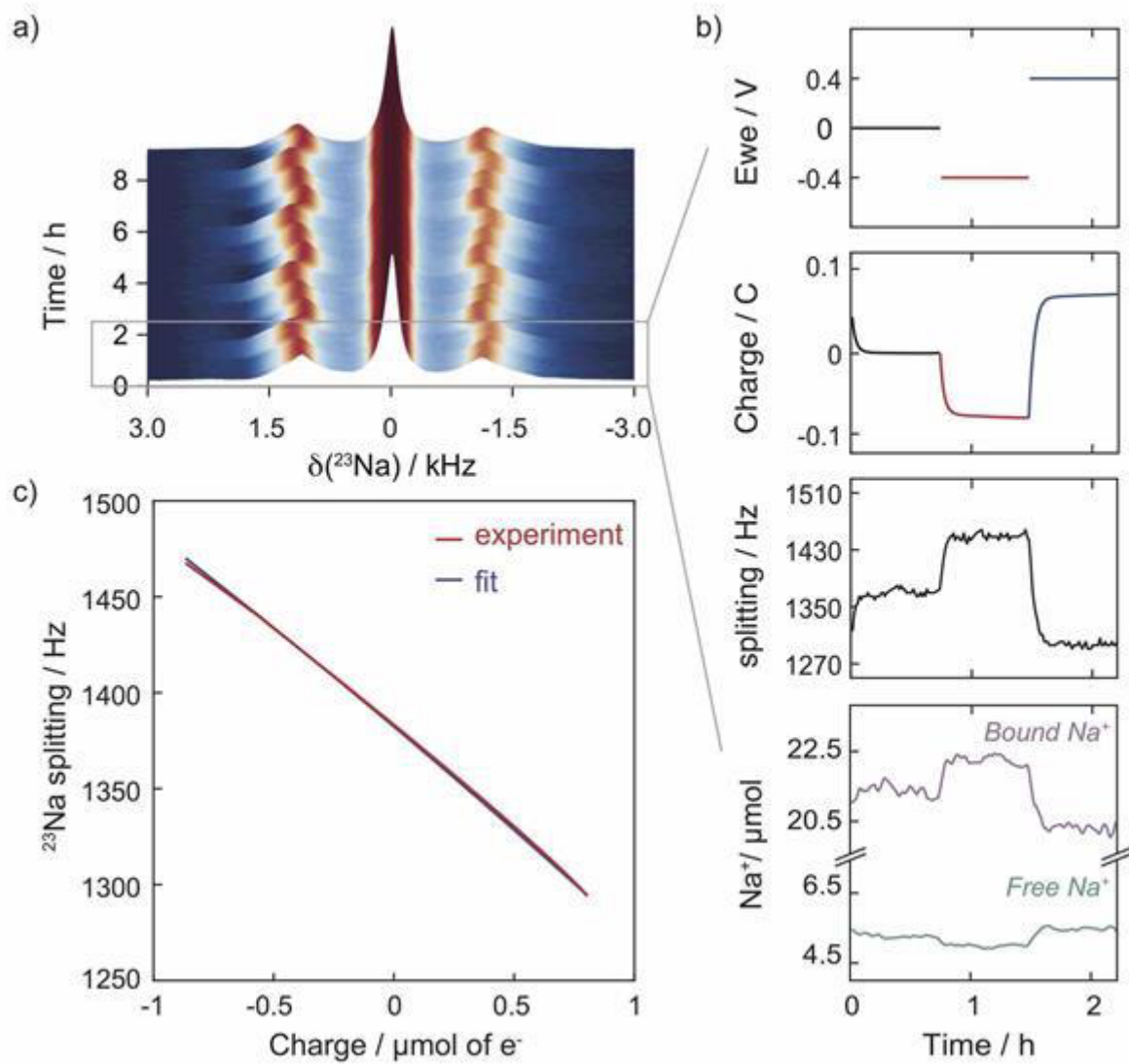
Here, I demonstrate that operando NMR spectroscopy possesses unique advantages in selectively probing and quantifying ion transport and ionic-electronic coupling during doping/dedoping of poly(3,4-ethylene dioxythiophene):polystyrene sulfonate (PEDOT:PSS) films, the most widely used organic mixed conductor.

Results

By exploiting the quadrupolar nature of the ²³Na nucleus, the anisotropy of sodium environments in heterogeneous polymer films is measured through the quadrupolar interaction between ²³Na quadrupole moments and local EFG tensors, which manifests itself as a quadrupolar splitting. Operando ²³Na NMR studies show that the observed ²³Na quadrupolar splitting is negatively correlated with the number of ions stored in the film due to the injection/extraction of sodium ions near PEDOT/PSS interfaces. The ion-to-electron coupling efficiency, measured via ²³Na NMR intensity changes, is close to 100%.

Conclusions

The operando NMR method opens up new routes to obtain quantitative insights into alkaline-ion dynamics and ion uptake on electrical gating in a wide range of OMIECs. These findings shed light on the working principles of organic mixed conductors and demonstrate the utility of operando NMR spectroscopy in revealing structure-property relationships in electroactive materials.



PT034 - Solid-State NMR Spectroscopy Investigation of Mixed-Metal MIL-53

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Parallel 13: Materials, Alsh, July 12, 2023, 15:45 - 17:45

There is an increasing need to better understand MOF structures because of their important and diverse applications. MIL-53, prepared using a benzene-1,4-dicarboxylate linker, is known as a “breathing MOF” because of the variation in pore size it displays upon interaction with guest molecules or with a variation in temperature or pressure. The phase transitions seen vary with the metal cation present, raising interest in “mixed-metal” materials, where breathing behaviour can be fine tuned.

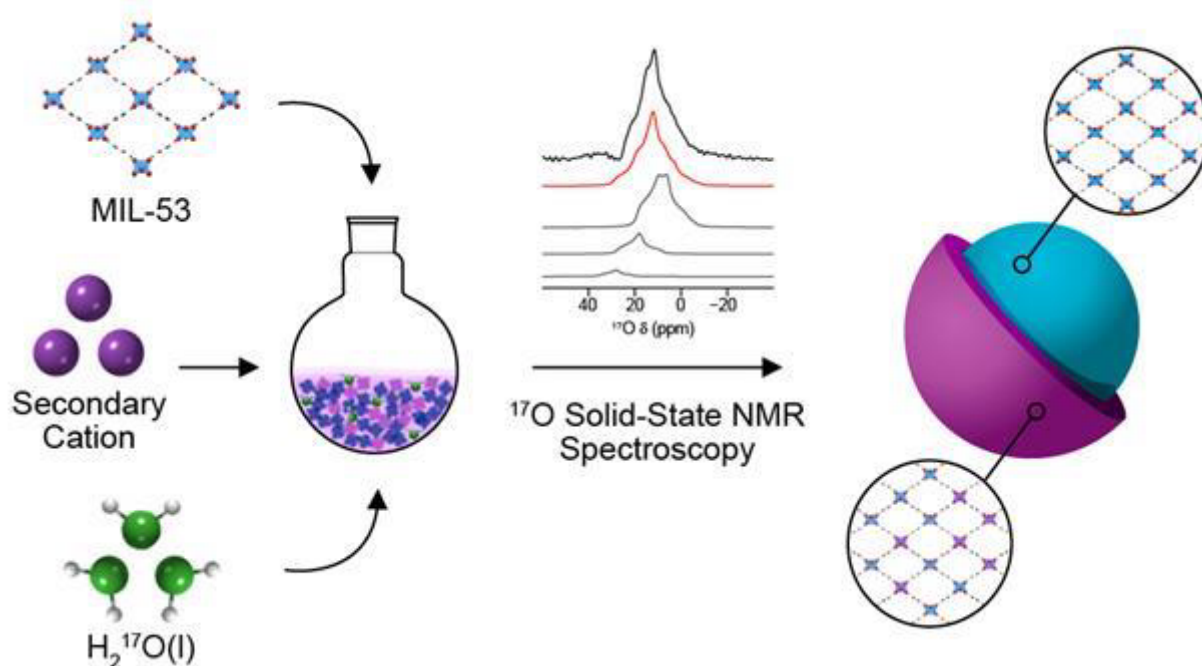
The bridging nature of oxygen in most MOFs makes ^{17}O NMR spectroscopy a potentially useful technique for investigating structural changes, such as metal cation substitution and variation in pore shapes. However, ^{17}O NMR is challenging, primarily owing to its extremely low natural abundance (0.037%). The high cost of ^{17}O -enriched reagents requires cost-effective and atom-efficient approaches to isotopic enrichment, minimising the enriched material needed but maximising its incorporation into the final product.

Here, we compare (Al,Ga)-MIL-53 prepared using different routes; dry gel conversion (DGC) reactions and two post-synthetic ion exchanges. We demonstrate (using ^{17}O MAS and MQMAS) that when using DGC reactions[1] there is preferential incorporation of Al into the framework, and a preference for clustering of like cations. A similar material is obtained using framework-framework ion exchange[2] but, surprisingly, a very different material is obtained using a framework-salt ion exchange. The particles have a core-shell structure, with a $\sim 3\ \mu\text{m}$ shell of 50:50 (Al,Ga)-MIL-53 around a $\sim 18\ \mu\text{m}$ core of Al/Ga-MIL-53.[2] The breathing behaviour of the mixed-metal materials is studied using ^{13}C and ^{17}O NMR, showing the formation of an unusual mixed-pore form rather than the open pore or narrow pore forms that are exhibited by the end members.[3]

[1] Bignami et al., Chem. Sci., 2018, 9, 850

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[3] Rice et al., Phys. Chem. Chem. Phys., 2020, 22, 14514



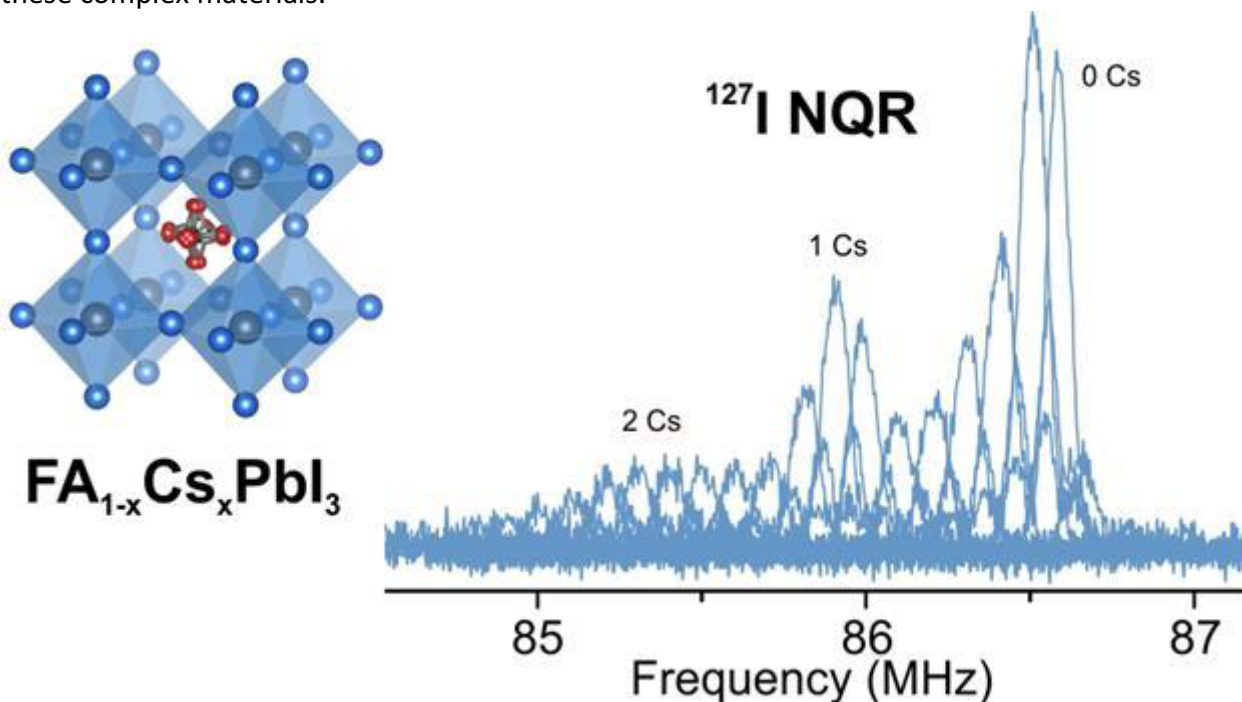
PT035 - Cesium Distribution in Lead Halide Perovskites revealed by NQR

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Parallel 13: Materials, Alsh, July 12, 2023, 15:45 - 17:45

Formamidinium-based hybrid lead iodide perovskites (FAPbI₃) have recently been implemented in high-performance photovoltaics. One of the pressing issues lies in the instability of the black polymorph, α -FAPbI₃, with the desired three-dimensional cubic perovskite phase. This phase is metastable and often rapidly converts into a non-perovskite one-dimensional hexagonal lattice. Partial substitution of FA with Cs is known to stabilize the material's cubic perovskite structure, as shown by X-ray diffraction. Here we interrogate the same research problem with ¹²⁷I nuclear quadrupole resonance (NQR), which has been shown to resolve structural changes with accuracies commensurate with synchrotron X-ray diffraction and scattering. We report the ¹²⁷I NQR spectra of FA_{1-x}Cs_xPbI₃ ($x = 0 - 0.1$) crystals showing not only the averaged but all the local iodide structures. Already minute quantities of Cs ions drastically change the observed ¹²⁷I NQR spectra. 5% of cesium ion incorporation leads to immense inhomogeneous line broadening and an additional species observable at lower frequencies. This new species could be assigned to iodide environments with one of the four FA neighbours being replaced by Cs, showing reduced quadrupolar coupling constants and increased asymmetry. For higher amounts of ion substitution, iodide environments with two cesium neighbours can be observed. This species is highly overrepresented with respect to a homogeneous halide distribution. This could be attributed to cesium clustering, foreshadowing the complete phase segregation occurring upon further cesium addition. These findings showcase the great potential of halide NQR for characterizing local structures of perovskite-based materials, enabling improved models for property calculations, as well as allowing for a better atomic picture of these complex materials.



PT036 - Improving accuracy and accessibility in ^1H - ^1H coupling measurements by using ^{13}C satellites

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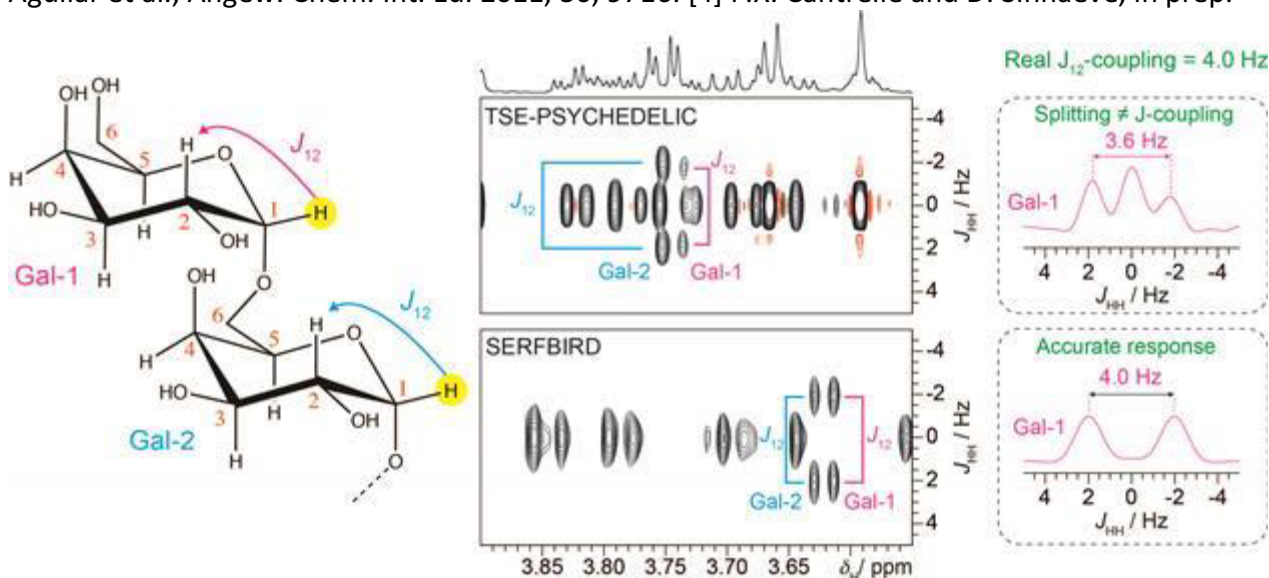
Parallel 14: Solution NMR Methods, Lomond, July 12, 2023, 15:45 - 17:45

In organic molecules, ^1H - ^1H couplings are both abundant and valuable for constitutional, configurational and conformational structure elucidation studies. The downside of their abundance is that it results in complex and broad multiplets that may overlap, obstructing extraction of individual couplings. Pure shift experiments alleviate the overlap problem by delivering fully homodecoupled ^1H spectra, boosting spectral resolution by an order of magnitude. They can elegantly be combined with selective 2D J-resolved experiments, such as SERF, G-SERF or PSYCHEDELIC, allowing individual coupling measurement with a minimum of spectral overlap [1].

However, all of these experiments fail when coupling partners have very close chemical shifts. First, the multiplets of these coupling partners may overlap, preventing selective inversion of one coupling partner. Second, the strong coupling condition between these protons affects the accuracy of coupling measurements with third protons. Here, a new selective 2DJ experiment based on the BIRD element [2,3] is presented that avoids this issue by exploiting the property that, at natural ^{13}C abundance, one-bond ^1H - ^{13}C couplings resolve the chemical shift degeneracy. The new experiment, coined SERFBIRD, thus allows measurement of ^1H - ^1H couplings that were inaccessible using established methods, or with an improved accuracy. Although applicable to any compound, it is a particularly powerful tool for carbohydrate analysis [4].

An important limitation of BIRD is that it cannot deal with protons coupled to a geminal proton, which has hindered homodecoupling of ^1H - ^{13}C experiments of methylene protons in general. Strategies that overcome this issue will be presented, making individual ^1H - ^1H coupling measurement also possible on the ^{13}C satellites of methylene protons.

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PT037 - How to stop convection ruining pure shift NMR experiments

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Parallel 14: Solution NMR Methods, Lomond, July 12, 2023, 15:45 - 17:45

Convection is known to occur in all liquid NMR samples, at a rate depending on solvent, temperature but also on NMR tube and probehead design. While its effects on diffusion experiments are well documented and can be corrected for, convection can also degrade signals in 1D pure shift NMR experiments, in extreme cases making it impossible to obtain good spectra.

Pure shift experiments typically include a hard spin echo followed by “active spin refocusing” (ASR), with the coherence transfer pathway (CTP) enforced by gradient pulses. For ASR elements that require a long selective pulse, such as the band-selective, Zangger-Sterk or PSYCHE elements, significant flow encoding occurs during this second spin echo, causing the gradient pulses to attenuate the pure shift signal acquired. Sensitivity losses can be large, especially when using cold probeheads, in which thermal gradients and hence convection, are particularly severe.

Fortunately, flow encoding during the ASR can often be compensated for by adjusting the relative strengths of the CTP gradient pulses. The optimal gradient strength balance is as a function of the experimental parameters (selective pulse duration, gradient pulse timing and amplitudes). Correcting for flow effects allows the sensitivity of pure shift experiments to be maintained even when a sample is convecting strongly. This can improve sensitivity by more than an order of magnitude (see Figure 1).

Convection-compensated pure shift NMR experiments should maximise the benefit of using cold probes, and increase the range of uses for flow-NMR devices.

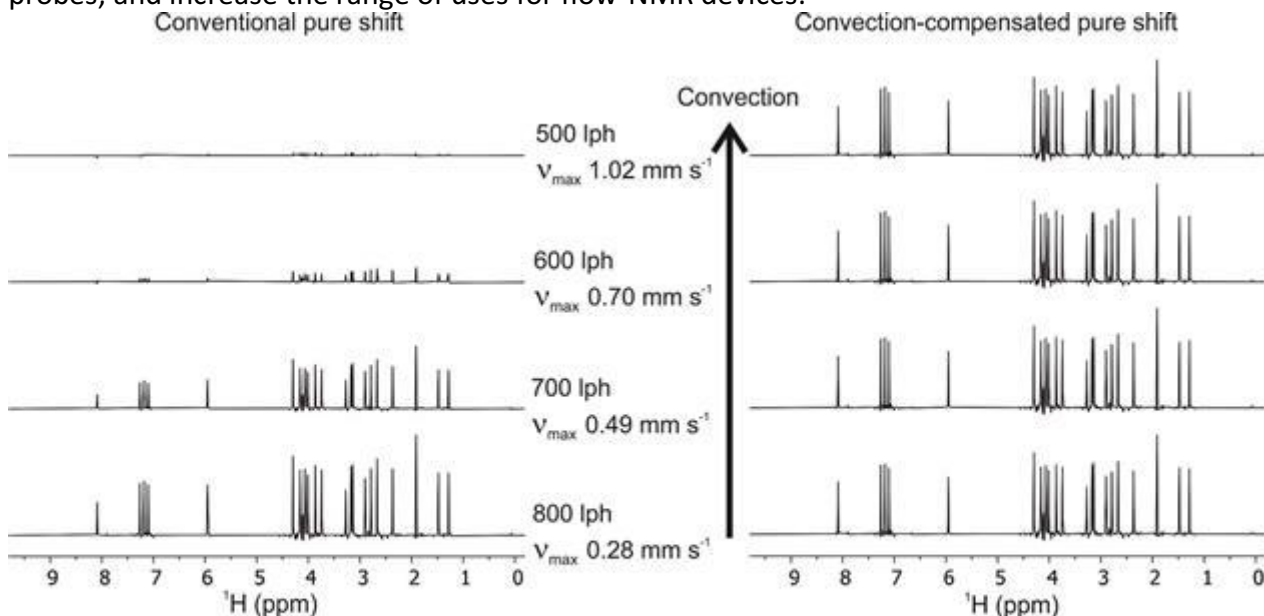


Figure 1: Zangger-Sterk pure shift spectra for strychnine in CDCl_3 at 288 K with different VT gas flow rates on a TCI cryoprobe. v_{max} values are maximum convection speeds. (Left) typical CTP gradient strengths (+47% and +31%); (right) optimized values (+47 and -41.2%).

PT038 - Development of ultrafast 2D NMR pulse sequences for the study of hyperpolarized complex mixtures

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Parallel 14: Solution NMR Methods, Lomond, July 12, 2023, 15:45 - 17:45

NMR is well suited for the analysis of complex mixtures as it provides both structural and quantitative information, but it suffers from a low sensitivity and from peak overlap, especially in 1D ¹H NMR. 2D NMR can improve both the peak discrimination and the assignment of mixture constituents, by spreading the chemical information over two orthogonal axes, but it retains the low sensitivity of NMR experiments. This limitation can be overcome by dissolution Dynamic Nuclear Polarization (d-DNP), which increases the liquid state NMR sensitivity by 4 orders of magnitude, in a single-shot experiment fashion. It has successfully been applied to 1D ¹³C hyperpolarized metabolomics studies. To benefit from the above-mentioned 2D NMR advantages while being compatible with the single-shot nature of d-DNP, we evaluate the potential of Ultrafast (UF) NMR for such applications, since its single-scan nature makes it the perfect candidate for d-DNP applications.

The coupling of UF 2D NMR and d-DNP has already been demonstrated but at an early stage of development and several improvements are needed to apply it to complex metabolite mixtures at natural abundance. Here, we report the development and optimisation of an UF heteronuclear pulse sequence called long-range HETCOR, designed for its coupling with d-DNP.

This pulse sequence capitalises on ¹³C hyperpolarized states and long-range scalar couplings to observe correlations involving quaternary ¹³C nuclei. Experimental conditions were adapted to minimize convection effects after sample injection, that can highly impact the quality of spatial encoding. The analytical performances of UF d-DNP experiments to study complex mixtures were assessed, through the repeatability and sensitivity of the technique.

After careful optimizations, a repeatability of 10% and a LOQ of 20 mM can be achieved for heteronuclear 2D experiments, which open promising perspectives for the study of concentrated biofluids or extracts in metabolomics.

PT039 - Hydrogen bond continuum in multicomponent pharmaceutical solids

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¹IOCB Prague, Prague, Czech Republic

Parallel 15: Theory and Computation, Boisdale, July 12, 2023, 15:45 - 17:45

The small mass of hydrogen atom means that it is intrinsically quantum mechanical and that nuclear quantum effects (NQEs), such as nuclear delocalization and tunneling may be of critical importance. NMR spectroscopy is very sensitive to the local environment of the observed nuclei and a combination of NMR experiments with quantum-chemical computations provides a detailed information about the positions and dynamic behavior of hydrogen nuclei. NQEs are, nevertheless, not included in most current computational methods. One route to including NQEs is provided by Feynman's path integral (PI) approach. The understanding and correct description of intermolecular hydrogen bonds are crucial in the field of multicomponent pharmaceutical solids, such as salts and cocrystals. The experimental distinction between these solid forms is often challenging.

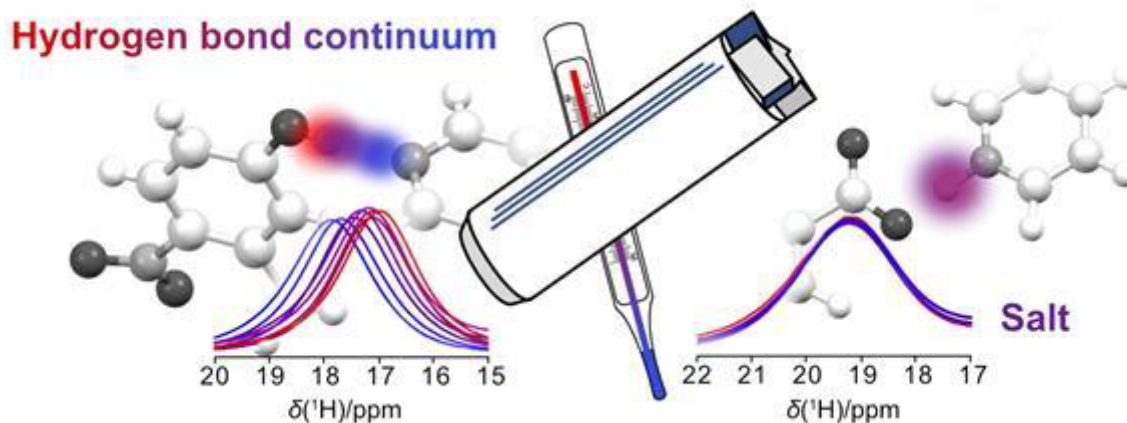
Aims

Get an insight into the salt-cocrystal problem using solid-state NMR spectroscopy and PIMD simulations.

Results

We found that the transformation of a salt into a cocrystal can occur as a smooth shift of the positional probability of the hydrogen atoms. Experimental solid-state NMR has revealed a remarkable temperature dependence and deuterium-isotope-induced changes of the chemical shifts of the atoms involved in the intermolecular hydrogen bond. A combination of solid-state NMR spectroscopy with DFT-PIMD simulations provides evidence of temperature-induced hydrogen atom shift in cocrystals with short hydrogen bonds (J. Am. Chem. Soc. 2022, 144, 7111, J. Magn. Reson. 2022, 345, 107334). When NQEs are included in the calculations, the hydrogen atom can be significantly delocalized between the acid and the base, thus forming a hydrogen-bond continuum. Furthermore, the calculations show that the average position of the hydrogen atom can shift from the acid towards the salt with decreasing temperature. Experimental NMR data may also serve for an evaluation of the accuracy of computational methods used for modelling NQEs in molecules.

Acknowledgement: Czech Science Foundation (grant No. 22-15374S).



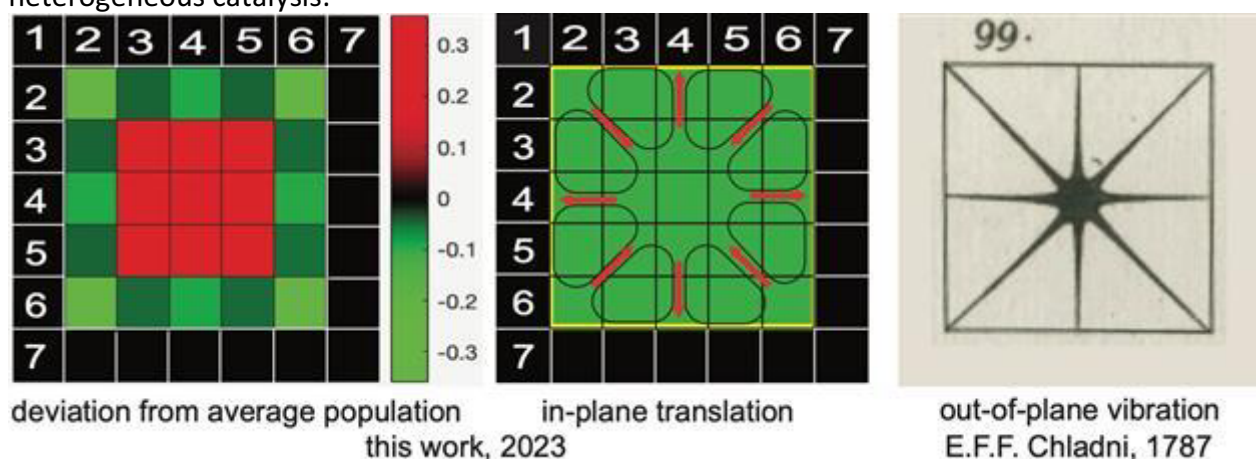
PT040 - Asymmetry in Three-Site Relaxation-Exchange NMR

Mr Bernhard Blümich¹, Matthew Parziale², Dr. Matthew Augustine²

¹RWTH Aachen University, Aachen, Germany, ²Department of Chemistry, UC Davis, Davis, USA

Parallel 15: Theory and Computation, Boisdale, July 12, 2023, 15:45 - 17:45

Asymmetry of peak integrals in 2D relaxation maps of exchange between three sites reports circular flow between the relaxation sites. This disagrees with detailed balance according to which the exchange between any pair of sites must be balanced in thermodynamic equilibrium. Confined diffusion of particles jumping randomly on a 2D checkerboard grid to any of their eight neighbor positions and confined gas diffusion were modelled in Monte Carlo simulations to explore the impact of topological constraints on particle motion. Both models produce density variations across the pore and reveal that up to 1% of the molecules move in circular paths between the relaxation pools. This motion is driven by different features of either algorithm. It is silent in thermodynamic equilibrium, so that multisite exchange maps are symmetric in equilibrium. The coherent flux is argued to result from stochastic pore resonance related to diffusion eigenmodes. If it can be driven experimentally by external oscillating electric, magnetic or ultrasonic fields, this may be a way to enhance heterogeneous catalysis.



PT041 - Easy to state, difficult to answer: Some basic questions in magnetic resonance theory.

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¹Southampton University, Southampton, United Kingdom

Parallel 15: Theory and Computation, Boisdale, July 12, 2023, 15:45 - 17:45

Magnetic resonance theory has a long history. Nevertheless, there are still some rather basic questions which might not have been addressed fully, or which are at least a matter of debate. We will try to highlight some of these questions, and discuss some possible approaches to resolve them.

Question 1: It is widely understood that the spin density operator describes the state of the spin ensemble, and that the density operator may be expressed as a linear superposition of spin operators. But are any superpositions physically possible? Or, to put it another way: what is the physical boundary of Liouville space?

Question 2: Suppose that the spin system has a certain state A at a certain time. The spin system evolves under a spin Hamiltonian. Which states of the density operator are physically accessible from state A ?

Question 3: This is a variant of question 2. Suppose that all spin Hamiltonian terms are permutation-symmetric, meaning that certain exchanges of spins leave the Hamiltonian unchanged. In this case, which states of the spin density operator are physically accessible?

Question 4: Suppose that the spin system is exposed to a set of Hamiltonians, some of which are coherent (the same for all ensemble members) and some of which are stochastic (different for different ensemble members, and randomly time-dependent). What are the long-lived states? Can the long-lived states be predicted from the Hamiltonians? If so, how?

Question 5: The phenomenon of hyperpolarization is very important. NMR signals may be enhanced by many orders of magnitude. But what is the definition of hyperpolarization?

Discussion of these questions involves the topic of Lie algebra and its relationship to spin dynamics. We will take the opportunity to introduce some new SpinDynamica routines, such as `Commutant`, `LieAlgebra`, and `SymmetryAdaptedBasis`.

PT042 - Elucidation of the mechanism of CLIC1 membrane insertion and its inhibition

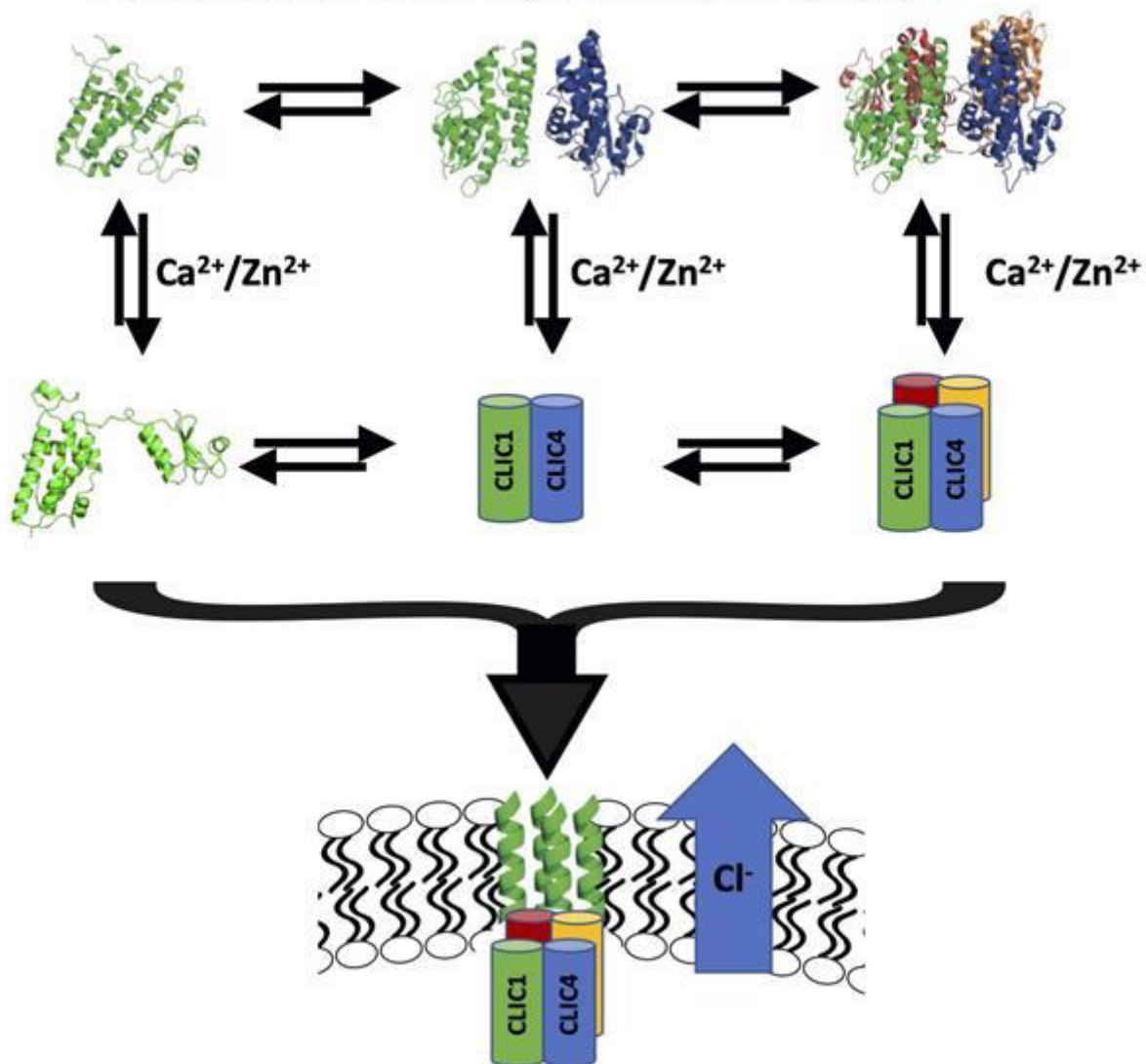
Mr Joseph Cassar¹, Dr Lorena Varela-Alvarez¹, Mrs Angela Serrano-Sanchez¹, Dr Fisayo Olotu², Dr Vahitha Abdul-Salam³, Mr. José Luis Ortega Roldan¹

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Parallel 16: BioNMR, Lomond, July 13, 2023, 10:45 - 12:45

The CLIC protein family displays the unique feature of altering its structure from a soluble form to a membrane-bound chloride channel. CLIC1, a member of this family, is found in the cytoplasm or in internal and the plasma membranes, with membrane relocalisation linked to endothelial dysfunction, tumour proliferation and metastasis. We have recently elucidated the mechanism of CLIC1 membrane insertion, involving Zn²⁺ binding and channel activation at low pH (Varela et al, JCS 2022). We have used an integrated structural biology approach combining solution NMR, X-Ray crystallography and SAXS to elucidate the mechanism of CLIC1 membrane insertion with structural detail. This mechanism involves a complex equilibrium between a major closed state and a minor open state in different oligomeric species which, upon binding to Zn²⁺, leads to tetramerization and insertion in the membrane (Medina-Carmona et al, Chem Comms, 2020). We have obtained structural models and dynamics information of the different states of CLIC1 in solution, including the low populated open state, providing the first molecular detail of the membrane insertion process. Using the structural models and the dynamics obtained for the opening equilibrium of the CLIC1 structure, we have adopted an AI based in-silico screening approach to identify a set of inhibitors targeting specific areas of CLIC1, as well as other CLIC proteins (Olotu et al, CSBJ, 2023). Our top hits are able to bind CLICs and halt its insertion in the membrane in HUVEC cells. In summary, we have elucidated the mechanism of activation and membrane insertion of CLIC1 with molecular detail and have used this information to develop novel inhibitors of CLICs membrane insertion, paving the way for the development of new treatments of glioblastoma and other types of endothelial dysfunction.

A model for CLIC membrane insertion



Varela & Hendry et al. *JCS*, 2022

PT043 - High-resolution and time-resolved insights into an RNA-cleaving DNA catalyst

Dr Manuel Etzkorn¹

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Parallel 16: BioNMR, Lomond, July 13, 2023, 10:45 - 12:45

DNAzymes are specific DNA-sequences that have been identified by in vitro selection and are capable of catalyzing a variety of reactions. RNA-cleaving DNAzymes arguably carry the highest therapeutic potential. However, the DNAzyme technology is facing a number of limitations, which coincide with an insufficient understanding of their mode of action.

Using an integrative approach that combines tailored NMR-based methods with FRET, EPR and MD simulations, we could obtain detailed insights into different states of one of the most active DNAzymes(1–4).

Our data capture an unexpected but highly efficient fold of the precatalytic DNAzyme:RNA complexes and provide information about the essential role of metal-ion cofactors. Using time-resolved NMR we could further follow the catalysis in real-time and identify new rate-limiting intermediate states during the catalytic cycle.

Exploiting our new insights into structure, dynamics, metal-ion binding, and catalysis, we could rationally design a single-atom replacement that strongly increases catalytic activity.

Overall, our data highlight the importance of dynamic processes such as transient interactions and conformational plasticity in these highly dynamic systems. In this respect we also demonstrate the need for high-resolution techniques, in particular NMR, to provide the required mechanistic insights for rational-design strategies with the aim to unravel the full potential of the DNAzyme technology.

1. Borggräfe, J. et al. Time-resolved structural analysis of an RNA-cleaving DNA catalyst. *Nature* (2022)
2. Borggräfe, J., Gertzen, C. G. W., Viegas, A., Gohlke, H. & Etzkorn, M. The architecture of the 10-23 DNAzyme and its implications for DNA-mediated catalysis. *FEBS J.* in press (2022)
3. Rosenbach, H. et al. Influence of monovalent metal ions on metal binding and catalytic activity of the 10–23 DNAzyme. *Biol. Chem.* (2020)
4. Borggräfe, J. & Etzkorn, M. Solution NMR Spectroscopy as a Tool to Study DNAzyme Structure and Function. *Methods Mol. Biol.* (2022)

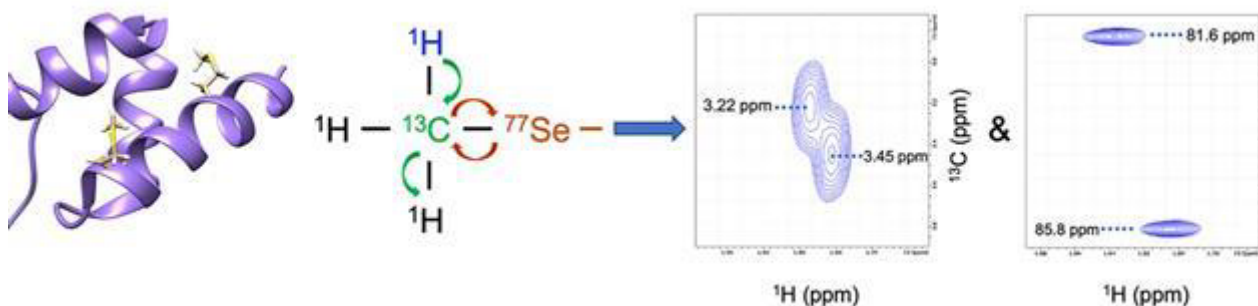
PT044 - Exploring sulphur sites in proteins via triple-resonance ^{77}Se NMR

Ms Jess Li¹, Dr Janusz Koscielniak², Dr Deepak Sail³, Fr Rolf Swenson³, Dr Clemens Anklin⁴, Dr Sharon Rozovsky⁵, **Dr. R. Andrew Byrd¹**

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Parallel 16: BioNMR, Lomond, July 13, 2023, 10:45 - 12:45

NMR has been applied to virtually all sites within proteins and biomolecules; however, the observation of sulfur sites remains very challenging. Recent studies have examined ^{77}Se as a replacement for sulfur and applied ^{77}Se NMR in both the solution and solid states. As a spin-1/2 nuclide, ^{77}Se is attractive as a probe of sulfur sites, and it has a very large chemical shift range (due to a large Chemical Shift Anisotropy), which makes it potentially very sensitive to structural and/or binding interactions, as well as dynamics. Despite being a spin-1/2 nuclide, there have been rather limited studies of ^{77}Se , and the ability to use ^1H -indirect detection has been sparse. Some examples exist, but in the absence of a directly bonded, non-exchangeable ^1H , these have been largely limited to smaller molecules. We develop and illustrate approaches using double-labeling of ^{13}C and ^{77}Se in proteins that enable more sensitive triple-resonance schemes via multi-step coherence transfers and ^1H -detection. These methods require specialized hardware and decoupling schemes, which we have developed and will be discussed. The requisite three spin ^1H - ^{13}C - ^{77}Se systems can be created for both methionine and cysteine. The triple resonance ^1H - ^{13}C - ^{77}Se experiments may be acquired as 2D or 3D, and NUS may be readily incorporated. The sensitivity gains over direct 1D ^{77}Se detection are on the order of 40-fold (~1600-fold in time). Applications to protein:protein and ligand:protein interactions at low (300 μM) concentration will be presented, illustrating the extreme sensitivity of ^{77}Se chemical shifts and the potential to reveal subtle structural shifts.



PT045 - Stopped-flow NMR reaction monitoring on a sub-second timescale

Dr Andrew Hall¹, Miss Annabel Flook¹, Mr Edward King², Professor Guy Lloyd-Jones¹

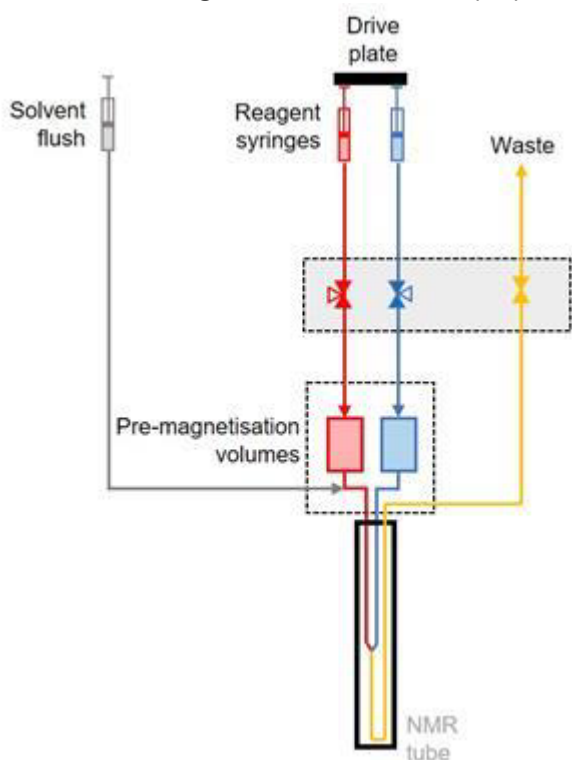
¹University Of Edinburgh, Edinburgh, United Kingdom, ²TgK Scientific, Bradford-on-Avon, United Kingdom

Parallel 17: Hardware, Alsh, July 13, 2023, 10:45 - 12:45

NMR is an extremely versatile and powerful tool for studying reaction kinetics and mechanisms. Many techniques exist for studying slow irreversible reactions, including ex-situ sampling, in-situ monitoring and continuous FlowNMR.¹ In contrast, fast irreversible reactions pose many challenges, as the reaction may be significantly faster than the time taken to initiate the reaction and load the sample into the spectrometer. For reactions with half-lives on a millisecond to second timescale, stopped-flow techniques are a convenient way of initiating the reaction inside the NMR spectrometer by mixing two or more reagent streams, before rapidly stopping the flow and acquiring the spectra.

Previous work in the Lloyd-Jones group, in collaboration with TgK Scientific, resulted in the development of a stopped-flow NMR instrument that rapidly combines up to three reagent streams, initiating reactions in under 150 milliseconds.² We now present a next generation stopped-flow NMR instrument with a reduced dead-time between initiation of the reaction and acquisition of the first spectrum of 10 milliseconds, allowing reactions to be studied on the tens of millisecond timescale. We demonstrate the utility of this instrument for studying a variety of rapid reactions, including approaches for acquiring, processing and interpreting spectra where the reaction is evolving on the NMR timescale.

1. Prog. Nucl. Magn. Reson. Spectrosc. 2022, 129, 28-106.
2. Eur. J. Org. Chem. 2021, 2021 (17), 2331-2342.



PT046 - New advances toward development of a 1.3 GHz NMR system: 1.01 GHz NMR system and MAS beyond 150 kHz

Dr. Yoshinori Yanagisawa², Dr. Yu Suetomi², Dr. Renzhong Piao², Dr. Toshio Yamazaki², Dr. Michitaka Ono³, Mr. Saito Kazuyoshi⁴, Dr. Hamada Mamoru⁴, Mr. Yoshikawa Masatoshi⁴, Dr. Hideaki Maeda², Dr. Tatsuya Matsunaga², Dr. Junpei Hamatsu⁵, Mr. Yuki Endo⁵, Mr. Takahiro Nemoto⁵, Dr. Kenichi Hachitani⁵, Dr Yoshitaka Ishii¹

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Parallel 17: Hardware, Alsh, July 13, 2023, 10:45 - 12:45

We discuss two major development milestones recently achieved toward development of a 1.3 GHz NMR system, which is an on-going project supported by the Japan Science and Technology Agency (JST). The first milestone is development of an ultra-compact 1.01 GHz NMR magnet (see Figure) and its preliminary NMR applications. The new ultra-compact 1 GHz NMR magnet utilizes high-temperature superconducting (HTS) coils made of bismuth-based cuprates besides conventional low-temperature superconducting coils. Because of the high current density of the HTS coil, the magnet weighs only 1.6 tons and its footprint is the smallest among the existing 1 GHz NMR systems. The cryogenic refrigerator mounted on the magnet eliminates needs of regular liquid-helium refilling. We have successfully collected multi-dimensional solution NMR and solid-state NMR (SSNMR) data for proteins at a ¹H frequency of 1.01 GHz. The second milestone is development of a magic-angle-spinning (MAS) probe that allows for spinning at ~160 kHz. Some preliminary SSNMR data will be presented with prospects and challenge in spinning at a faster rate. Other research progress using ultra-fast MAS and high-field NMR from the ongoing project to develop 1.3 GHz NMR will be also discussed with applications for amyloid proteins and other systems.

PT047 - A dual-core NMR system with RF and 3D-gradient capabilities in low-field

Dr Thomas Robertson¹, Mrs Topaz Cartlidge¹, Dr Thimo Hugger², Dr Sebastien Brehm², Miss Rose Bannister¹, Dr Sam Thompson¹, Dr Klaus Zick², Dr Frank Engelke², Dr Giuseppe Pileio¹

¹University Of Southampton, Southampton, United Kingdom, ²Bruker Biospin GmbH, Silberstreifen, Germany

Parallel 17: Hardware, Alsh, July 13, 2023, 10:45 - 12:45

Introduction

Many materials of key interest for industrial applications, such as porous media and battery electrodes, possess large internal heterogeneous magnetic fields originating from magnetic susceptibility mismatches between the porous structure and the pore-filling medium. This results in rapid local fluctuating magnetic fields whose magnitude is proportional to the static field and that drastically reduce the lifetime of transverse magnetization and make pulse sequences which rely on multiple echoes, such as the conversion of magnetization to singlet, very challenging at high magnetic fields.

Aims

This work describes the development of hardware which seeks to address this challenge and thus enable the investigation of structural parameters such as porosity, pore size distributions and tortuosity in porous media.

Methods

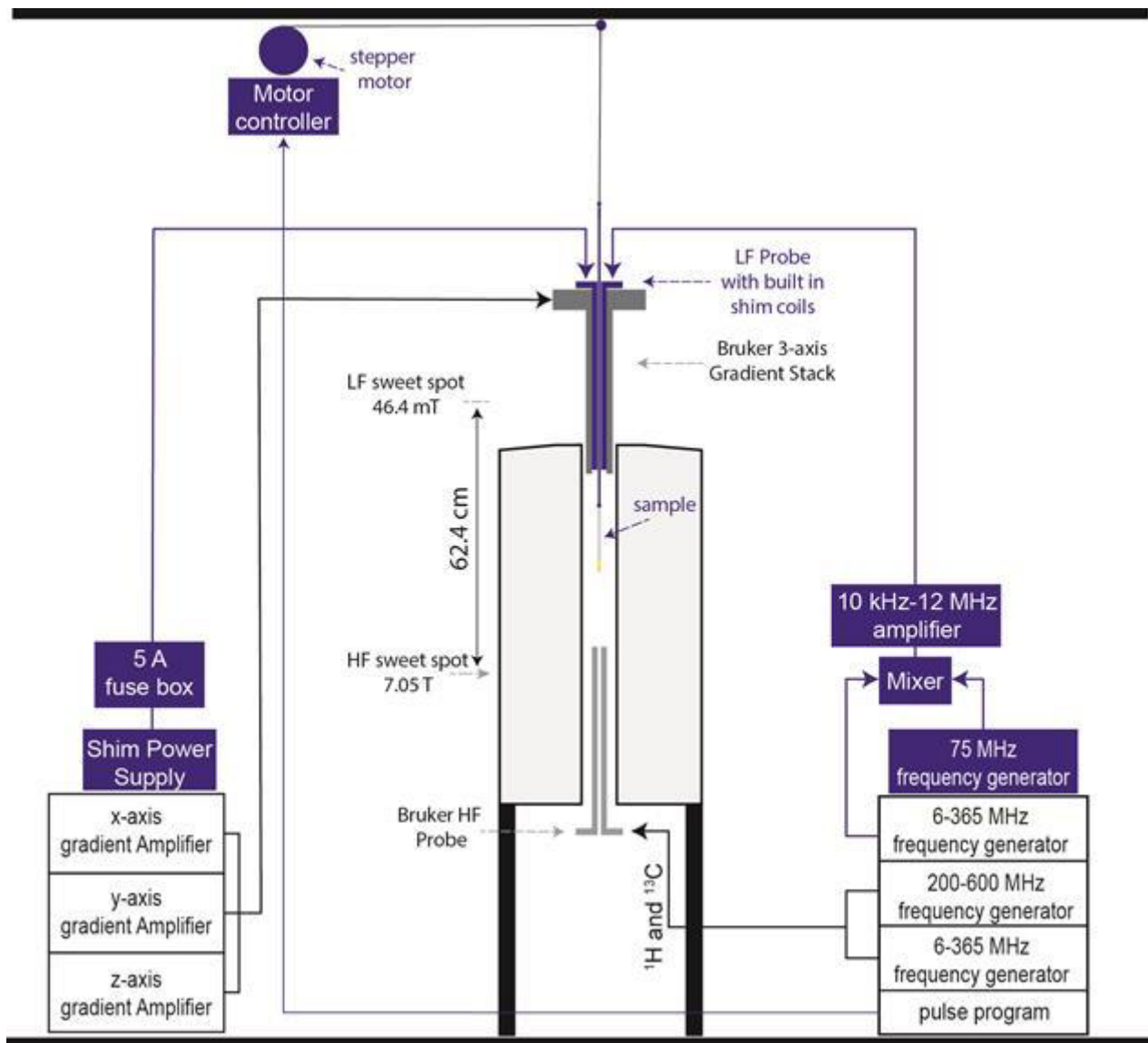
This is practically accomplished through a dual-probe system and a sample shuttle able to move a conventional valved NMR tube between high field (7.05 T) and low field (46 mT, 500 kHz for ¹³C). During transport the sample is kept in alignment with the x and y axis, enabling repeatable gradient pulses when heterogeneous samples are studied. At low field, a 3-axis gradient system is available in combination with radiofrequency pulses to implement a variety of (field-cycled) pulse sequences including those to measure T₁, T₂, long-lived spin order decay, and diffusion tensors.

Results

Applications such as access to long lived singlet lifetimes and diffusion tensor imaging (DTI) in porous media with large magnetic susceptibility inhomogeneities are of particular interest to us since these will enable unique insights into diffusion within those media.

Conclusions

This talk will describe hardware design, development and validation for the application of a range of pulse sequences with field cycling and the application of radiofrequency pulses and 3D gradients in low field.



PT048 - Application of lanthanoid chelating tags to exploit PCS on GPCRs via tagged nanobodies (GPS-PCS)

Prof. Daniel Häussinger¹, Mr Pascal Rieder¹, Dr. Feng-Jie Wu², Dr. Philip Rößler³, Dr. Layara Akemi Abiko, Dr Alvar Gossert³, Prof. Stephan Grzesiek²

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Parallel 18: "ParaNMR", Boisdale, July 13, 2023, 10:45 - 12:45

Introduction

Pseudocontact shifts (PCSs) induced by lanthanoid ions that are conjugated to biomacromolecules by lanthanoid chelating tags (LCTs) have proven to be a versatile tool in biomolecular NMR spectroscopy. The application of LCTs is often limited in larger or very cysteine rich proteins, as most conjugation techniques rely on single cysteine techniques. In addition, the required single cysteine mutants can alter the structure and / or the function of the investigated protein.

G protein-coupled receptors (GPCRs) are a highly relevant class of protein targets that are responsible for the recognition of extracellular ligands and the triggering of crucial signaling cascades within human cells. The dynamic interaction of GPCRs with various ligands results in delicate conformational equilibria which are not yet well understood.

Aims

We have developed a new approach to assign the resonances of the β_1 -adrenergic receptor (YY- β_1 AR) using PCSs in order to characterise the inactive, preactive and fully active conformations of the GPCR and monitor the structural changes between them [1].

Method

Because our efforts to produce suitable point mutations for YY- β_1 AR to apply LCTs were of only limited success, we tagged instead of the GPCR itself, two different nanobodies (Nb60 and Nb80) that form high affinity complexes with YY- β_1 AR.

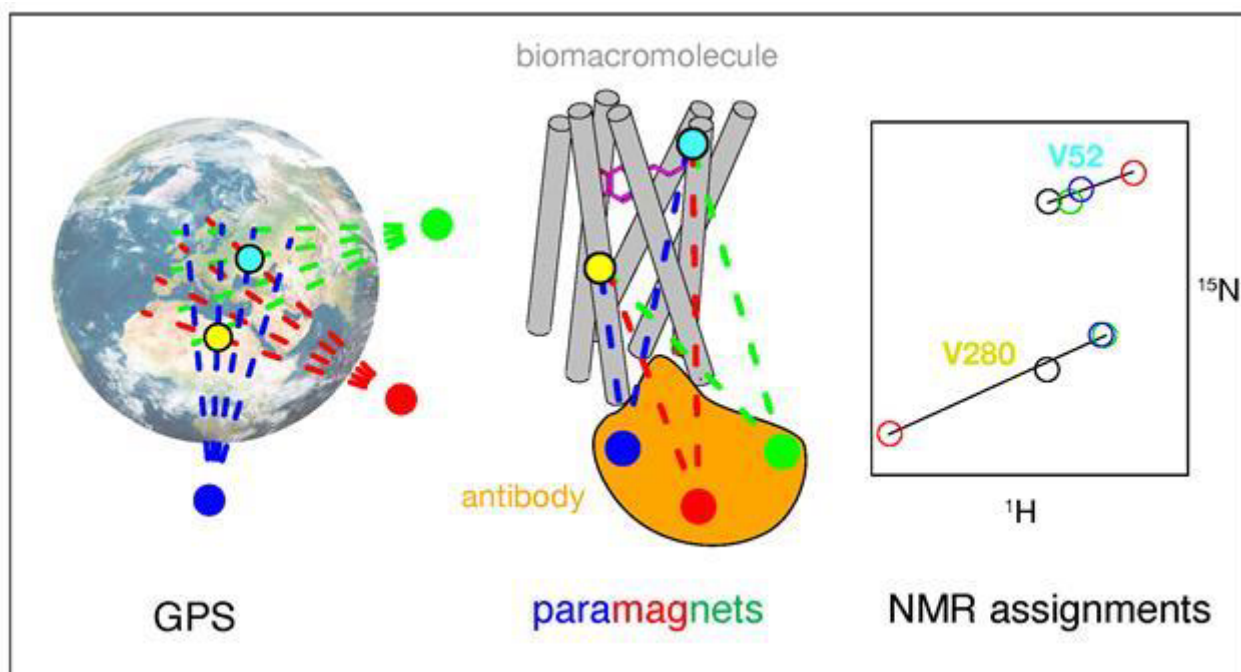
Results

The observed PCS on the GPCR allowed for the assignment of all Val and Tyr resonances in selectively ¹⁵N labelled YY- β_1 AR constructs in binary and ternary complexes with agonists or antagonists and with / without NBs. A method to predict suitable mutation sites on the NBs was also developed.

Conclusions

A ~100 kDa sized, detergent solubilised GPCR was characterised in various conformational states by a new method termed GPS-PCS. The approach is not limited to GPCRs but could be extended to all biomacromolecules for which suitable stable binders exist.

[1] F.J. Wu, et al. JACS 2022 144(47), 21728-21740.



PT049 - Solid-state NMR study of fluoride ion conductivity in paramagnetic CeF_3

Dr Miwa Murakami¹, Prof Atsushi Mineshige²

¹Kyoto University, Uji, Japan, ²University of Hyogo, Himeji, Japan

Parallel 18: "ParaNMR", Boisdale, July 13, 2023, 10:45 - 12:45

Introduction

The high mobility of fluoride ion in metal fluorides with the tysonite-type structure such as LaF_3 and CeF_3 has been investigated by various methods. Long-standing controversies on conduction pathways among the three crystallographical sites in tysonite LaF_3 (F1, F2, and F3) have been settled down by using ^{19}F magic angle spinning (MAS) NMR; [1] F1–F1 exchange occurs first, followed by F1–F3, and F1–F2 exchange is most restricted. Unfortunately, similar analysis is difficult for CeF_3 due to the paramagnetic effect of Ce^{3+} .

Aims

To unravel the conducting pathways among the three sites in CeF_3 by ^{19}F NMR.

Methods

To reduce paramagnetic broadening, we examined solid-state ^{19}F NMR of CeF_3 at low magnetic field (4.7 T) under MAS (23 and 35 kHz). The observed ^{19}F MAS NMR spectra at $-40 \sim 240^\circ\text{C}$ are assigned to F1~F3 on the bases of the calculated dipolar coupling tensor between each ^{19}F spin and the paramagnetic electron spins at Ce^{3+} .

Results

Clearly resolved sideband manifolds for F1~F3 at 240°C indicate that ion exchange among the three sites is not fast enough to affect the ^{19}F spectrum, whereas F1 motion is suggested by its broader linewidth.

Further, we show that addition of only 0.1 % Sr^{2+} in CeF_3 brings significant effects on F-ion mobility. While the F2 and the F3 manifolds at 0°C are not affected by the Sr-doping, the F1 manifold is affected appreciably (Fig). At 240°C , a part of the F2 and most of the F3 manifolds are merged into the F1 signal.

Conclusions

The preference of the exchange pathways in CeF_3 is consistent with that postulated for LaF_3 . [2]

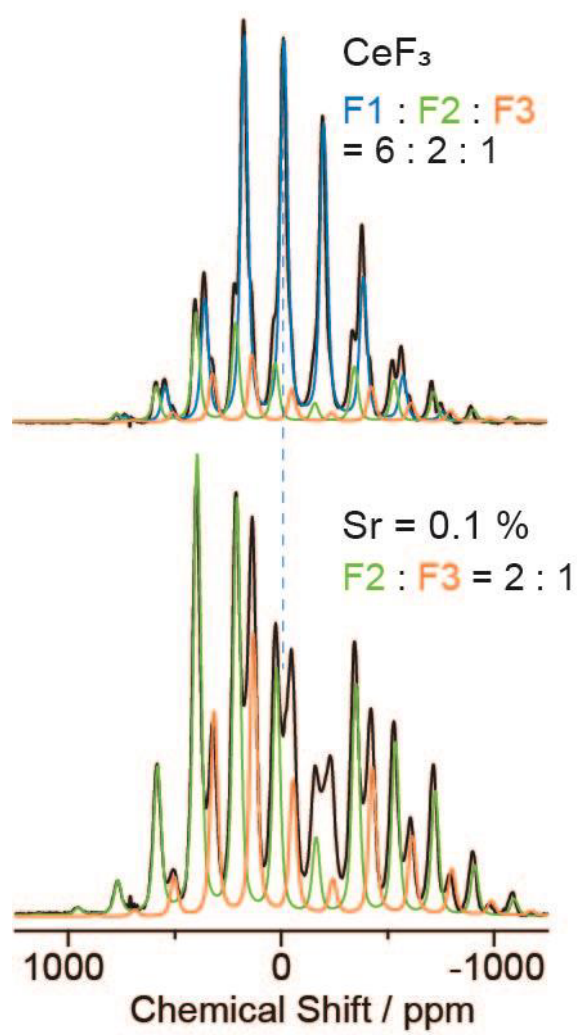
[1] Chem. Mater. 9, 1068 (1997).

[2] J. Phys. Chem. Solids 161, 110432 (2022).

Acknowledgment: This article is based on results obtained from a project, RISING3, JPNP 21006, commissioned by NEDO, Japan.

^{19}F MAS NMR spectra

0 °C, 4.7 T, MAS 23 kHz



PT050 - USING FLUORINATED PROLINE AND ^{19}F NMR TO PROBE THE SPECIFIC ROLE OF PROLINES IN PROTEIN INTERACTIONS AND FOLDING

Dr Bruno Kieffer¹, Abir Ben-Bouzaene¹, Mariam Sai¹, Pr Vladimir Torbeev¹, Dr Davy Sinneave², Pr Bruno Linclau³

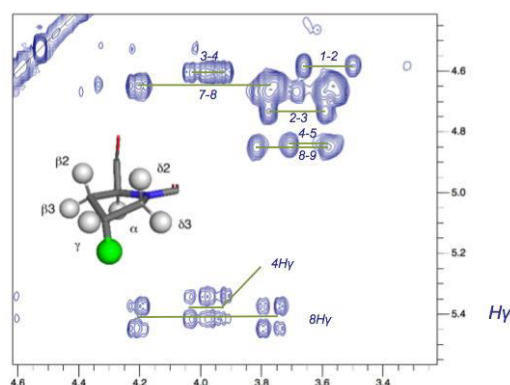
¹Université de Strasbourg, Illkirch, France, ²Université de Lille, Lille, France, ³Ghent University, Ghent, Belgium

Parallel 18: "ParaNMR", Boisdale, July 13, 2023, 10:45 - 12:45

Addition of fluorine atoms on biomolecules is widely used to modify their properties, according specific applications. This chemical modification enables fluorine NMR spectroscopy offering a very sensitive tool to explore the structure and dynamics of the system of interest. Proline was one of the earliest fluorinated amino acid made available for such approach. For this specific case, the fluorination was shown to modulate both the peptide's backbone conformation by favouring either the cis or trans isomers of the Xaa-Pro peptide bond as well as the kinetics of this conformational exchange. Given the importance of these properties in intrinsically disordered regions of proteins where proline residues are over-represented, we took advantage of ^{19}F NMR to get insights into the recognition mechanisms between cognate polyproline sequences and SH3 protein domains. We will report our findings on the interaction between two distinct SH3 domains from Retinoic Acid Receptor and Bin1 and cognate PRM (Proline Rich Motifs). We will show how fluorine signal lineshape analysis allows quantitative binding kinetics data to be measured and how fluorination affects binding rates [1]. The repertoire of fluorinated prolines has been extended [2,3] offering a wide range of spectroscopic and molecular properties to explore the peculiar role of prolines in protein sequences.

References:

1. Sinneave D. et al., 2021, Magn. Reson. 2,
2. Hofman G. J., et al., 2018, Chemical Communications 54 5118-5121
3. Hofman G. J., et al., 2019, J Org Chem 84 3100-3120



PT051 - A ^2H approach towards the Role of Methyl Dynamics under DNP

Mr Thomas Biedenbänder^{1,2}, Dr. Victoria Aladin^{1,2}, Prof. Dr. Liliya Vugmeyster³, Mr Björn Corzilius^{1,2,4}

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Parallel 19: Solid State NMR and AI, Lomond, July 13, 2023, 13:45 - 15:45

Methyl NMR studies have become highly popular in NMR spectroscopy during the last decades. The fast three-fold reorientation of the methyl group around its symmetry axis ($\text{H}_3\text{C}-\text{C}$) yields advantageous relaxation properties and thus well-resolved NMR spectra.[1] Moreover, it was recently shown that the three-fold reorientation is still active under DNP conditions being exploited in SCREAM-DNP (Specific Cross Relaxation Enhancement by Active Motions under DNP).[2] Here, the cross-relaxation-promoting methyl dynamics drive the polarization transfer from the hyperpolarized ^1H spins to ^{13}C . The specifically hyperpolarized methyl- ^{13}C can then be used, for example, for extracting information about the interface between an RNA and a protein.[3]

This study aims to gather a more detailed molecular understanding of methyl dynamics at low temperatures, particularly their behavior under DNP conditions, which have not been extensively described so far. Therefore, selectively deuterated methyl groups of methyl-bearing molecules are used as a model system to investigate methyl dynamics inside a glassy matrix and in the presence of polarizing agents. The applied ^1H ^2H CPMAS yields high DNP enhancements facilitating the determination of ^2H -R1 relaxation rates at a range of 100 to 165 K. Our results suggest a distribution of ^2H R1 relaxation rates represented by a stretched exponential, as shown in wetted protein powder,[4] yielding an activation energy for the three-fold reorientation of 3 kJ/mol in the case of ethanol- d_3 . However, the presence of the radical impacts the underlying spectral density of the measured relaxation rate as observed by comparison of a doped with an undoped sample. This indicates the necessity to establish a general and reliable approach to measure methyl dynamics under DNP.

References:

- [1] S.Schütz, R.Sprangers, MethylTROSYspectroscopy: A versatile NMR approach to study challenging biological systems, Progress in Nuclear Magnetic Resonance Spectroscopy 116(2020)56-84.
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- [3] V.Aladin, A.K.Sreemantula, T.Biedenbänder, A.Marchanka, B.Corzilius, Specific Signal Enhancement on an RNA-Protein Interface by Dynamic Nuclear Polarization, Chemistry – A European Journal n/a(n/a)(2022).
- [4] L.Vugmeyster, D.Ostrovsky, K.Penland, G.L.Hoatson, R.L.Vold, Glassy dynamics of protein methyl groups revealed by deuterium NMR, Journal of Physical Chemistry B 117(4)(2013)1051-1061

PT052 - Investigating polymorphism with Solid-State Dynamic Nuclear Polarization NMR

Dr Samuel F. Cousin¹, Dr. Fabio Ziarelli⁴, Dr. Colan E. Hughes³, Prof. Stéphane Viel^{1,5}, Dr Giulia Mollica¹, Prof. Kenneth D. M. Harris³, Dr Arthur C. Pinon², **Dr Pierre Thureau¹**

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³Cardiff University, Cardiff, United Kingdom, ⁴Aix Marseille Univ, CNRS, Centrale Marseille, FSCM,

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Parallel 19: Solid State NMR and AI, Lomond, July 13, 2023, 13:45 - 15:45

- Introduction

Polymorphism is key to understand the properties of solids and optimizing their applications. Therefore, it is essential to characterize polymorphic forms that are accessible to a molecule of interest and to understand the transformations that may occur between them. Unfortunately, the low sensitivity of NMR may limit the opportunity to detect small amounts of a minor polymorphic phase in a mixture with a major polymorphic phase during, for example, solid-state phase transformations between polymorphs.

- Aims and Methods

The goal of this study is to exploit DNP enhanced solid-state NMR to detect the presence of structurally distinct domains within inhomogeneous materials. We propose, here, to establish NMR contrast, which is based on non-uniform transport of DNP-enhanced polarization, between a minor polymorphic phase within a major polymorph. Numerical simulations of non-uniform transport of DNP-enhanced polarization can be achieved through ¹H spin diffusion to explore the spatial distribution of the different polymorphic phases within the solid particle.

- Results

Our method can detect a minor amount (<4%) of polymorph III of m-aminobenzoic acid within a powder sample of polymorph I at natural isotopic abundance. Based on proposed models of the spatial distribution of the two polymorphs, simulations of ¹H spin diffusion allow transport of DNP-enhanced polarization to be calculated for each model as a function of particle size and the relative amounts of the polymorphs.

- Conclusions

Overall, these results demonstrate the ability of solid-state DNP NMR experiments to reveal the presence of a minor polymorphic phase within a major polymorph in a powder sample at natural isotopic abundance and yield quantitative information on the spatial distribution of the two polymorphs. Clearly, the method presented here may be applied in the future to identify the early onset of transformations between polymorphic forms in a wide range of other systems.

PT053 - Integrated Assessment of the Structure and Dynamics of Solid Proteins

Mr Benedikt Söldner¹, Dr Kristof Grohe², Dr Peter Neidig², Mrs Jelena Auch¹, Mr Sebastian Blach³, Dr Alexander Klein¹, Dr. Suresh K. Vasa¹, Prof Lars Schäfer³, Prof. Dr. Rasmus Linser¹

¹Technical University Dortmund, Dortmund, Germany, ²Bruker BioSpin GmbH, Ettlingen, Germany,

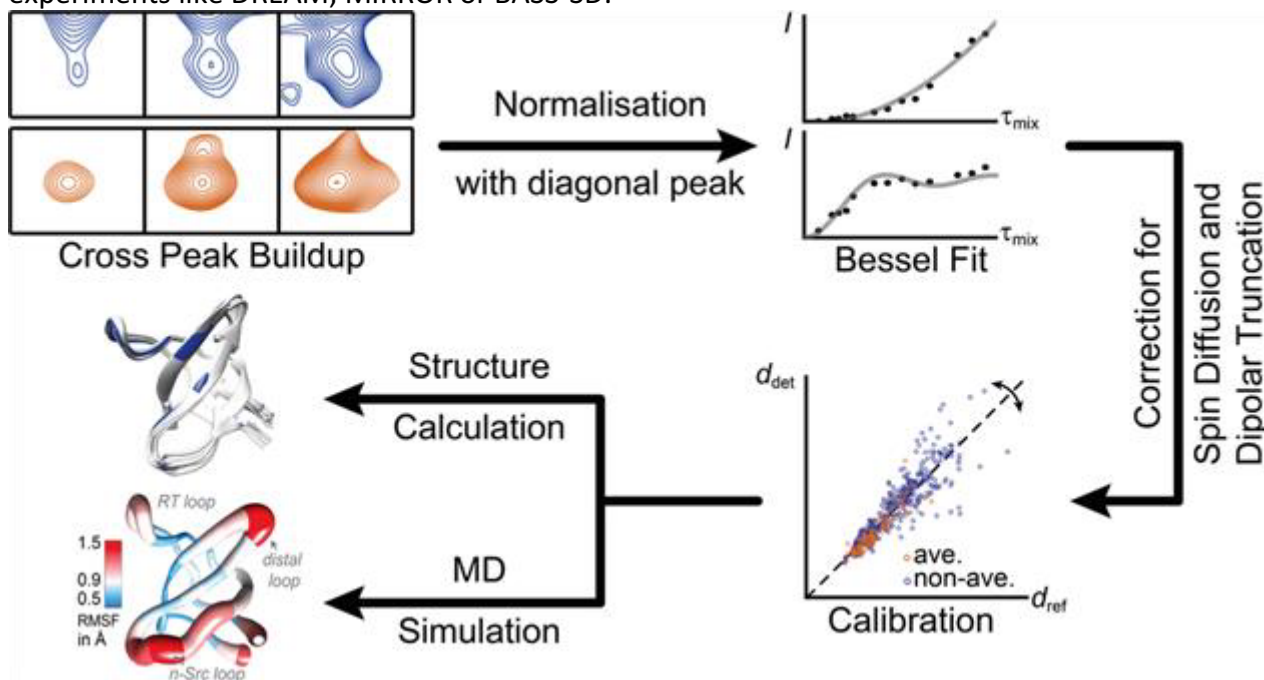
³Center for Theoretical Chemistry, Ruhr University Bochum, Bochum, Germany

Parallel 19: Solid State NMR and AI, Lomond, July 13, 2023, 13:45 - 15:45

Magic-angle spinning rates exceeding 100 kHz facilitate the usage of protons as detection nuclei in solid-state NMR spectroscopy even for fully-protonated proteins and enables a variety of new methods for investigating proteins under physiological temperatures. Although the measurement of fully-protonated proteins at such high MAS rates provides a dense network of internuclear proton-proton connectivities, structure elucidation has so far been limited by low reliability of the internuclear distances determined. In this work, we demonstrate that time-resolved analysis of internuclear magnetisation transfer in combination with new methods for correction of dipolar truncation and spin-diffusion enables structural restraints with unprecedented precision. Spin diffusion is heuristically taken into account by assuming a merely additive effect to the observed magnetization flow, as has been done in the framework of NOE buildup in solution. In contrast, for correction of dipolar truncation, a simulation-based method hinging on nearest-neighbor interactions was developed. It is based on an extensive data set of RFDR buildups for 2- and 3-spin scenarios with different geometries simulated with SIMPSON.

In addition to high-resolution structure determination, the dense network of exact solid-state NMR distances now enables restrained ensemble-averaged MD simulations for solid protein preparations. Such MD simulations facilitate detailed insights into protein dynamics and site-specific conformability without the necessity of reproducing the crystal lattice, which is often unknown and challenging to implement.

This method was developed for and applied to RFDR experiments but will be adaptable to other experiments like DREAM, MIRROR or BASS-SD.



PT054 - Fully Automatic Pulse EPR

Mr Hugo Karas¹, Prof. Stefan Stoll², Professor Gunnar Jeschke¹

¹ETH Zürich, Zürich, Switzerland, ²University of Washington, Seattle, USA

Parallel 20: EPR/ESR, Alsh, July 13, 2023, 13:45 - 15:45

Pulse EPR spectroscopy as a technique is currently mostly constrained to specialist research laboratories. One reason for this is the complexity of performing an experiment. Here we present an approach to reduce this complexity and open pulse EPR up to less-experienced users.

AutoEPR is a Python-based spectrometer-independent automation toolbox. It was originally designed as the back end for automating DEER experiments (autoDEER) but it is also readily expandable to a large variety of other pulse EPR experiments.

Features included in autoEPR:

- **Automated Control:** Fully automated experiments from pulse tuning and set-up experiments (Field-Sweeps, Resonator profiles, etc...) to final measurement and analysis. This can all be done such that the user only needs to press a single button and walk away.
- **Integrated Analysis:** Whilst the experiment is running, the toolbox can actively process and analyse the data. This analysis can be used to either set future experimental parameters or to intelligently end the experiment once specific criteria have been satisfied. It is possible to expand to all Python-based data analysis packages.
- **Generalised Sequences:** Experimental sequences are written and designed in an easy-to-use object-based approach. Full support exists for both shaped and chirped pulses, including resonator and TWT compensation. Experiments and procedures written on one spectrometer are readily transferable to other spectrometers, even if they are of a different design or model.
- **Hardware independence:** This toolbox is spectrometer independent and supports both home-built spectrometers and modern Bruker Elexsys-II (using the XeprAPI). Both traditional systems with multiple pulse forming units (MPFU) and modern AWG-based systems are supported. For Bruker systems, autoEPR works by writing PulseSpel files itself.

For more information, please visit <https://github.com/JeschkeLab/autoDEER>

PT055 - Electron Paramagnetic Resonance Spectrometer for In-situ Investigation of Dynamic Nuclear Polarization Mechanisms at 14T

Dr Ilia Kaminker¹, Orit Nir-Arad¹, Dr Alexander Fialkov¹, Eyal Laster¹, Dr Nurit Manukovsky¹

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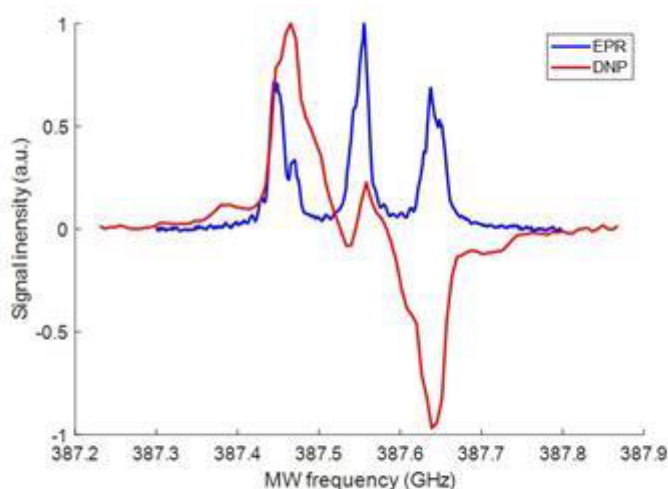
Parallel 20: EPR/ESR, Alsh, July 13, 2023, 13:45 - 15:45

Dynamic Nuclear Polarization (DNP) is a hyperpolarization technique that rose to prominence in the past two decades due to its ability to address the most pressing issue of NMR spectroscopy – its limited sensitivity. DNP is the most general hyperpolarization approach that allows for orders of magnitude signal enhancements in a wide variety of NME applications ranging from materials through chemistry to biology.

In the core of DNP lies polarization transfer from highly polarized electron spins to nuclear spins of interest. This is a complex quantum mechanical process, which has been the subject of intense research, one clear conclusion of which is that Electron Paramagnetic Resonance (EPR) data, providing information about the electron spin dynamics, is indispensable for the theoretical understanding of DNP, and must be collected under conditions that are as close to those of DNP as possible.

At low (0.3-1.2 T) fields, where EPR detection is readily available, multiple insights on DNP mechanisms have been obtained, and new DNP experiments, e.g. pulsed-DNP, have been developed. At higher (3-7 T) fields, several groups have constructed dedicated dual DNP/EPR instruments specially tailored for the investigation of DNP mechanisms.

In contrast, at higher (>7 T) field DNP experiments, only the nuclear spins have been studied so far, and electron spin dynamics has been investigated only theoretically using quantum mechanical simulations. I will present the new dual DNP/EPR instrument operating at 14 T / 400 GHz constructed in our group. This instrument is tailored for the detailed investigation of DNP mechanisms "from the electron spin perspective" at high fields and low temperatures. The highlights include multinuclear DNP, CW and pulsed (including pulse shaping) EPR for static samples in the 8-300 K temperature range and EPR under magic angle spinning.



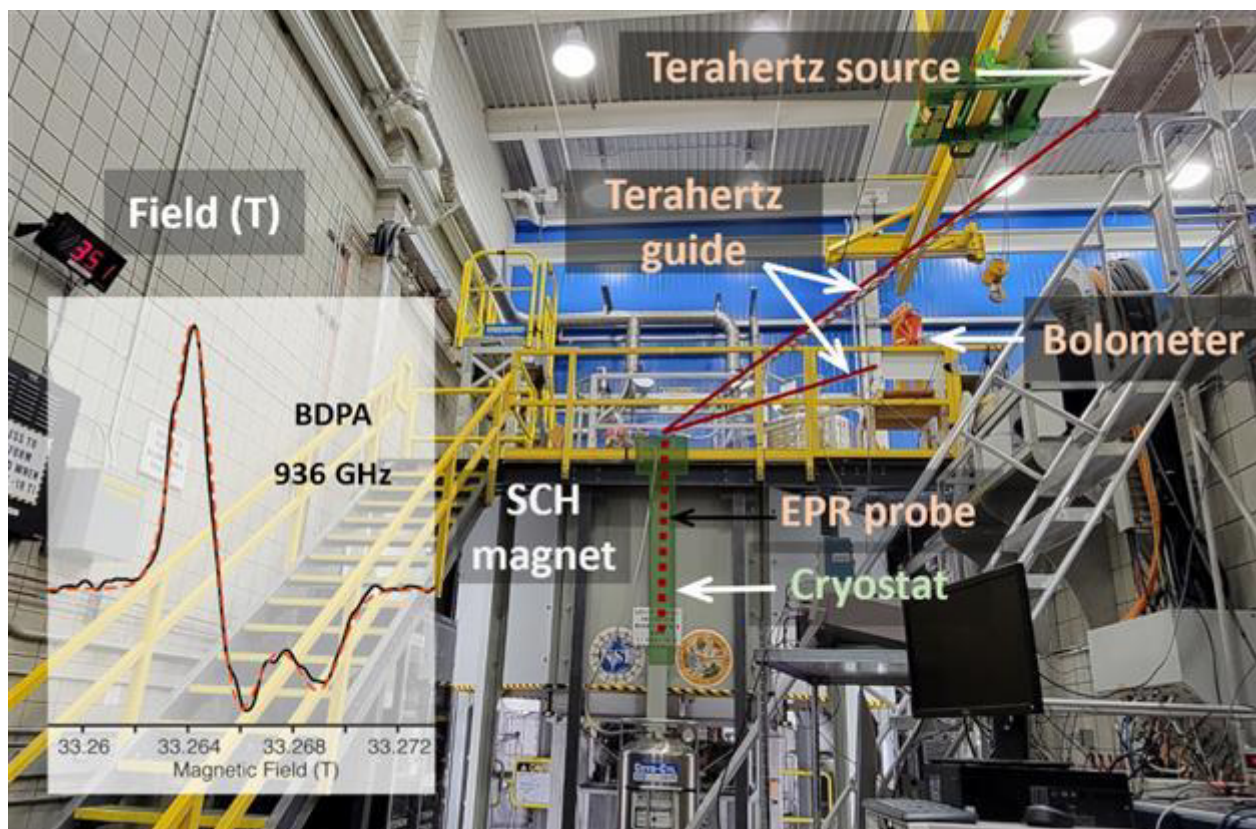
PT056 - Terahertz EPR Spectroscopy Using a 36-Tesla High-Homogeneity Series-Connected Hybrid Magnet

Dr Thierry Dubroca¹, Dr. Frederic Mentink-Vigier¹, Prof. Stephen Hill¹, Dr. Jurek Krzystek¹

¹National High Magnetic Field Lab / FsU, Tallahassee, United States

Parallel 20: EPR/ESR, Alsh, July 13, 2023, 13:45 - 15:45

Electron Paramagnetic Resonance (EPR) is a powerful technique to study materials and biological samples on an atomic scale. High-field EPR in particular enables extracting very small g -anisotropies in organic radicals and half-filled 3d and 4f metal ions such as MnII ($3d^5$) or GdIII ($4f^7$). It also allows one to resolve EPR signals from unpaired spins with very close g -values, both of which provide high-resolution details of the local atomic environment. The recent commissioning of the high homogeneity Series Connected Hybrid magnet (SCH, superconducting + resistive) at the National High Magnetic Field Laboratory (NHMFL) allows the highest-field EPR spectroscopy in existence with high-resolution. Herein, we report the first EPR experiments performed using the SCH magnet capable of reaching the field of 36 T, corresponding to an EPR frequency of 1 THz for $g = 2$. The magnet's intrinsic homogeneity (25 ppm, that is 0.9 mT over 1 cm diameter, 1 cm length cylinder) was previously measured by NMR. We characterized the magnet's temporal stability (0.1 mT over 5 minutes) using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and fully resolved the very weak g -anisotropy of another model radical, 1,3-bis(diphenylene)-2-phenylallyl (BDPA), $\Delta g = 0.00025$ obtained from measurements at 932 GHz and 33 T. Subsequently, we recorded EPR spectra at multiple frequencies for two GdIII complexes with potential applications as spin labels. We demonstrated a significant reduction in line broadening due to second-order zero-field splitting, and a resolution enhancement of g -tensor anisotropy for half-integer spins in an unprecedented high-field regime. This new instrument is now part of the NHMFL user program. Interested parties are encouraged to contact the authors to learn about the process to obtain access to this new spectrometer. Funded by the National Science Foundation (DMR-2128556) and the State of Florida.



PT057 - Isotropic Mixing and fast DOSY Experiments

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Parallel 21: Small Molecule/Drug Discovery, Boisdale, July 13, 2023, 13:45 - 15:45

With the advent of fast pulsing experiments like the ASAP sequences [1], equally fast experiments with significantly reduced relaxation delays are also searched for in the homonuclear case. A particularly intriguing experiment is diffusion-ordered spectroscopy, DOSY. It will be shown, which problems arise when the conventional DOSY is transformed into a β -excited experiment and how to solve them. A very critical component in this transformation is the avoidance of coupling evolution during the long delay Δ , for which we propose isotropic mixing as a viable solution. While the power requirements are high for TOCSY-like periods, fast DOSY experiments significantly below 1 minute total duration are easily possible. To reduce the power requirements, we also looked into a pulse-delay sequence as an implementation of isotropic mixing, similar to the use of the perfect echo to approximate planar mixing (PM), which, combined with BURBOP broadband pulses, leads to very broadband PM-TOCSY experiments. The result of a thorough optimization using average Hamiltonian theory in the interaction frame resulted in the so-called isotropic perfect echo, which leads to an isotropic average Hamiltonian, albeit the isotropic interaction is scaled by a factor three compared to homonuclear TOCSY experiments. First applications of the IPE sequence are shown.

[1] D. Schulze-Sünninghausen, J. Becker, M.R.M. Koos, B. Luy, "Improvements, extensions, and practical aspects of rapid ASAP-HSQC and ALSOFAST-HSQC pulse sequences for studying small molecules at natural abundance", *Journal of Magnetic Resonance* 281, 151–161 (2017) and references cited therein.

PT058 - Long-lived coherences in chains of methylene protons

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Parallel 21: Small Molecule/Drug Discovery, Boisdale, July 13, 2023, 13:45 - 15:45

Long-lived coherences (LLCs) are zero-quantum coherences with lifetimes that are longer than transverse relaxation times T_2 . For a pair of two chemically inequivalent spins, LLCs can be excited between the singlet S_0 and central triplet T_0 states [1,2]. Here we present methods to excite LLCs involving 6 proton spins of three CH_2 spin pairs (geminal protons in each group are chemically equivalent) in aliphatic chains in various biomolecules. The LLCs can be excited by polychromatic spinlock-induced crossings (poly-SLIC) using a superposition of 2 or 3 weak radio-frequency fields [3]. The resulting LLCs correspond to coherent superpositions between the levels spanned by the $S_0 T_0 T_0 \leftrightarrow T_0 S_0 T_0 \leftrightarrow T_0 T_0 S_0$ states and between the levels spanned by $S_0 S_0 T_0 \leftrightarrow S_0 T_0 S_0 \leftrightarrow T_0 S_0 S_0$ states, which evolve under the free precession Hamiltonian, and have lifetimes longer than T_2 . The method allows recording 2D spectra that are reminiscent of J spectra with narrow peaks with linewidths on the order of 0.1 – 0.2 Hz in the F1 dimension, and frequencies that are determined by differences of vicinal $3J$ couplings. Such coherences may be of interest for ligand-based drug screening.

[1] G. Pileio, M. Carravetta, and M. H. Levitt, Extremely Low-Frequency Spectroscopy in Low-Field Nuclear Magnetic Resonance, *Phys. Rev. Lett.* 103, 083002 (2009).

[2] R. Sarkar, P. Ahuja, P. R. Vasos, and G. Bodenhausen, Long-Lived Coherences for Homogeneous Line Narrowing in Spectroscopy, *Phys. Rev. Lett.* 104, 053001 (2010).

[3] A. Sonnefeld, G. Bodenhausen, and K. Sheberstov, Polychromatic Excitation of Delocalized Long-Lived Proton Spin States in Aliphatic Chains, *Phys. Rev. Lett.* 129, 183203 (2022).

PT059 - Fragment Screening and Fast Nanomolar Detection on a Benchtop NMR Spectrometer Boosted by Photo-Induced Hyperpolarization

Ms Gabriela Stadler¹, Dr. Takuya Segawa¹, Mr Matthias Bütikofer¹, Dr Barbara Czarniecki², Dr Sandra Loss², Dr. Felix Torres^{1,3}, Mr Roland Riek¹

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Parallel 21: Small Molecule/Drug Discovery, Boisdale, July 13, 2023, 13:45 - 15:45

The low sensitivity of NMR spectroscopy in drug discovery applications can be overcome by using photo-chemically induced dynamic nuclear polarization (photo-CIDNP). We established the proof-of-concept by screening a 212-compound fragment library on a high-field (600 MHz) NMR spectrometer, using low micromolar concentrations and single-scan experiments of a few seconds. [1] The polarization yield obtained by photo-CIDNP increases inversely proportional to the magnetic field, facilitating the use of low-field benchtop magnets. Benchtop NMR spectrometers are about 20-fold cheaper than high-field spectrometers, require little maintenance, and their permanent magnets do not require cryogenic or helium cooling.

We show that photo-CIDNP-based fragment screening is possible on a cryogen-free low-field benchtop NMR spectrometer. The performance of screening in comparison to state-of-the-art high-field NMR is discussed and reveals the advantages of our approach regarding costs and simplicity of execution.

We present a photo-CIDNP miniscreen against the cancer target PIN1 with 28 compounds measured on an 80 MHz NMR spectrometer in only 3 minutes per sample using 500 μ M compound and 10 μ M protein concentrations.

The miniscreen verified the fast detection of low-millimolar binders at a benchtop NMR spectrometer. While the concentrations used are comparable to a state-of-the-art NMR screening on high-field, the measurement time could be reduced by 5 to 10-fold. Binding could also be observed at lower concentrations down to 50 μ M ligand and 1 μ M protein. The detection limit for one compound was 100 nM after 9 minutes, while without hyperpolarization no signal was detected after days of continuous measurement.

These results demonstrate the potential of photo-CIDNP to overcome the sensitivity limitation of NMR spectroscopy, enabling life science applications on cheap low-field permanent magnets and opening the door to broader use of NMR in the drug discovery community.

[1] Torres, Stadler et al. Ultrafast NMR fragment screening using photo-hyperpolarized (CIDNP) NMR. (under review)

PT060 - Imaging NMR for the quantitative study of protein-ligand interactions and enzymatic reactions in a single NMR tube

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Parallel 21: Small Molecule/Drug Discovery, Boisdale, July 13, 2023, 13:45 - 15:45

- Introduction

NMR is a powerful tool to study protein-ligand interactions as it unveils atomic details of the interactions in solution. Imaging NMR is a new technique enabling us to condense any titrations in a single NMR tube containing a gradient of the analyte of interest.

- Aims

Imaging NMR has the huge potential of accelerating drug discovery and fundamental research. We aimed at the development of: 1) K_D and binding specificity, through the newly implemented Imaging Saturation Transfer Difference (STD) NMR experiment; and 2) the determination of enzymatic parameters K_M and V_{max} from fitting the Michaelis-Menten equation.

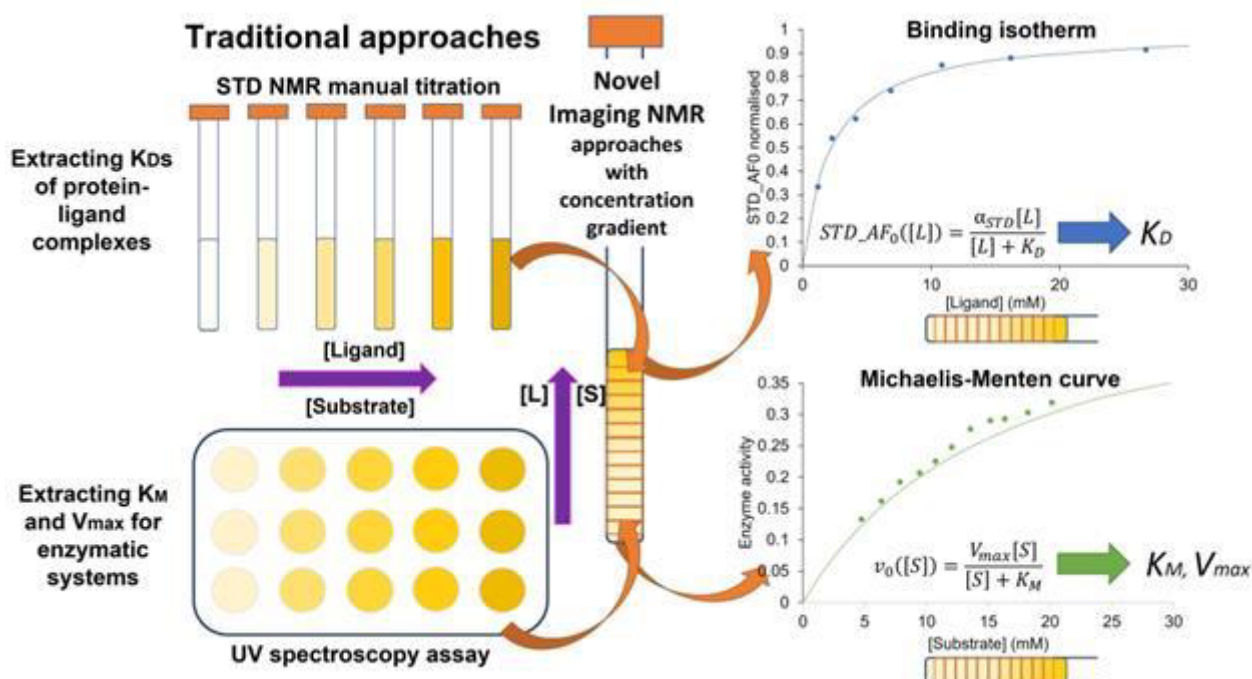
- Methods

Both implementations are performed in a single NMR tube, with a huge reduction of time and resources compared to conventional methodologies. We prepare samples containing a controlled gradient of ligand against homogeneous concentration of protein, and developed for the first time Imaging STD NMR to obtain the K_D of the complexes in a single tube. For 2) we prepared substrate gradients against homogeneous enzymes to extract K_M and V_{max} .

- Results and conclusions

We prove that 1) Imaging STD NMR is an effective methodology to extract dissociation constant, obtaining values in good agreement with literature in 10%-20% of the experimental time relative to the manual STD NMR titration. Using the same approach, we can also assess the specificity of binding, following the evolution of the binding epitope upon increasing ligand excess.

We also demonstrated that 2) by Imaging NMR we can condense enzymatic assays in a single NMR tube. Whereas UV spectroscopy is the established technique for this, its main limitation is the need of a chromogen, often precluding direct detection and/or excluding physiological substrates. By Imaging NMR we manage to obtain accurate K_M and V_{max} in a single sample and with no limitation on the nature of substrate.



Poster Abstracts

P-121 - Strategies for acquisition and processing of serial NMR data

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¹Centre of New Technologies, University of Warsaw, Warsaw, Poland, ²Department of Mathematical Methods in Physics, University of Warsaw, Warsaw, Poland

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

NMR measurements are often performed in series and under varying conditions, either controlled (temperature, pH, concentration) or uncontrolled (reaction progress). As a result, peak signals in the spectra move from spectrum to spectrum according to some physical model. One can then use Maximum Likelihood Estimation (MLE) to find the best parameters that fit the data in agreement with the model.

Aims

Given that several NMR signals need to be averaged in order to improve the inherently low sensitivity of the technique, and that spectrometer time is usually limited or expensive, the following question raises: for fixed spectrometer time for serial measurements, is it better to acquire less spectra with more averages or vice versa? Where “better” means to improve the fitting with MLE. Furthermore, could other processing methods, like Radon transform [1, 2], improve the results? We aim at giving concise answers to these research questions.

Methods

We extend on the existing mathematical formulation of the problem [3, 4] and compare our findings with computational simulations.

Results & Conclusions

In general terms, it is usually preferred to acquire more spectra with less averages. Although, this statement is only true for as long as peak signals can be distinguished from noise. The use of Radon transform can help circumvent this impediment.

P-061 - Switching off a GPCR: different facets of receptor desensitization studied at atomic resolution

Ms Arnelle Loebbert

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

G protein-coupled receptors (GPCRs) represent the largest family of signaling proteins and are targeted by one third of all clinically used drugs. To control the signaling process of GPCRs, cells have developed a tightly regulated system of desensitization where GPCR kinases (GRKs) play a vital role. By selectively phosphorylating the C-terminus and/or intracellular loops of activated receptors, GRKs initiate the recruitment of arrestins to GPCRs, leading to a plethora of cellular responses. Within this project, our aim is to study the allosteric activation of the kinase, its interaction with effector molecules and the resulting phosphorylation pattern in time-resolved manner. We performed selective ^{13}C -methionine labeling of GRK1 (60 kDa) and GRK2 (80 kDa) in insect cells. By recording characteristic fingerprint spectra, we can determine distinct conformational changes of the kinase upon binding of physiological effectors (ATP, C-terminal peptides) in contrast to allosteric ($\beta\gamma$ -subunit, anionic phospholipids) or competitive modulators (paroxetine, sangivamycin). Resonance assignment by mutagenesis and the determination of KD values by NMR titration experiments were used to characterize these interactions in unprecedented detail. Additionally, phosphorylation events catalyzed by GRK1/2 were monitored in real-time on the isolated ^{15}N -labeled C-terminal tails of various GPCRs (hs β_1 AR, hs β_2 AR, bovine rhodopsin). Thus, we were able to determine the individual phosphorylation rates for differing sites and could further investigate the influence of allosteric modulators and activated receptor preparations on kinase activity. Moreover, we could demonstrate the high regulatory importance of the dynamic N-terminal stretch of the kinase. Unraveling details of GPCR desensitization in turn may aid in the discovery of new therapeutic agents targeting specific GRK functions.

P-155 - Advances in Spherical Rotors for Dynamic Nuclear Polarization

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¹ETH Zurich, Zurich, Switzerland

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Spherical rotors are an alternative to the current cylindrical rotors used for MAS NMR. Initial implementation used 3D printed ABS-like plastic stators which are ideal for prototyping but wear during long-term experiments and repeated spin up/spin down procedures due to softness or crack under the cryogenic conditions required for MAS DNP due to a large thermal shrinkage coefficient.

Aims/Methods

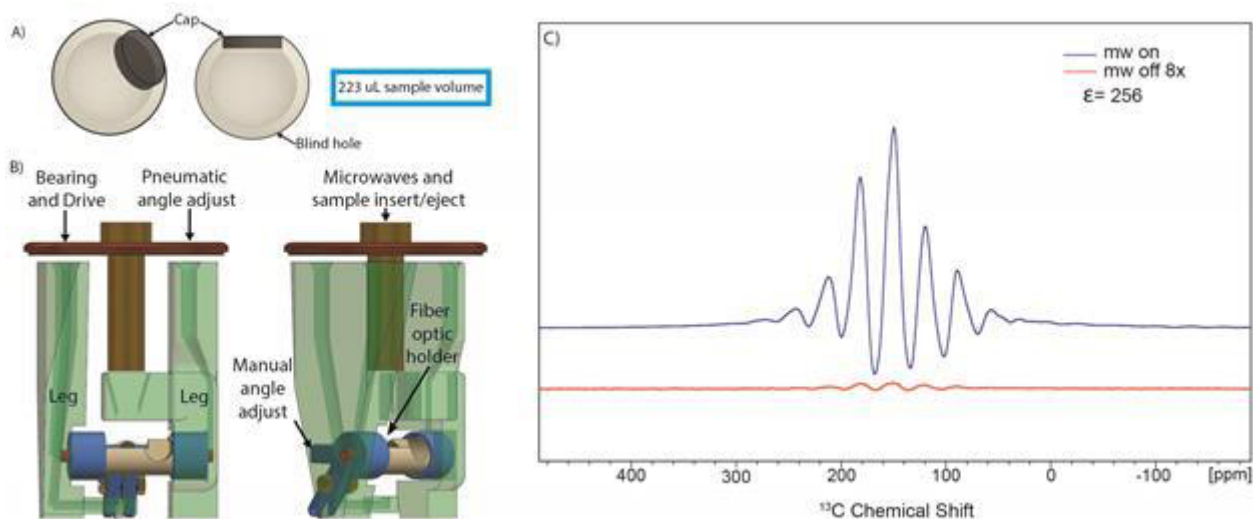
Here we introduce a probe assembly for cryogenic temperatures which was used to perform the first MAS DNP using spherical rotors. The use of a stator machined from the ceramic Macor® in combination with a “blind hole” sapphire sphere preserves the room temperature fluid flow at cryogenic temperatures due to the identical thermal shrinkage of the two materials. Spinning is achieved using a single gas stream at the complement of the magic angle and a second gas stream provides pneumatic magic angle adjustment at cryogenic temperatures. Cooling utilizes cold variable temperature (VT) and spinning gas with sample temperatures reaching 90 K as measured using KBr. A saddle coil provides direct vertical access to the sample for microwaves and sample insert/eject while giving sufficient radio frequency performance for ssNMR experiments.

Results

Preliminary DNP results have been obtained using 9.5 mm spherical rotors on a standard of 20 mM AMUPol, 4 M ¹³C 15N Urea in 60/30/10 d-8 glycerol/D₂O/H₂O at temperatures below 110 K, sample sizes up to 223 µL and a spinning frequency of 2 kHz. A spherical rotor hollowed to a 1 mm shell creates a spherical-shell rotor holding 223 µL of sample.

Conclusions

This probe and spherical rotor design is also being scaled for 6 mm spherical rotors which is projected to increase the spinning frequency to above 5 kHz. In the future this system can be used to reach temperatures of 5 K using helium without major modifications.



P-203 - Bullet-Dissolution Dynamic Nuclear Polarization and Ligand Binding

Ms Pooja Pooja¹, Mr Nils Lorz², Dr Masoud Minaei¹, Dr Michael Jurkutat¹, Dr Karel Kouřil¹, Professor Sami Jannin³, Dr Alvar Gossert², Dr Benno Meier^{1,4}

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

In bullet-DNP, a sample is hyperpolarized at cryogenic temperatures and rapidly transferred to a second magnet where it is dissolved and liquid-state nuclear magnetic resonance (NMR) spectra are recorded [1,2]. Bullet-DNP has advantages for observing ligand-binding since it is scalable to small solvent volumes and thus reduces the required amount of protein. Here we present ligand-binding experiments, in which the sensitivity of the hyperpolarized signal detection is improved further via a ¹³C-¹H reverse INEPT polarization transfer from carbon to methyl protons. As of now, we have achieved substrate concentrations of 4 μM with a significant enhancement in signal.

References:

- [1] Kouřil, K., Kouřilová, H., Bartram, S., Levitt, M. H., and Meier, B.: Scalable Dissolution-Dynamic Nuclear Polarization with Rapid Transfer of a Polarized Solid, *Nat. Commun.*, 10, 1733, <https://doi.org/10.1038/s41467-019-09726-5>, 2019.
- [2] Kouřil, K., Gramberg, M., Jurkutat, M., Kouřilová, H., Meier, B.: A cryogen-free, semi-automated apparatus for bullet-dynamic nuclear polarization with improved resolution, *Magnetic Resonance*, 2, 815, <https://doi.org/10.5194/mr-2-815-2021>, 2021.

P-263 - Rapid Continuous Bacterial and Mammalian Cell Metabolomic Profiling by Chemical Shift Imaging NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Probing the metabolomic profile of human primary colonocytes and commensal bacteria has been gathering a lot of scientific attention in recent years for its ability to highlight early stages of lower gastrointestinal (GI) pathologies, such as ulcerative colitis, inflammatory bowel disease, leaky gut syndrome, as well as primary colorectal carcinomas. Several studies have highlighted the importance of small molecular metabolites, such as short-chain fatty acids (SCFAs), on the interplay between human primary colonocytes and gut bacterial species. The primary SCFAs produced in the large intestine are acetate, butyrate and propionate, with all three having important physiological roles in hepatic gluconeogenesis, influencing metabolic homeostasis markers, such as hormones PYY and GLP-1, improving B-cell function, insulin secretion, as well as intracellular signaling molecules. Dysregulation of the production of these SCFAs has been linked to cardiovascular disease (CVD), metabolic syndrome, and diabetes mellitus.

However, most current in vitro methods for probing this cell-cell crosstalk involve complex and expensive set-ups, featuring multiple vessels, frequent sampling, and non-reusable technologies (e.g., organ-on-chip, TIM-1,2 and SHIME models).

In this work, we demonstrate a new, rapid semi-automated method for the spatially resolved quantification of small molecular metabolites directly in an NMR tube, with minimal sample preparation or sampling. Our method is based on the chemical shift imaging (CSI) methodology, developed by Wallace et al., and allows for the dynamic and accurate determination of spatially resolved exchange of small molecular metabolites between bacterial and mammalian cells across a gradient, as well as pH.

P-043 - Lectin-glycan interactions: exploring the role of sugar presentation and cell-like contexts through NMR

Miss Sara Bertuzzi¹, Miss Maria Pia Lenza², June Ereño-Orbea¹, Ana Ardà^{1,3}, Mr Jesus Jimenez-barbero^{1,3,4}

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

The comprehensive elucidation of the features regulating sugar-lectin recognition in natural contexts is elusive and far from being completely understood. Indeed, the deep knowledge of these biologically significant interactions is a great challenge in the glycoscience field.

Aims

The environment in which the interaction takes place strongly influences protein and glycan presentation. Focusing on the sugar counterpart, the orientation, dynamics, flexibility, accessibility, and density are aspects that have to be considered and evaluated. Herein, our main objective was to acquire knowledge on these factors using diverse methodologies, not only in vitro but also in the cell-like contexts.

Methods

We have employed a panel of different NMR-based protocols.

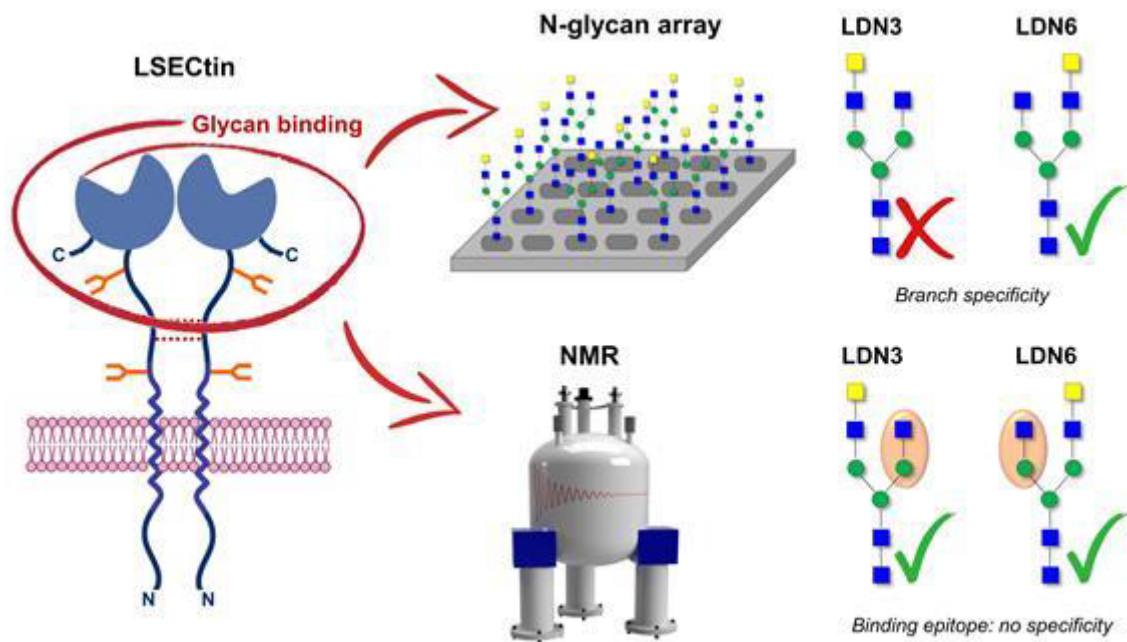
Results

Firstly, we have highlighted how two different techniques, NMR and microarrays, provide different results on the recognition of a pair of positional N-glycan isomers (LDN-3 and LDN-6) by a C-type lectin, LSEctin. Indeed, the obtained results depend on whether the glycans are free in solution or anchored to the microarray surface.

We have also employed NMR to scrutinize glycan-lectin recognition in-cell and on-cell using intact cells. Specifically, an in-cell NMR approach using *Danio rerio* oocytes has been optimized to detect the binding of an exogenous microinjected lectin (galectin-7) to the non-hydrolysable lactose analogue thiodigalactoside (TDG). Moreover, an on-cell NMR strategy has been applied to study the recognition features of siglec-10, overexpressed in mammalian cells (HEK293), when interacting with a selective glycomimetic.

Conclusions

From our results, it is demonstrated how the presentation of the interacting sugar epitope influences the outcome of the recognition, thus translating the difficulty of comparing results obtained from different techniques. In addition, our proposed NMR protocols using intact cells are a good approximation to detect (i) intracellular recognition events involving lectins and sugars in a cellular-like microenvironment and (ii) extracellular binding processes involving receptors embedded into cellular membranes.



P-369 - Quantifying Distance Measurements in Solids using Rotational Resonance (R2) Experiments

Miss Neelam Sehrawat

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Development of radio-frequency pulse-based efficient recoupling/decoupling methods is key to the accurate measurement of orientation-dependent structural constraints in chemical, materials, and biological samples using solid-state nuclear magnetic resonance (ssNMR) spectroscopy. Nevertheless, the accuracy of these constraints is often compromised due to poor spectral resolution and sensitivity of the ssNMR spectra, and lack of theoretical models to quantify such experimental data. Multiple time-modulations, increased dimensionality due to multi-spin contributions, and the presence of a non-commuting set of operators in the nuclear spin interaction Hamiltonian are the main difficulties associated with the theoretical models.

Aim

To develop a theoretical framework based on the concept of the effective Floquet Hamiltonian for quantifying the distances in a multi-spin network using the Rotational Resonance (R2) - based recoupling experiments in solid-state NMR.

Method

In the present framework, multi-spin contributions to the effective Hamiltonian are incorporated in a reduced subspace (2x2 block diagonal matrix) and the perturbation corrections are evaluated up to the third-order using the contact transformation method.

Results

Both the demagnetization effect and broadening of R2 -exchange profiles in the presence of neighboring protons are mainly governed by the operators resulting from the cross-terms (CSA)protons \times (Dipolar)hetero, (Dipolar)protons \times (Dipolar)hetero and (CSA)protons \times (Dipolar)protons in the effective Floquet Hamiltonian. While the second-order perturbation corrections to the effective Floquet Hamiltonian are sufficient to explain the magnetization exchange profiles for N = 1 R2 condition, corrections up to the third-order are essential to describe the N = 2 R2 exchange dynamics.

Conclusions

The analytical simulations being computationally less intense, economical, and less time-consuming compare extremely well with the exact numerical simulations. Integration of the proposed framework into a routine program code would be extremely beneficial in the iterative fitting of the experimental data and for the extraction of multiple distance constraints in larger biomolecular systems.

P-277 - Preoperative Prediction of Oligodendroglial Tumors Pathological Grading Using Apparent Diffusion Coefficient Histogram Analysis in the Parenchymal Region of the Tumor

Mr Xianwang Liu^{1,2,3}

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Purpose: To explore the value of preoperative prediction of pathological grading of oligodendroglial tumors (OG) using apparent diffusion coefficient (ADC) histogram analysis in the parenchymal region of the tumor.

Methods: The clinical, MR, and pathological data of 25 oligodendrogliomas (OD, WHO grade 2) and 26 anaplastic oligodendrogliomas (AOD, WHO grade 3) confirmed by surgical pathology were retrospectively analyzed. The parenchymal region of the whole tumor was outlined and histogram analysis was performed on the axial ADC images using MaZda software. Nine histogram parameters were obtained, including mean, variance, skewness, kurtosis, 1st, 10th, 50th, 90th, and 99th percentiles. The differences in histogram parameters between OD and AOD groups were compared, and operating characteristic curves (ROC) were drawn to analyze the differential diagnostic efficacy of each histogram parameter for OD and AOD.

Results: The mean, 1st, 10th, and 50th percentiles of the histogram parameters in the AOD group were smaller than those in the OD group, and the differences between the parameters were statistically significant ($P < 0.05$), while the differences in variance, skewness, kurtosis, 90th and 99th percentiles between the OD and AOD groups were not statistically significant ($P > 0.05$). The best grading diagnostic efficacy was achieved at $108.00 \times 10^{-6} \text{ mm}^2/\text{s}$, with an area under the curve (AUC), sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of 0.840, 76.92%, 84.00%, 80.39%, 83.30%, and 77.80%, respectively.

Conclusion: Histogram analysis based on ADC in the parenchymal region of the tumor is valuable for preoperative prediction of OG pathological grading, and the 1st percentile has high diagnostic efficacy for grading.

Table 1. Comparison of ADC histogram parameters between OD and AOD

Parameters	OD (n=25)	AOD (n=26)	P value
Mean	151.07 ± 19.50	145.56 ± 9.11	0.005942
Variance	472.20 ± 376.19	457.54 ± 143.47	0.856422
Skewness	0.21 ± 0.43	0.43 ± 0.45	0.077142
Kurtosis	-0.18 ± 0.95	0.34 ± 0.84	0.076533
ADC1	115.94 ± 8.03	102.78 ± 10.72	0.000009
ADC10	130.90 ± 9.06	118.64 ± 11.12	0.000080
ADC50	151.00 ± 17.00	143.67 ± 9.50	0.003152
ADC90	176.00 ± 43.00	170.50 ± 13.75	0.291208
ADC99	201.49 ± 28.56	199.17 ± 18.32	0.732386

Notes: OD, oligodendrogliomas; AOD, anaplastic oligodendrogliomas; ADC, apparent diffusion coefficient

Table 2. Diagnostic performance of ADC histogram parameters in differentiating OD and AOD

Parameters	AUC (95%CI)	Cutt-off value	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
Mean	0.725 (0.528,0.840)	148.70	80.77	66.00	74.51	72.40	77.30
ADC1	0.840 (0.710,0.928)	108.00	76.92	84.00	80.39	83.30	77.80
ADC10	0.815 (0.682,0.910)	125.00	80.77	76.00	78.43	77.80	79.20
ADC50	0.741 (0.599,0.853)	145.00	73.08	76.00	72.55	76.00	73.10

Notes: CI, confidence intervals; OD, oligodendrogliomas; AOD, anaplastic oligodendrogliomas; ADC, apparent diffusion coefficient; AUC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.

P-079 - Ubiquitin's structural heterogeneity visualized directly within cells at atomic resolution

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Numerous atomic-level in vitro structural studies suggest that ubiquitin's (Ub's) structural heterogeneity drives selective binding partner recognition facilitating its vast regulatory scope.[1–4] However, structural in vivo evidence to this end is lacking. Here we attempted to visualize Ub's structural heterogeneity at atomic resolution directly within vitrified HeLa cells by utilizing in-cell high field dynamic nuclear polarization solid-state NMR.[5,6] Comparison of our multidimensional NMR data with computational predictions on the basis of over 1,200 Ub-containing structures revealed a remarkable correlation between in-vitro Ub plasticity and in-cell NMR spectroscopic signatures. Similar experiments conducted on proteolytically stressed cells suggested a functional and time-dependent dependence of ubiquitin's structural heterogeneity with especially marked variation in previously reported highly dynamic β 1- β 2 and α 2- β 5 loop regions.[1,3] Taken together, we present the first native atomic insights into Ub's structural heterogeneity in cells and reveal how the induction of stress modulates Ub's conformational space in-vivo.

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P-041 - Structural study of non-canonical hairpins formed by repetitive CGAG-rich sequences found in the promoter of the neurodevelopmental regulator AUTS2 gene

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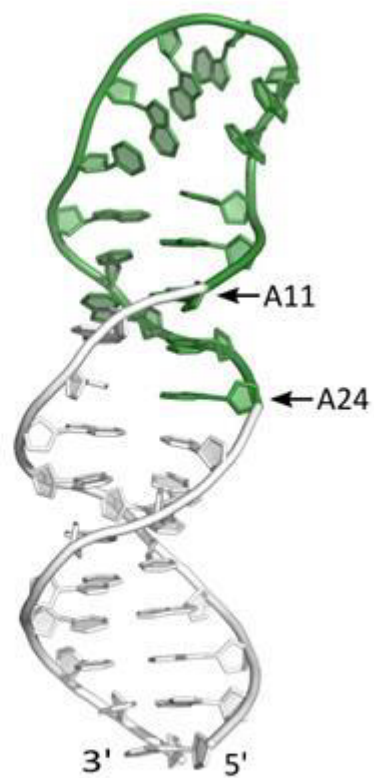
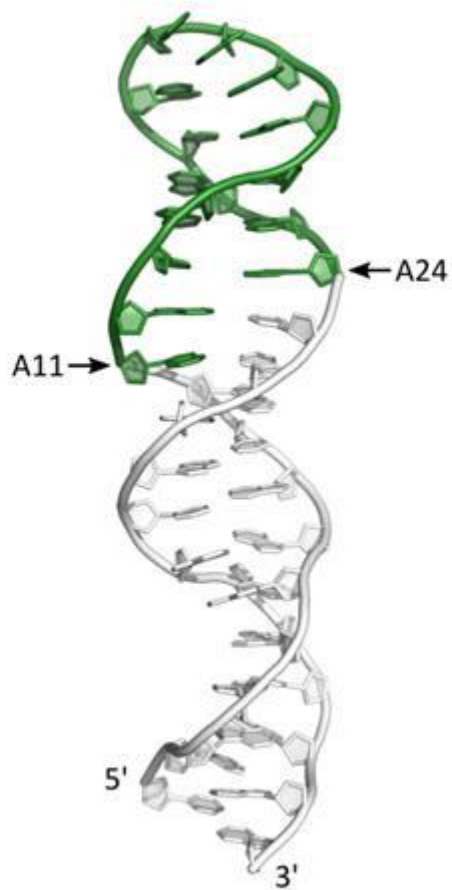
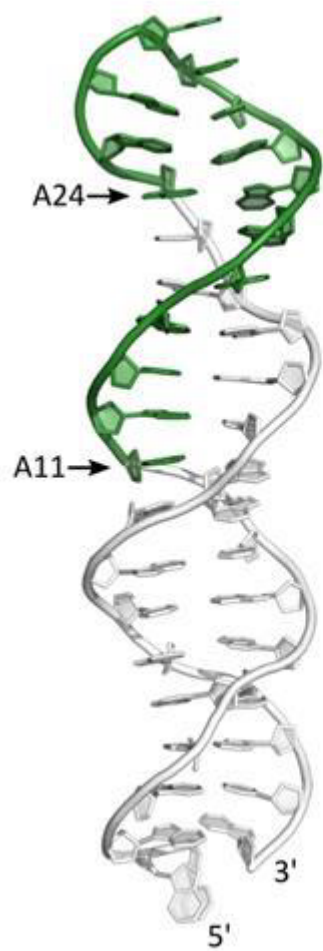
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Either a short or a long isoform of AUTS2 protein is predominantly expressed depending on different stages of brain development (1). Misregulations in AUTS2 isoform expression have been correlated to developmental delay and intellectual disability (2). How this expression switch is regulated on the molecular level is still unknown. We focused our structural study on a CGAG-rich region located approximately 150 base pairs upstream of the transcription start site of the long isoform. As expected, based on the literature (3,4), the NMR spectra showed that the oligonucleotides from the CGAG-rich region are structurally polymorphic which could play a role in the expression switch. To overcome the polymorphic nature of the CGAG-rich region we focused on three truncated variants to explain the general sequence-structure relationship. Using NMR we show that all the variants form thermally stable non-canonical hairpins stabilized predominantly by G:C and G:A base pairs. The different number of CGAG repeats contained in each variant affected the arrangement of the loop, whereas the stems were dominated by G:C and sheared G:A base pairs in all three constructs. The loop region architecture is biologically very relevant since it contains the predicted protein binding site. In general, we reveal important driving forces that govern the structural landscape of the CGAG-rich region of AUTS2 promoter. Additionally, the presented approaches will aid in future structural studies of highly repetitive sequences and characterization of their complicated conformational landscapes with the use of NMR.

Acknowledgments: This work was supported by Slovenian Research Agency (ARRS, grants P1-0242 and Z1-3192 as well as Young researcher grant for A.N.).

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P-235 - Structural Studies of Fluorine-Doped Lithium-Rich Anti-Perovskite Solid Electrolytes for All-Solid-State Batteries

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

All-solid-state batteries (ASSBs) have the potential to improve the safety and performance of current lithium-ion (Li-ion) battery technology [1], which is unable to deliver the performance, safety and cost requirements of next-generation energy storage devices [2]. Lithium-rich anti-perovskites (LiRAPs) are a promising class of solid electrolyte material owing to their reportedly high ionic conductivities (10^{-3} S cm⁻¹) their and tuneable crystal structure, which may be manipulated through chemical substitution (i.e., doping) to enhance ion transport mechanisms [3]. The hydrated LiRAP Li₂OHCl is just one composition within this family that is currently of considerable interest. It is widely known to undergo an orthorhombic to cubic phase transition at ~ 35 °C, which appears to correlate with a significant increase in ionic conductivity. However, the precise mechanisms underpinning this structural transformation are still the subject of much debate. It has been shown that the cubic phase of Li₂OHCl can also be formed by the substitution of OH⁻ for F⁻, forming the solid solution Li₂(OH)(1-x)F_xCl [4]. To the best of our knowledge, the Li₂(OH)(1-x)F_xCl ($x = 0 - 0.2$) series and the associated structural changes have not been investigated experimentally to determine the complex structure-functionality relationship within this important class of materials. Here, we present our recent powder X-ray diffraction (XRD) and multinuclear solid-state NMR (SSNMR) results in conjunction with our first-principles density functional theory (DFT) calculations. Our recent findings suggest that the true composition of Li₂(OH)(1-x)F_xCl-based systems differs from what has previously been reported, and that the precise location for F⁻ doping may vary depending on composition. In addition, the synthetic strategy adopted appears to play a considerable role in the precise phases formed. Our combination of experimental methods with DFT enables us to relate local and long-range structural effects to distinct compositional changes, thereby allowing us to unravel the complex structure-function relationships in Li₂(OH)(1-x)F_xCl-based solid electrolytes, which will aid us in designing better functioning electrolytes in the future.

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P-055 - NMR-Pack, NEF-Pipelines & ARIAXC Install, Convert and Analyse... A Trio of Software Tools for the Biological NMR Spectroscopist

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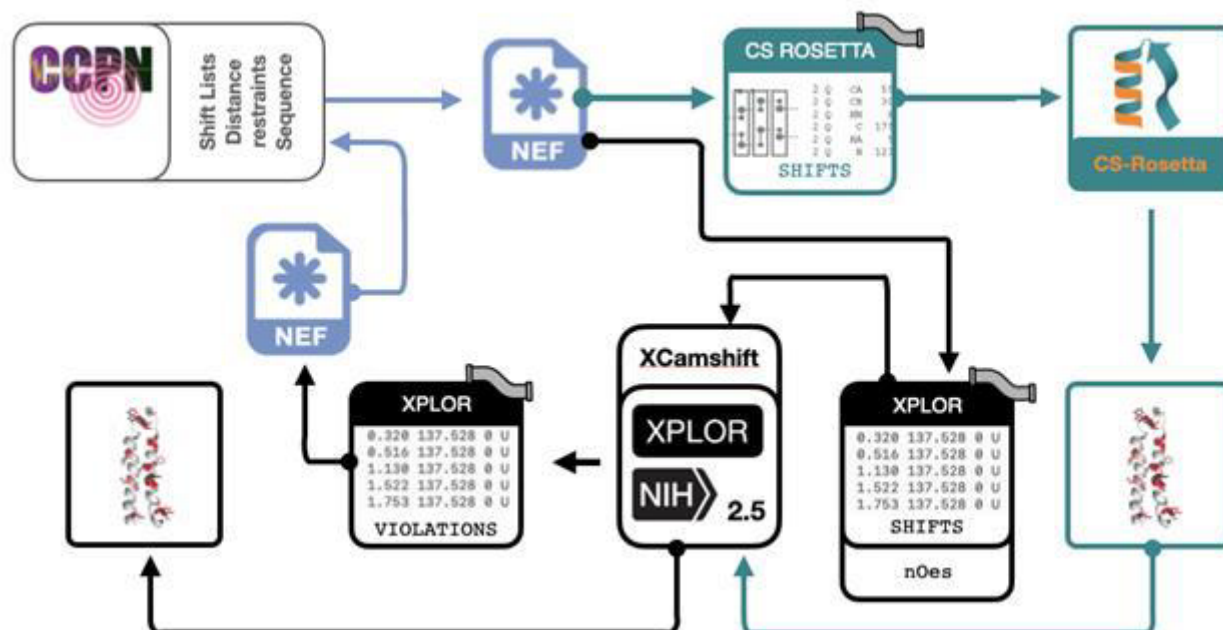
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

An army marches on its stomach! Biological NMR in much the same way due to the quantity and diversity of the data used can claim to march on the software that analyses the data we measure. Here we showcase 3 projects that are designed to improve the biological NMR toolset, and which play off each other's strengths.

NMR-Pack: Installing Biological NMR software across the currently supported platforms can be challenging. NMRPack provides a package manager for NMR software installation based on the supercomputing package manager SPACK. By codifying installation processes in Python it can use the knowledge of the community to ease software distribution. Currently supported software includes NMRPipe, ARIA2 (including GUI tools) and XPLOR-NIH.

NEF-Pipelines: The NMR Exchange Format is a new file format for biological NMR data exchange. NEF-Pipelines takes advantage of the regularity of the NEF file format to create a pipeline of commands that can manipulate a NEF file as a Unix pipeline. The tools include foreign file IO for programs such as NMRView/NMRfx, Sparky, XPLOR-NIH, ARIA and PINE and more general tools such as ones for renaming and renumbering peaks and chemical shift tables, merging NEF files and validating their content to name a subset. NEF-Pipelines provides the software glue for an NMR project and can minimise the need for custom data conversion scripts. However, when needed it provides a framework inside which customs scripts and tools can coexist, reducing the biological NMR spectroscopists workload.

ARIAXC: ARIA is one of the main programs used for NMR structure calculation. Unfortunately, the underlying dynamics engine CNS is no longer developed and doesn't support interesting and useful restraints such as chemical shifts and PREs. ARIAXC is a working implementation of ARIA2 that uses XPLOR-NIH as its molecular dynamics engine. Use of modern forcefield terms will be demonstrated.



P-353 - New lanthanoid chelating tags with novel scaffolds for PCS NMR spectroscopy

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Pseudocontact shift (PCS) NMR spectroscopy has gained a significant impact on protein structure determination over the last decades. Several lanthanoid chelating tags (LCTs) have been developed that allow rigid attachment to a protein. Most often, they are based on a DOTA framework, which allows strong binding of the lanthanoid cation. Attachment to the protein can be achieved via reaction of the proteins' cysteine residue with a linker on the Ln-DOTA complex. The recently published DOTA-M7-Nitro tag reached the limit of the achievable anisotropy parameters with DOTA-type LCTs. To further extend the size of the anisotropy parameters, the development of a new scaffold is necessary. We have, therefore, explored new scaffolds for LCTs, that have intrinsic susceptibility anisotropy parameters significantly exceeding those of DOTA based chelators. Based on these new scaffolds, corresponding LCTs were synthesized. Preliminary results for selected examples will be presented. The new LCTs might open the door to a new class of high-performance PCS tags.

P-021 - Excursion of the Hepatitis B Virus Capsid C-terminal Domain Probed by Paramagnetic solid-state NMR

Mr Louis Brigandat

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The hepatitis B virus consists of a capsid that surrounds the viral genome and is composed of 120 core protein dimers (Cp). Here, we investigate the functional C-terminal domain (CTD) of Cp, which for instance interacts with partner proteins for cellular trafficking. To do this, it has to expose its signal sequence on the outside of the capsid shell. How this is regulated by phosphorylation remains largely unknown, mostly because this 34-amino acid peptide is invisible in cryo-EM, X-ray diffraction, or classical solid-state NMR (ssNMR) experiments due to its short proton T1 ρ , preventing efficient transfer during cross-polarization times. Here we use a spin-labelling strategy in which we introduce a nitroxide radical onto the C-terminal cysteine of Cp after mutating all other cysteines. We then use a combination of proton and carbon-detected ssNMR to obtain paramagnetic relaxation enhancements (PRE) by comparing the 3D peak intensities of paramagnetic and diamagnetic samples. This allowed us to obtain site-specific distance information for over 90 % of the residues of Cp, which we used to compare the excursions of the CTD under three different conditions: in the unphosphorylated, RNA-filled capsid; in the phosphorylated, empty capsid; and in the presence of a capsid assembly modulator that induces abnormal capsid assembly. Our results show that in unphosphorylated capsids, the capsid surface is completely unaffected, indicating that the CTD remains largely inside the capsid. In contrast, in phosphorylated empty capsids, the CTD can protrude through the triangular pores of the capsid, which we therefore identify as the signaling exposure site, contrary to what was previously postulated. Our work provides, for the first time, a molecular view of the CTD localisation in different Cp forms and more generally positions paramagnetic tags and PREs as an important tool for elucidating flexible domains in complex assemblies such as viral capsids.

P-289 - An NMR-based assay informs on the substrate scope of the Pseudomonas Ethylene-forming enzyme

Mr Siddhant Dhingra

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Ethylene is a phytohormone contributing to leaf senescence and fruit ripening. Several plant-infecting microorganisms have been reported to produce ethylene to alter plant metabolism, including *Pseudomonas* which utilises ethylene-forming enzyme (PsEFE) to catalyze the formation of ethylene from 2-oxoglutarate (2-OG) via a Grob-type oxidative fragmentation reaction (Fig.1(i)) 1,2. Concomitantly, PsEFE catalyses the hydroxylation of L-arginine which is coupled to the oxidative decarboxylation of 2-OG to succinic acid¹.

Aims & Methods

An NMR-based assay was optimised under varied pH conditions and concentrations of the different assay components using initially 2-OG as substrate. To explore the possibility of PsEFE to catalyse the formation of substituted olefins, the optimized NMR assay was used to monitor the PsEFE-catalysed conversion of C3 and/or C4 substituted 2-OG derivatives.

Results

The optimized ¹H NMR assay revealed that C-3 or C-4 substituted 2-OG derivatives are alternative PsEFE substrates. However, the formation of the corresponding substituted olefin products (2, Fig. 1(ii)) was not detected; instead, the corresponding ω-hydroxy acids (1, Fig 1(ii)) and diacid (3, Fig. 1(ii)) were observed as the only reaction products. These alcohols and diacids are of biological and industrial importance, broadening the scope of PsEFE for large-scale production of relevant compounds. For example, γ-hydroxybutyric acid (GHB), which is formed from 2-oxoadipate is a neuroactive natural product known to induce euphoria.

Conclusion

The combined results highlight the scope of PsEFE in enzymatic synthesis of not only ethylene but also various diacids and ω-hydroxy acids. NMR assays were particularly powerful to investigate the PsEFE substrate-scope because they enable quantification of substrate depletion and product formation and inform on the identity of the product(s) formed thus providing mechanistic insights.

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P-089 - What NMR tells us about Ficoll

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

Ficoll™ is a synthetic polymer obtained by copolymerization of sucrose and epichlorohydrin. It has a broad range of applications in biochemistry, e.g., as a constituent in a centrifugation media for blood cells separation[1] or as an agent mimicking crowded environments[2]. Although its physicochemical properties are well described, a detailed molecular structure has not yet been established.

Aims:

We aim to obtain more detailed information on Ficoll™ PM70 structure, including site-specific information on branching degree and terminal glycerols/polyglycerols occurrences. The obtained information will support the generation of atomistic models of the polymer in molecular dynamics simulations.

Methods:

We performed numerous high-resolution 2D NMR experiments for peak assignment, including 1H-13C HSQC, 1H-13C qHSQC, 1H-13C HSQC-TOCSY, 1H-13C HSQC-NOESY, 1H-13C HMBC, and 1H-13C H2BC. Moreover, we investigated 1H-13C HSQC chemical shifts at different temperatures and solvents, monitored the oxidation of Ficoll™ by sodium periodate using serial 1H-13C HSQC experiments, and supported data analysis by chemical shift simulations.

Results and conclusion:

We propose a polymer structure for the Ficoll™ with probability-weighted, site-specific information on the occurrence of branching/terminus in the sucrose mer. The project is in the advanced stage, but still, some analyses are undergoing to refine the proposed structure.

Our work exploits a variety of NMR experiments and proves the usefulness of NMR spectroscopy in the structural analysis of synthetic, highly branched copolymers.

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P-069 - SPICY: A method for single-scan rotating frame relaxometry

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The rotating frame relaxometry which is indicated by the relaxation time T1p can be utilized to quantify macromolecular changes in tissues [1]. Especially T1p relaxation dispersion measurements can give information about low frequency biochemical motions, such as chemical exchange or protein dynamics. T1p relaxation is used as an MRI contrast as well. In both T1p NMR spectroscopy and MRI, the long scan times are the major limitations of the experiments. For that reason, in this study, we developed a novel, fast method called SPICY (SPIn lock CYcle) [2] for the determination of the T1p relaxation time in a single scan, and we showed that the method can also be applied to one-dimensional imaging. In conventional T1p methods, the experiment has to be repeated several times while increasing the duration of the spin locking period. In SPICY, the spin locking is repeated by utilizing a loop structure, allowing the determination of T1p time in only one scan. We demonstrated the performance of the new method by measuring T1p dispersion in liquid samples and protein hydrogels modelling the extracellular matrix of articular cartilage and comparing the results with reference values measured with the conventional T1p method. We also showed that the imaging version of SPICY gives comparable results with the reference imaging method. The single-scan nature of the SPICY not only shortens the scan time but gives plenty of opportunities to expand the method to, for example, the multidimensional Laplace-NMR or MRI, or to the hyperpolarized NMR and MRI studies.

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P-025 - Software to aid in DNA-Ligand Structure Determination using Solution NMR and Restrained Molecular Dynamics Simulations.

Mr Ivan Yankov

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction. Understanding the structure and dynamics of DNA-ligand complexes in solution is valuable in designing novel drug-like scaffolds. For example, structural information of minor groove binders is key to optimising the DNA binding domain of artificial transcription factors used in treating triplet expansion diseases. Coupling solution NMR with molecular dynamics simulations provides a powerful method for structural characterisation, but the complexity of the calculations and their dependence on model parameters can be a barrier to adoption.

Aims. Our aim is to transform the manual step-by-step NMR analysis process into a seamless start-to-finish uninterrupted calculation, in order to expedite the elucidation of DNA-ligand atomic coordinate ensembles.

Methods. We present a software package that combines readily available tools to automate NOESY intensity-to-distance analysis and couple it with the AMBER molecular dynamics program. The software package reduces unnecessary user interactions such that key inputs can be defined at the start of the workflow. CPU parallelisation reduces the NOESY-to-distance calculation time enabling users to receive live data during the assignment process. A template with default parameters and a tutorial on how to incorporate the workflow in your NMR analysis is presented.

Results. The application of the software is demonstrated for a palindromic dodecamer DNA duplex in complex with a novel iPr-polyamide ligand. The impact of NOESY restraints on the simulated structure of the DNA-polyamide complex is illustrated by contrasting a restrained and an unrestrained molecular dynamics simulation. The other factors that impact reproducibility of the NOESY intensity to distance conversion workflow are discussed.

Conclusion. The automated NOESY-to-distance analysis improves reproducibility and enables scalability of the process. Precious user time is reallocated from terminal manual interaction to expert analysis of the results. The tool is provided as open-source software and can be incorporated into NMR assignment software (e.g. Sparky) to aid the assignment process.

P-109 - Conformational dynamics and liquid-liquid phase separation of the endocytic protein Eps15

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

During clathrin-mediated endocytosis, a dynamic network of proteins participates in cargo selection, clathrin recruitment and bending of the plasma membrane. One of the early initiators, Eps15, is responsible for locally concentrating downstream components on the membrane surface and it permits dynamic rearrangement of proteins, within the budding vesicle.

Oligomerization of Eps15 promotes the assembly of Eps15 into liquid-like protein droplets that catalyzes endocytosis. Eps15 consists of 3 Eps15 homology (EH) domains, a coiled-coil region and an intrinsically disordered region (IDR). The disordered region is characterized by the presence of multiple Asp-Pro-Phe motifs, supposed to interact with the endocytic proteins AP-2 and Fcho1/2. They have also been speculated to interact with the EH domains enabling the formation of larger networks of Eps15 dimers that are connected to each other via this interaction. EH domains are involved in intracellular trafficking and cell signaling. They bind with particularly low affinities and specificity to Asn-Pro-Phe motifs, that are present in many endocytic proteins.

To understand the working mechanism of Eps15 in its diverse physiological functions including liquid-liquid phase separation, we analyze its conformational dynamics and binding to its partners by NMR spectroscopy and single molecule Förster resonance energy transfer (smFRET).

P-111 - Biosynthetic incorporation of CF₃-methionine into large protein complexes for ¹⁹F NMR-based analysis of their structure and dynamics

Mr Satoshi Kishigami

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

This research investigates the genetic incorporation of fluorinated chemical groups and the use of fluorine-19 (¹⁹F) NMR techniques to study the structure and dynamics of large biomacromolecules. Despite the widespread use of NMR in studying the chemical structures of biomolecules, detecting the structural changes of large macromolecules in complex biological mixtures remains challenging. The increase in molecular size and structural complexity in a target substrate causes significant signal broadening and spectral crowding. To overcome these challenges, we employed a site-specific trifluoromethyl (CF₃) incorporation technique to produce CF₃-labeled protein oligomers and applied ¹⁹F NMR techniques to study these biomacromolecules. Fluorine nuclei are absent within biological systems, leading to background-free ¹⁹F NMR spectra. In addition, the three chemically equivalent fluorine atoms of CF₃ enhance the signal intensity. We synthesised an unnatural methionine analogue with a CF₃ chemical group in high yield via two different pathways, which allowed us to use the reagent in gram scale for protein labelling. Then, we successfully optimised the expression protocol to produce recombinant proteins labelled with CF₃-methionine in a methionine biosynthesis deficient (auxotrophic) *Escherichia coli* strain and confirmed the incorporation of CF₃ by mass spectrometry and NMR. Using ¹H and ¹⁹F diffusion NMR, we confirmed the self-assembly of the chaperone sHsp16.5 (*Methanococcus jannaschii*) into monodisperse complexes (24 subunits, 396 kDa), which indicates the oligomerisation is unaffected by the labelling with the fluorinated unnatural amino acid. The CF₃ probe allowed us to examine the stability of these complexes by variable temperature NMR. We have also studied the 2.5 MDa Q-beta virus-like particles formed by labelled coat proteins (180 subunits) and detected a very fast and complex dynamic equilibrium using ¹⁹F chemical exchange saturation transfer (CEST) experiments. In conclusion, this study opens the door to background-free biomolecular ¹⁹F NMR methodologies in the analytical probing of high molecular weight proteins.

P-167 - Pulse compensation and signal decomposition for parallel NMR experiments

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

One approach to increasing NMR measurement sample throughput is to implement multiple, independent NMR detection sites. Radiofrequency interference becomes a significant problem in multi-detector NMR systems due to inductive coil coupling together with matching network enhancement, especially among open coil systems without excellent electrical isolation [1]. This issue may cause pulse sequence coupling and signal transfer between multiple detection sites.

Aims

The ultimate objective of this work is to explore methods to handle RF coupling in parallel NMR, i.e., the parallel pulse sequences calibration and parallel signal decomposition.

Methods

The EM simulation was conducted with COMSOL Multi-Physics 6.0 while only considering the coil array with water samples insert. Spin dynamics calculation and single-channel pulse optimization were implemented with the Spinach package [2]. The SOBI method [3] was used to split the simulated and experimental data.

Results

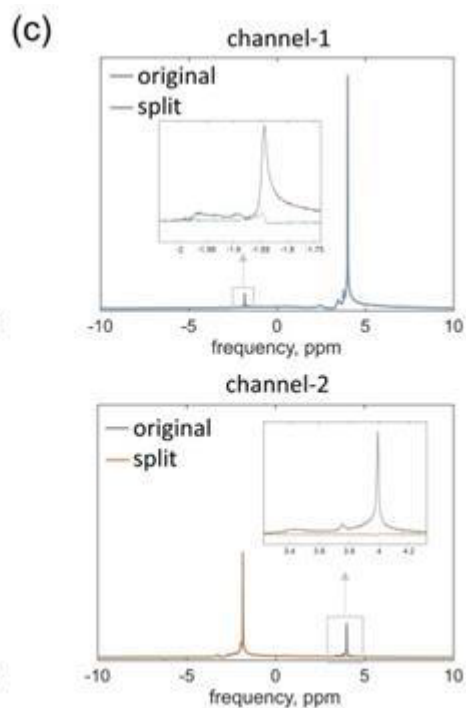
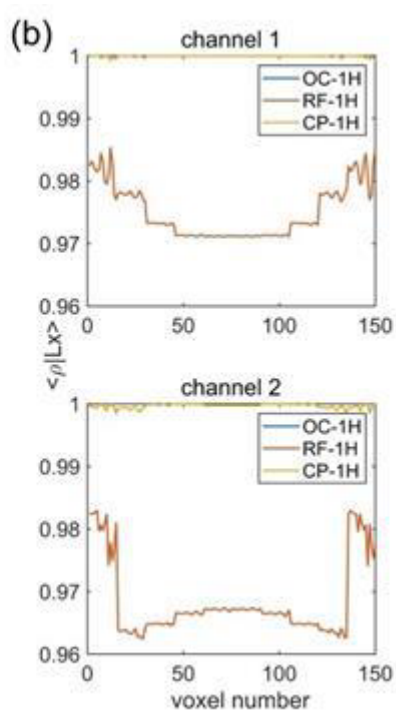
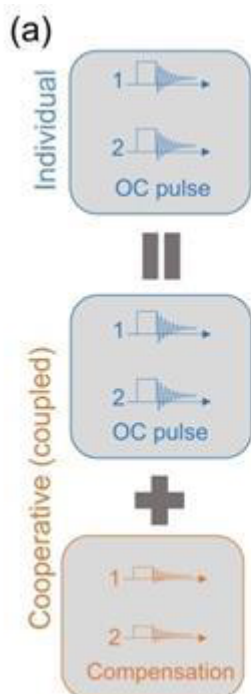
A theoretical framework combining electromagnetic simulation and spin dynamics calculations was developed for parallel NMR simulation, the coupling matrix was used to design cooperative pulse sequences which realize the equipment transfer of individual excitation, and the signal decomposition removed over 90% of coupled components regards the experiment data.

Conclusions

The coupling coefficients extracted from parallel signals benefit signal decomposition as well as parallel pulse sequence calibration. These results will help design parallel NMR probes and explore the parallelization capacity limit in a fixed magnet system.

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P-255 - Improving Li-ion interfacial transport in hybrid solid electrolytes

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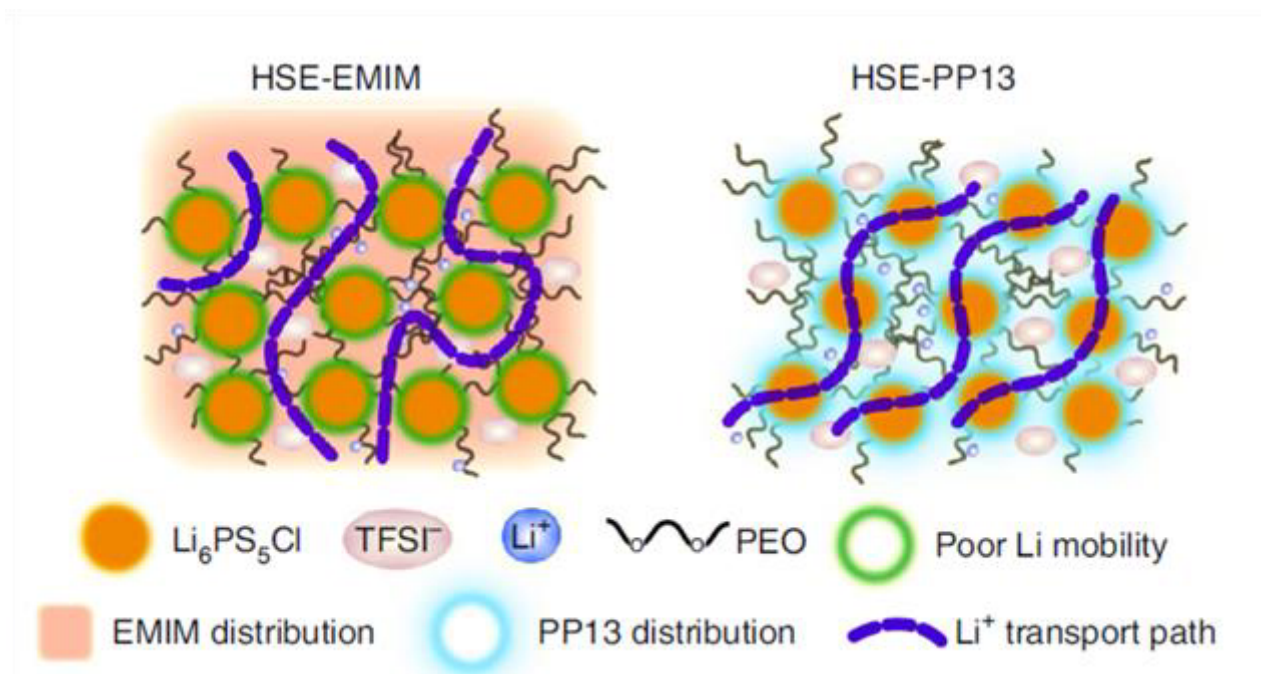
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The development of commercial solid-state batteries has to date been hindered by the individual limitations of inorganic and organic solid electrolytes, which has motivated research into hybrid concepts. Unfortunately, the room-temperature conductivity of hybrid solid electrolytes is still insufficient to support the required battery performance. A key challenge is to assess the Li-ion transport over the inorganic and organic interfaces and relate this to surface chemistry.

Here we study the interphase structure and the Li-ion transport across the interface of hybrid solid electrolytes using solid-state nuclear magnetic resonance spectroscopy. In a hybrid solid polyethylene oxide polymer–inorganic electrolyte, we introduce two representative types of ionic liquid that have different miscibilities with the polymer. Using ⁶Li/⁷Li 2D exchange spectroscopy, ¹H-⁶Li 2D Hetcor experiments and variable contact ¹H-⁷Li CP the samples were analysed and the results correlated with their performance as a solid electrolyte material.

The poorly miscible ionic liquid wets the polymer–inorganic interface and increases the local polarizability. This lowers the diffusional barrier, resulting in an overall room-temperature conductivity of 2.47×10^{-4} S/cm. A critical current density of 0.25 mA/cm² versus a Li-metal anode shows improved stability, allowing cycling of a LiFePO₄–Li-metal solid-state cell at room temperature with a Coulombic efficiency of 99.9%.

Tailoring the local interface between the inorganic and organic solid electrolyte components in hybrid solid electrolytes seems to be a viable route towards designing highly conducting hybrid solid electrolytes.



P-319 - Solvent signal suppression in pure shift NMR

Miss Emma Gates

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The presence of large solvent signals in solution-state NMR degrades spectral quality and hinders spectral analysis. Line broadening due to radiation damping often overlaps neighbouring signals and causes baseline distortion, while the high dynamic range between the intense solvent signal(s) and weak solute signals can make the latter difficult to observe.[1] These problems are often encountered with pharmaceutical formulations, metabolites, and biological samples, which are commonly analysed in non deuterated water.

Solvent suppression in NMR is used routinely in many laboratories. Typically, presaturation methods are employed as these are straightforward, efficient, and readily available for common NMR experiments. However, lengthy continuous-wave irradiation prior to application of the pulse sequence prevents the use of short recovery delays, making the method incompatible with fast pulse experiments. Furthermore, proton exchange between solute and solvent can cause the signals of exchanging nuclei to be attenuated or lost completely. WATERGATE[2] is an attractive alternative solvent suppression technique, as it can be integrated into a pulse sequence, avoiding the need for long recovery delays and limiting attenuation of the signals of exchangeable spins.

Here, WATERGATE is integrated into pure shift NMR experiments, providing ultra-high resolution spectra with negligible solvent signal remaining. The new method is compared to previously published techniques,[3-5] achieving comparable or improved suppression of the solvent signal, with the added advantage of retaining exchangeable signals. The benefits of the new method are demonstrated with peptides and pharmaceutical formulations, including a commercial atropine eye-drop preparation.

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P-099 - Probing the ubiquitin-like modifier FAT10 for non-covalent binding sites to the adaptor protein NUB1L by MAS NMR at 800 MHz

Ms Charlotte Weiss¹, Mr Nicola Catone², Dr Salima Bahri³, Mr Leon Schoewe¹, Dr Venkata Subbarao Redrouth¹, Dr Annette Aichem², Professor Dr Marcus Groettrup^{1,2}, Dr Guinevere Mathies¹

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

FAT10¹ plays an important role in the adaptive and innate immune response in mammals.[1] The protein consists of two ubiquitin-like domains (referred to as N- and C-domains), which both show the typical ubiquitin β -grasp fold. FAT10 gets covalently attached to substrate proteins and beside ubiquitin, it is the only ubiquitin-like modifier that targets substrates for degradation by the 26S proteasome. In presence of the adaptor protein NUB1L², degradation of FAT10 and respective conjugates is accelerated.[2] Via its N-domain, FAT10 can bind non-covalently to the three ubiquitin-associated domains of NUB1L. FAT10 itself is prone to precipitation and for this reason, only the structure of a stabilized, cysteine-free (Cys-free) mutant could be determined so far.[3] Here, we report the first MAS NMR structural study of FAT10. We were able to record highly resolved ¹³C-¹³C DARR and ¹⁵N-¹³C-¹³C ZF TEDOR-DARR correlation spectra at 800 MHz for the microcrystalline, uniformly ¹³C-¹⁵N-labelled Cys-free N-domain of FAT10. Site-specific assignment of backbone and sidechain carbon and nitrogen resonances was possible for about 70 % of the residues. ¹H-¹³C CP and ¹³C-¹³C DARR spectra at 800 MHz of a precipitate of the wild type (WT) N-domain of FAT10 showed poor resolution indicating structural disorder. We are currently preparing a sample containing a NUB1L construct as well as (Cys-free) N-FAT10 for sedimentation into a MAS rotor. Chemical shift perturbations will identify binding sites of (Cys-free) N-FAT10 to NUB1L. These binding sites can be verified in vitro and in cellulo for stabilized and WT FAT10, respectively.

¹ human leukocyte antigen (HLA)-F adjacent transcript 10

² NEDD8 ultimate buster-1 long

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P-115 - Unravelling the secrets of an aptamer-small molecule complex using NMR spectroscopy, the case of the testosterone binding TESS.1 aptamer

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Since their discovery in 1990 [1,2], DNA aptamers have shown to be promising bioreceptors for small molecule detection. Numerous articles have appeared describing newly raised aptamers capable of binding with the desired small molecule target with high affinity and selectivity. These aptamers can then be converted into a biosensor by using a fairly simple and logical design that exploits the conformational change of the aptamer induced by the target. Although a number of these biosensors appear successful, there is still a general lack of knowledge about the underlying molecular events taking place during an aptamer-target interaction. Such knowledge could aid in further optimisation towards real-world applications. We describe our efforts to apply NMR based strategies to this end, using the structure-switching testosterone binding TESS.1 DNA aptamer, developed and extensively characterized by the Stojanovic group, as model system [3].

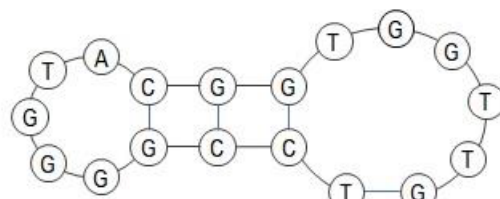
While NMR spectroscopy is a uniquely suited technique to acquire molecular level information about conformational changes and intermolecular interactions, the TESS.1 aptamer, being 51 nucleotides long, did present some challenges when attempting to go towards an assignment. Therefore, the sequence has been truncated and further optimized, generating a more 'NMR optimal' 30-nucleotide long construct, labelled TESS.1_s_mod, which interacts with the testosterone in a similar fashion as the originally sized TESS.1 aptamer. Continuing with this new construct, partial assignments provided a preliminary insight into the conformation of the aptamer and interaction with the testosterone. Finally, analysis of single-nucleotide ¹³C and ¹⁵N labelled sequences allowed a more complete assignment leading to an improved analysis and interpretation of the aptamer-target interaction.

We will present and discuss recent results that provide the first detailed molecular view on this aptamer and its interaction with the testosterone target.

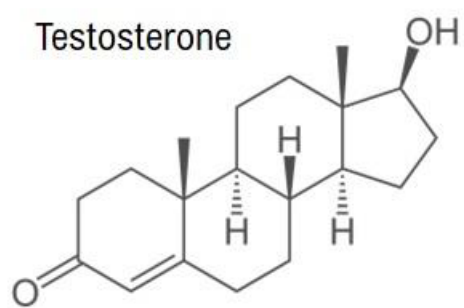
[1] <https://doi.org/10.1126/science.2200121>

[2] <https://doi.org/10.1038/346818a0>

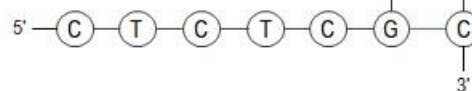
[3] <https://doi.org/10.1021/acscchembio.7b00634>



TESS.1 aptamer



Testosterone



P-273 - Metabolomics of an in vitro model of steatohepatitis using Nuclear magnetic resonance

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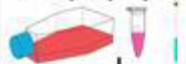
Tracer-based metabolic profiling using isotope-enriched feedstocks for NMR studies enables tracking the fate of important metabolic precursors. The liver plays a vital role in various metabolic processes. Non-alcoholic fatty liver disease (NAFLD) is a prevalent health concern characterized by lipid accumulation in hepatocytes (hepatic steatosis). Investigating the metabolomics of liver steatosis can provide insights into the underlying mechanisms and diagnostic markers for personalized medicine strategies. However, NMR spectroscopy has not yet been applied to such studies, presenting a research opportunity.

The objectives of this study were to determine the altered metabolic pathways associated with hepatic steatosis using NMR in conjunction with different ¹³C-labeled energetic compounds and to optimize a suitable protocol for NMR-based metabolomic studies employing an in vitro model of steatosis.

To induce steatosis, HepaRGTM cells were treated with ¹³C-labeled lactate, pyruvate, and octanoate. Subsequently, a well-defined protocol was followed to extract the intracellular components of HepaRGTM cells. Various samples were prepared and subjected to NMR analysis. The acquired data were then processed and statistically analyzed to identify significant changes in key metabolites and metabolic pathways.

The findings revealed that the progression of steatosis was associated with changes in main lipid classes, including triglycerides containing unsaturated fatty acids, as well as changes in lactate, alanine, and other intermediates within the tricarboxylic acid cycle (TCA). These identified metabolites offer valuable insights into potential therapeutic targets for the treatment of steatosis.. In conclusion, through the utilization of ¹³C-labeled energetic compounds in combination with NMR spectroscopy, this study successfully identified key metabolites closely linked to the development of steatosis. The application of this approach holds great promise for enhancing our understanding of steatosis and may pave the way for the development of targeted therapeutic interventions. Additionally, this research contributes to the advancement of NMR-based metabolomics using an in vitro model of steatosis.

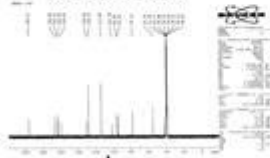
Sample preparation



Data Acquisition



Spectral data

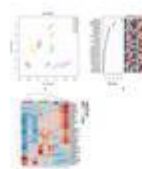


Spectral processing

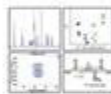


Biological interpretation

Pathway analysis



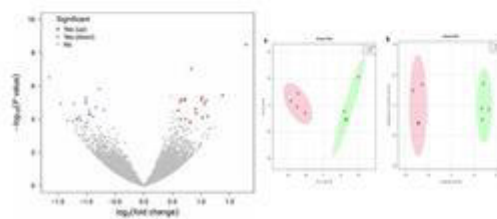
Metabolite identification



hmdb

BMRB
Biological Magnetic Resonance Data Bank

Data analysis



P-125 - Ensemble Based Calculation Of NMR Spin-Spin Couplings In Proteins

Miss Amina Gaffour¹, Mr Micheal Bakker¹, Mrs Jana Pavlikova Přecechtělová¹, Mr Vojtěch Zapletal¹, Mr Krishnendu Bera²

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

-Introduction

Understanding proteins' functions at the molecular level requires the knowledge of their structure characteristics. Computer-aided calculations and predictions of NMR observables, including the spin-spin coupling constants (SSCCs) have significantly helped to interpret the experimental nuclear magnetic resonance (NMR) data as well as validate MD force fields employed in protein structure characterization. Calculations of NMR parameters at the quantum mechanics (QM) level have become increasingly tractable with the availability of fragmentation techniques that automate the construction of molecular clusters representative of protein building blocks. Examples of fragmentation methods include the automated fragmentation quantum/mechanics/molecular mechanics method and the adjustable density matrix assembler (ADMA).

-State of art

Accuracy of both have been previously demonstrated for structured proteins only. This project aims to extend the application of fragmentation techniques to intrinsically disordered proteins (IDPs). For the first time, we show the performance of ADMA in SSCC calculations.

-Methods

The methodology is validated for both the structured proteins GB3 and Ubiquitin, and the disordered protein fragment Tau(210-240). We employ molecular dynamics simulations, Uniform Manifold Approximation and Projection (UMAP) dimensional reduction and K-means clustering techniques along with ADMA and density functional methods for NMR calculations.

-Results/Conclusion

The calculated SSCCs are in a good agreement with the experimental NMR data as well as with estimates made using empirically parametrized Karplus equations.

P-347 - NMR study of micelles formed by aggregation of aqueous surfactant mixtures

Dr Ritu Ghanghas¹, Dr Anne Selent¹, Pau Mayorga Delgado¹, Dr Perttu Lantto¹, Dr Jiri Mares¹,
Professor Ville-veikko Telkki¹

¹University Of Oulu, Oulu, Finland

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Surfactants are substances that lower surface tensions of liquids. At higher concentrations, they create self-assembled molecular clusters called micelles. Application of surfactants include their use in households as well as in industry as detergents, emulsifiers, and food ingredients. Surfactants are also common component of aerosols and can change the surface tension of cloud droplets.

Research on surfactant mixtures is of considerable interest for numerous technical applications, because surfactant mixtures enhance the performance of applications when compared to the use of neat surfactants. When mixing surfactants, one can combine properties that are of interest. Most studies on mixed surfactants have focused on the critical micelle concentration (CMC) of the system in order to discuss the miscibility of components¹. To get more insight into the molecular structure of mixed micelles it is important to have a deeper understanding of surfactants behavior and properties in aqueous solutions. Aggregation of pure surfactants has been widely studied by NMR² but aggregation of mixtures has not been investigated so much. Here, we study the mixed micelle formation in binary surfactant aqueous solutions of sodium decanoate/hexanol and SDS/hexanoate mixtures for different concentration of surfactants. We exploit relaxation and diffusion measurements to obtain insights into the microscopic structure of mixed micelles in surfactant solutions. The CMCs of each individual component in the mixed surfactant solutions were obtained by analyzing changes in relaxation times and by measuring the diffusion constants. Along with the NMR experiments, we have done large-scale atomistic molecular dynamics simulations of the surfactant mixture solutions corresponding to the experimental conditions. These clarify further details of the experimental results.

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P-225 - Showcasing Advanced NMR Approaches to Probe Li-Ion Dynamics in Several Crystal Structures

Mr Benjamin Duff¹, Miss Lucia Corti, Miss Bethan Turner, Dr Jacinthe Gamon, Dr Stuart Elliott, Dr Guopeng Han, Miss Alexandra Morscher, Mr Marco Farina, Dr Matthew S. Dyer, Dr Andrij Vasylenko, Professor Matthew J. Rosseinsky, Professor Frédéric Blanc

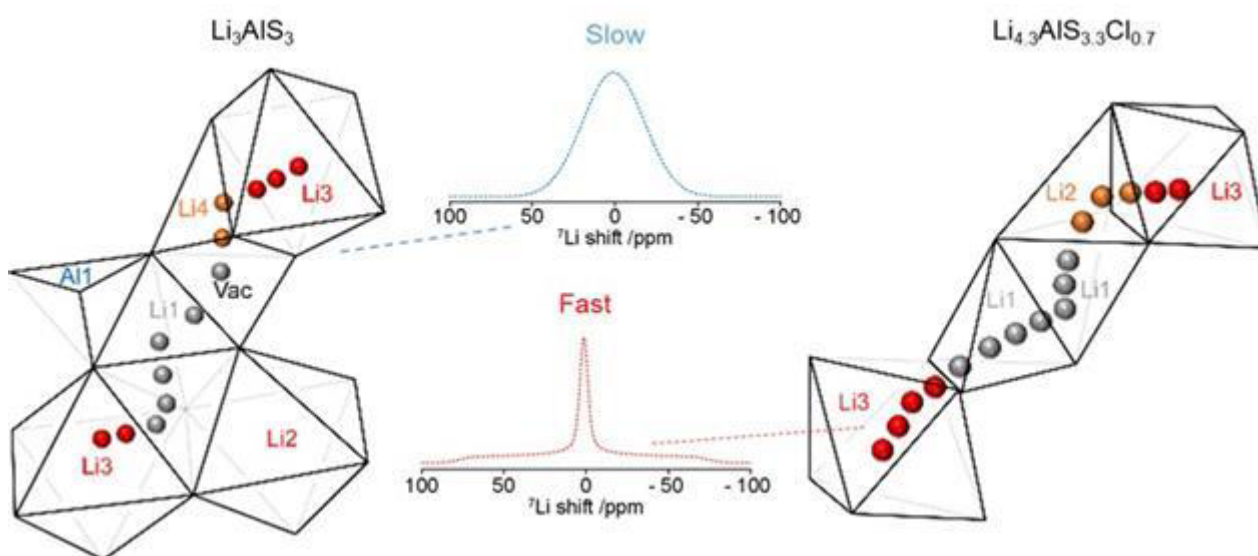
¹University of Liverpool, Liverpool, United Kingdom

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Li-containing materials providing fast ion transport pathways are fundamental in Li solid-state electrolytes (SSEs) and the future of all-solid-state batteries. Solid-state NMR is an invaluable analytical technique in the research of potential SSEs due to the capability of probing both the structures and ion dynamics on the atomic level and across a wide range of timescales that are often inaccessible by other experimental techniques.

An array of solid-state NMR approaches have been utilised in order to probe both the local structures, via 6Li, 27Al, 31P MAS NMR, as well as the Li-ion transport properties of a range of materials via complimentary 6,7Li variable temperature NMR techniques such as 6Li-6Li EXSY, 7Li line narrowing, relaxometry and spin-alignment echo NMR.

Some of our recent work into the structural elucidation of two potential solid electrolytes, Li₃AlS₃ and Li_{4.3}AlS_{3.3}Cl_{0.7} as well as the insights gained into the Li-ion dynamics in these materials, is summarised in Figure 1. A range of variable temperature 6Li and 7Li NMR approaches such as 6Li-6Li EXSYs, 7Li line narrowing and relaxometry were used in order to quantify Li-ion dynamics as well as determine the ion mobility pathways. 7Li NMR line narrowing spectra of Li₃AlS₃ revealed both mobile and immobile Li-ions, while in Li_{4.3}AlS_{3.3}Cl_{0.7}, a single type of fast-moving ion is present and responsible for the higher conductivity of this phase. 6Li-6Li EXSYs of Li₃AlS₃ reveal that the slower moving ions hop between non-equivalent Li positions in different structural layers. The same methodology has also been implemented in some of our previous work, including the lithium ultraphosphate Li₃P₅O₁₄, liquid electrolyte loaded MgMOF₇₄, and the tin sulphide Li_{3.3}Sn_{3.3}Cl_{0.7}. The identification of factors limiting translational Li-ion mobility in materials through the determination of Li-ion correlation times via NMR, provide a framework for the development of more highly conductive Li solid electrolytes.



P-081 - Insights into structural and dynamic properties of mitochondrial tRNA fragments

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Mitochondrial genome codes for 22 different mitochondrial tRNAs (mt-tRNAs) [1]. Both nuclear tRNAs and mt-tRNAs engage in the biogenesis of 10-35 nucleotides long tRNA fragments. It was shown that tRNA fragments participate in crucial cellular processes, such as gene silencing, RNA processing, translation, and epigenetic regulation. Additionally, dysregulation of tRNA fragments has been linked to cancer cell proliferation, invasion, and metastasis, which highlights tRNA fragments' potential to be used as biomarkers and therapeutic targets in cancer treatment [2]. However, mechanisms of mt-tRNA processing into fragments and their influence on other cellular processes remain unknown [3].

mt-tRNAs are structurally "plastic" and notoriously hard to crystalize, which makes X-ray crystallography studies less suitable for structural characterization. Consequently, high-resolution structural and dynamic data are rare [4]. Therefore, high-resolution NMR spectroscopy could provide the solution to this problem, enabling us to structurally and dynamically characterize mt-tRNAs as well as their fragments, which would in turn allow us to identify which structural motifs are necessary for interactions with different maturation enzymes, translation machinery, and other possible targets.

Herein, we characterize the structure of mt-tRNA fragment A2 and its mutant. Analyzing differences in 1D and 2D NMR spectra will reveal the influence of mutation on structural and dynamic characteristics of the mt-tRNA fragment A2. This will allow us to start unraveling the mysteries surrounding mitochondrial tRNA fragments and their role in cellular metabolism as well as in pathology of mitochondrial diseases and cancers.

Acknowledgments: The authors acknowledge the financial support from the Slovenian Research Agency (grants P1-0242 Z1-3192, Z1-3198).

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P-183 - Continuous Non-Destructive ^{13}C Hyperpolarization Monitoring Using an Atomic Magnetometer

Mr Sven Bodenstedt

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

The ability to “hyperpolarize” or furnish nuclear spin systems with large nonequilibrium spin polarization of unity order has enormous potential in applications of magnetic resonance spectroscopy (NMR) and magnetic resonance imaging (MRI). The emerging techniques of ultra-low field NMR provide a valuable toolset to quantify (e.g. the degree of polarization), investigate (e.g. the relaxation mechanisms) and control (e.g. via dynamical decoupling) these systems. In this presentation, I will outline the role of some of the techniques under development in our lab.

Methods & Results

(a) Real-time nuclear spin polarimetry using a zero-field magnetometer[1]: Rapid and continuous quantification of polarization is required for in vivo applications with substances hyperpolarized via dissolution dynamic nuclear polarization (dDNP). Here, we demonstrate direct, real-time and non-destructive quantification of the degree of polarization of $[1-^{13}\text{C}]$ -pyruvate (see Figure 1), a metabolite used in oncology for metabolic imaging, that is hyperpolarized via dDNP. In contrast to current single-time-point polarization measurements, our method does not rely on long calibrations procedures. It can tolerate small ^1H - ^{13}C J-couplings on the order of a few Hz.

(b) Error-tolerant spin control[2,3,4]: The concept of robust composite pulses is and has been used in conventional high-field NMR. In our lab, we have developed methods that translates this concept to the ultra-low to earth’s field regime allowing spin-manipulation without introducing significant losses of polarization or coherence.

Conclusion

Using DC magnetometers instead of inductive detection offers a non-destructive way to investigate hyperpolarized nuclear spins and that has the potential to outweigh state-of-the-art methods.

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P-107 - NMR-based structural and functional characterisation of the SARS-CoV-2 Stem-Loop 1

Mrs Sabrina Töws

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Since the end of 2019, the pandemic outbreak COVID-19 presents a constant aspect of everyday life, making it so important to study SARS-CoV-2 down to its smallest components. For this purpose, we present here our results for the study of the first RNA stem-loop present in the 5'-untranslated region of the virus, 5_SL1, regarding its structural and functional characteristics.

We report the solution structure of this RNA element solved by NMR spectroscopy. The viral stem-loop is characterised by a Y-rich apical loop and a bulge formed by an A-C mismatch and a stacking adenosine, A27. RDC and SAXS data confirm a nearly coaxial stacking of the lower and the upper helical part of 5_SL1. Collecting NMR data at varying pH values shows that in each of the two non-canonical regions, one nucleotide is sensitive to pH: namely A12 in the bulge and C21 in the loop. Such elevated pKa values for nucleotides at specific sites are a frequent phenomenon in regulatory RNA structures, influencing the local structure at these sites, and thus global RNA folding. However, prediction algorithms for protonation events are lacking, and force fields do not routinely include the topologies and potentials for protonated nucleotides. A potential pH-dependent RNA-based regulation of 5_SL1 is under current investigation using additional NMR measurements to complete the analysis of the protonated nucleotides and their population.

Together with the group at Columbia, we used the solution structure of 5_SL1 for the virtual screening of several small fragments. Two of these fragments were confirmed as hits of 5_SL1 by combining NMR-based fragment screenings and affinity estimation as well as NMR binding site mapping. Binding site mapping moreover indicated fragment binding to the bulge region of the viral RNA element. Additionally, a cell-free assay was established to screen the small molecules in a cell-mimicking environment.

P-103 - The Henry Wellcome Building for Biomolecular NMR Spectroscopy (HWB-NMR)

Dr Mark Jeeves^{1,2}, Dr Sara Whittaker¹, Dr John Kirkpatrick^{1,3}, Dr Nick Fine¹, Dr Megha Karanth^{1,2}

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

The Henry Wellcome Building for Biomolecular NMR spectroscopy (HWB-NMR) is a purpose-built facility, located at the University of Birmingham. We welcome UK-based and international researchers with ambitions to discover key breakthroughs in biomedical science to visit and collect high-resolution data using our optimised open access NMR spectrometers (500 - 1000 MHz with a 1.2 GHz spectrometer expected in 2025). Our users are pioneering research in the areas of structural biology, drug design, metabolism and metabolomics. A friendly dedicated team of NMR staff are available to support users needs (for both expert and non-expert) offering comprehensive training, consultation, collaborative expertise and mechanisms to integrate NMR into a structural biology program.

Equipment

HWB-NMR houses cryoprobe equipped 900 MHz and 1.0 GHz spectrometers, offering excellent sensitivity and resolution essential for studies of large proteins, RNA and complex molecular dynamics. Our 800 MHz spectrometer combined with 1.7 mm cryoprobe provides superb high-resolution data collected on samples where amounts are limited, such as cell extracts of metabolites derived from natural cell lines. Three 600 MHz spectrometers operate a range probes including 1.7 mm TCI (for metabolomics and drug discovery samples), 5mm TXO (indispensable for studies of IDPs and for direct observation of insensitive nuclei). Probes for biological solid state at 900 MHz and ¹⁹F detection (concurrent with ¹H decoupling) at 600 MHz will soon be available. Our facility thus caters for data collection of a wide range of samples at several magnetic fields. Magnets are equipped with Bruker SampleJet sample changers enable efficient remote spectrometer operation on multiple samples.

Conclusion

HWB-NMR warmly welcomes users to the facility and encourages scientists to access the state of the art equipment available. Access is generously supported by the Wellcome Trust allowing UK-based researchers free access to the 800 MHz, 900 MHz and GHz spectrometers.

P-267 - NMR Spectra for tracer analysis of metabolism

Dr Mark Jeeves¹, Ms Laura Ferrante², Dr Kashif Rajpoot³, Dr Christian Ludwig⁴

¹Institute of Cancer and Genomic Sciences, University Of Birmingham, Birmingham, United Kingdom,

²School of Computer Sciences, University of Birmingham, Birmingham, United Kingdom, ³University of Birmingham Dubai, Dubai International Academic City, Dubai, United Arab Emirates, ⁴Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, United Kingdom

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Healthy metabolism requires efficient functioning of the metabolic network. Perturbations within one compartment can instigate disease by spreading through and altering the entire metabolic network. Feeding cells with metabolic precursors enriched with low-abundance, stable isotopes, such as ¹³C, enables tracing of metabolic pathways.

Aims

It is crucial to understand the mechanisms by which cells are able to compensate for pathway disruptions and to facilitate the discovery of new drug targets. Through the experimental methods and data analysis strategies outlined here we will develop the next generation of tools to analyse the highly complex metabolism networks of cancer cells.

Methods

We use the information from signal multiplets arising from ¹³C-¹³C in 2D-¹H,¹³C-HSQC spectra scalar couplings to measure the relative incorporation of ¹³C nuclei into metabolites from a specifically labelled tracer molecule.

Results

Previously we developed the combined analysis of MS and NMR spectra (CANMS) and demonstrated the beneficial effects originating from integrating data analysis of these two orthogonal technologies. We have since then developed experimental protocols to optimise sensitivity and speed of data acquisition. To enable non-expert NMR users to be able to analyse such NMR spectra we developed the MetaboLab and MetaboLabPy software packages, which provides automated peak picking and multiplet analysis enabling this technology to be used by those with limited NMR experience.

Conclusions

We have designed NMR methods for collecting data from tracer based studies to complement Mass Spectrometry data and devolved software that performs automated analysis enabling the technologies to be used by the non-NMR specialist.

P-251 - NMR study of liquid eutectic mixtures: structural and thermophysical characterisation

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Deep Eutectic Solvent (DES) is an umbrella term that covers an immense variety of molecular liquids with a growing list of applications. Two specific subgroups of DES are attracting much interest in the pharmaceutical industry: Natural Deep Eutectic Systems (NADES) and Therapeutic Deep Eutectic Systems (THEDES) because they offer a totally biocompatible alternative to enhance drug solubility and bioavailability.¹ However, very little is known about their molecular structure, even though some examples are already marketed formulations such as EMLA (Eutectic Mixture of Local Anaesthetics). Our aim is to explore the full capabilities of NMR to better characterise eutectic mixtures, making use of a range the measurable properties and experimental conditions.²⁻⁴

In this communication we will demonstrate how the combination of several NMR experiments (such as ¹H, ¹³C, NOE, ROE, relaxation, diffusion), complemented with molecular dynamic simulations, can be used to obtain valuable information about the supramolecular structure, intermolecular interactions, molecular behaviour, dynamics, and eutectic properties of THEDES and hydrophobic drugs solved in NADES.

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P-377 - Solid-state NMR study of thermotropic phase transitions in Griebenschmalz

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Lard with crispy pieces of skin was the premier bread spread before being all but replaced by butter in the mid-1900s. The product is known as Griebenschmalz in German, smalec or smarowidło in Polish, and stegefedt in Danish. Global ambitions of energy conservation and resource utilization has led to a revival of lard and skin as raw materials for food. Consumer satisfaction depends not only on the taste, but critically relies on the mouthfeel which is largely determined by the gradual transitions between various solid and liquid fat mesophases during biting, chewing, and swallowing. While an individual batch of lard may comprise triglycerides with acyl chains lengths or degrees of unsaturation yielding suboptimal melting behavior, a product with improved customer appeal could be achieved by blending multiple batches. With the aim of enabling rational blending of lard-based spreads, we here apply a combination of solid-state and liquids NMR methods for detailed characterization of thermotropic phase transitions in commercial-grade lard products and model systems comprising mixtures of triglycerides, polar lipids, and keratin. MAS and high-power ¹H decoupling yields ¹³C spectra with sufficient resolution to identify multiple aliphatic, olefinic, and allylic carbons in the triglyceride acyl chains, as well as many amino acid residues in the collagen. Spectral editing by CP and INEPT, with interpretation of signal intensities within a two-step model of CH-bond reorientation under moderate MAS, allows identification of molecular segments having combinations of correlation times and order parameters that are classified as solid, intermediate, liquid, and liquid crystalline dynamics. MAS cryoprobe technology enables detection of minute amounts of compounds that through the thermodynamic mechanism of freezing point depression may have a decisive influence on the balance between liquid and solid phases. Our results illustrate that multicomponent fat blends extend the temperature interval of melting, yielding superior sensory properties.

P-385 - Using solid-state NMR spectroscopy to investigate the ADOR process

Dr Nicole Kelly¹, Dr Cameron Rice¹, Miss Deborah A Brako-Amofo¹, Professor Russell Morris¹, Professor Sharon Ashbrook¹

¹University Of St Andrews, St Andrews, United Kingdom

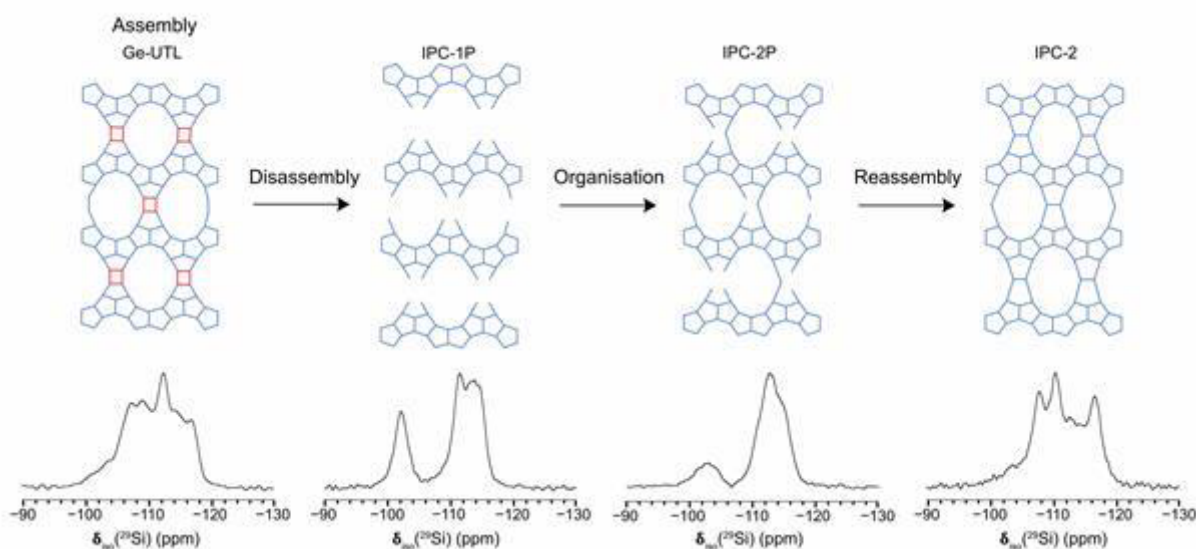
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The ADOR process is an effective way of producing zeolites that would not be feasible through traditional routes. The ADOR process consists of four stages, assembly-disassembly-organization-reassembly.¹ The structure and chemistry of the parent zeolite are an important consideration, with the current focus on zeolites with silica-rich layers linked by germanium-rich cubic units. Germanosilicate zeolites are ideal for ADOR as they have hydrolytically sensitive Ge–O bonds that are preferentially hydrolysed over more stable Si–O bonds.

²⁹Si solid-state MAS NMR spectroscopy has been utilised in previous studies to investigate the ratio of Q⁴/Q³ species (which would be 2.5 and 7 for idealized IPC-1P and IPC-2P, respectively). The Q⁴/Q³ ratio can be used to track the ADOR process both ex-situ and in-situ. ¹⁷O MAS NMR can be used to follow the hydrolysis and rearrangement steps in ADOR,² although the low natural abundance of ¹⁷O requires the use of isotopic enrichment. Due to the high cost of enriched H₂¹⁷O(l), low volumes have to be used in the reaction which affects which IPC intermediates are formed during an ADOR process. Other complementary techniques, such as powder X-ray diffraction and Raman spectroscopy, can assist in understanding the ADOR process.

Here we characterise a model set of 4 ADOR intermediates and products using ²⁹Si MAS NMR and ¹⁷O NMR (using a new cost-efficient enrichment process). The experimental work is supported by periodic DFT calculations, which provide information on how the NMR spectra are affected by the average bond length and angle of the crystal structure. We then use ²⁹Si and ¹⁷O NMR to follow an ADOR reaction to understand how the changes to the local structure that control the process.

1. S.E. Henkelis et al. Nature Protocols, 2019, 14, 781-794
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P-321 - Quantitative band-selective pure shift NMR at no additional cost in experiment time

Mr Howard Foster

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

NMR spectroscopy is often described as a quantitative analytical technique, but typically only the pulse-acquire experiment is intrinsically quantitative. Even then, signal overlap caused by the limited chemical shift dispersion and prominent signal multiplicity in ^1H NMR hinders quantitative analysis. Pure shift NMR techniques[1] suppress signal multiplicity and thus improve signal resolution, but at the cost of introducing site-dependent signal loss.

If each of the pulse sequence elements that cause this signal loss acts independently, then repeating each element a variable number of times before acquisition allows extrapolation back to the loss-free signal integral. This is the principle originally proposed in the HSQC₀ experiment;[2] we tentatively propose the name EXQUISITE (EXtrapolating QUantitative Integrals by Successive ITERation) for its more general application. Our initial application of the EXQUISITE method to the band-selective pure shift NMR experiment[3,4] gave relative signal integrals for model systems within 1 % of those afforded by equivalent pulse-acquire spectra.

In our original implementation of EXQUISITE, the experiment time may be prohibitively long as it is directly proportional to the number of iterations required to perform the extrapolation, each of which must be acquired under quantitative conditions. Here, we investigate a modification that measures each iteration sequentially within a single acquisition, allowing quantitative band-selective pure shift NMR data to be obtained at essentially no additional cost in experiment time over the conventional experiment. We believe that this has the potential to be a significant development in the application of multiple-pulse solution state NMR methods to quantitative analysis.

- 1 Prog. Nucl. Magn. Reson. Spectrosc., 2015, 86–87, 1–20.
- 2 J. Am. Chem. Soc., 2011, 133, 1662–1665.
- 3 Chem. - A Eur. J., 2013, 19, 17283–17286.
- 4 J. Magn. Reson., 2018, 293, 19–27.

P-395 - Exploiting ^{17}O Solid-State NMR Spectroscopy of Catalysts and Porous Solids

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¹University of St Andrews, St Andrews, United Kingdom, ²Johnson Matthey, Reading, United Kingdom

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Zeolites are aluminosilicate frameworks with applications in storage, separation and as industrial catalysts¹. These materials are challenging to characterise, owing to the high levels of disorder present, but NMR spectroscopy provides insight into local structure, disorder and reactivity.

Oxygen is a key linking component of zeolite frameworks, present as Brønsted acid sites and in the water and some of the guest molecules that fill the pores, and provides an alternative insight into zeolites in contrast to ^{27}Al and ^{29}Si NMR spectroscopy. However, ^{17}O has a low natural abundance (0.037%), and isotopic enrichment is usually required to obtain spectra on a reasonable timescale. We have recently shown that cost-effective and energy-efficient ^{17}O enrichment of zeolites can be achieved at room temperature using a “slurry” with $\text{H}_2^{17}\text{O}_{(l)}$ ², although the rate and selectivity of the process varies with the cations (e.g., H^+ , Na^+ , K^+) present, and the timescale of the enrichment can be long (1-100 days).

In this work, we demonstrate a different method for ^{17}O enrichment that is significantly faster than the slurrying approach. We use a joint experimental and computational approach to study ^{17}O enrichment of zeolites with the CHA framework. ^{17}O MAS and MQMAS NMR spectroscopy allows us to determine the rate and selectivity of ^{17}O enrichment, and compare how this varies for zeolites with different cations present, for zeolites prepared in different ways (e.g., using ion exchange) and with the conditions under which the reaction is carried out. This study highlights the complex interaction of zeolites with water and works to understand this interaction to further develop the use of zeolites in industrial processes.

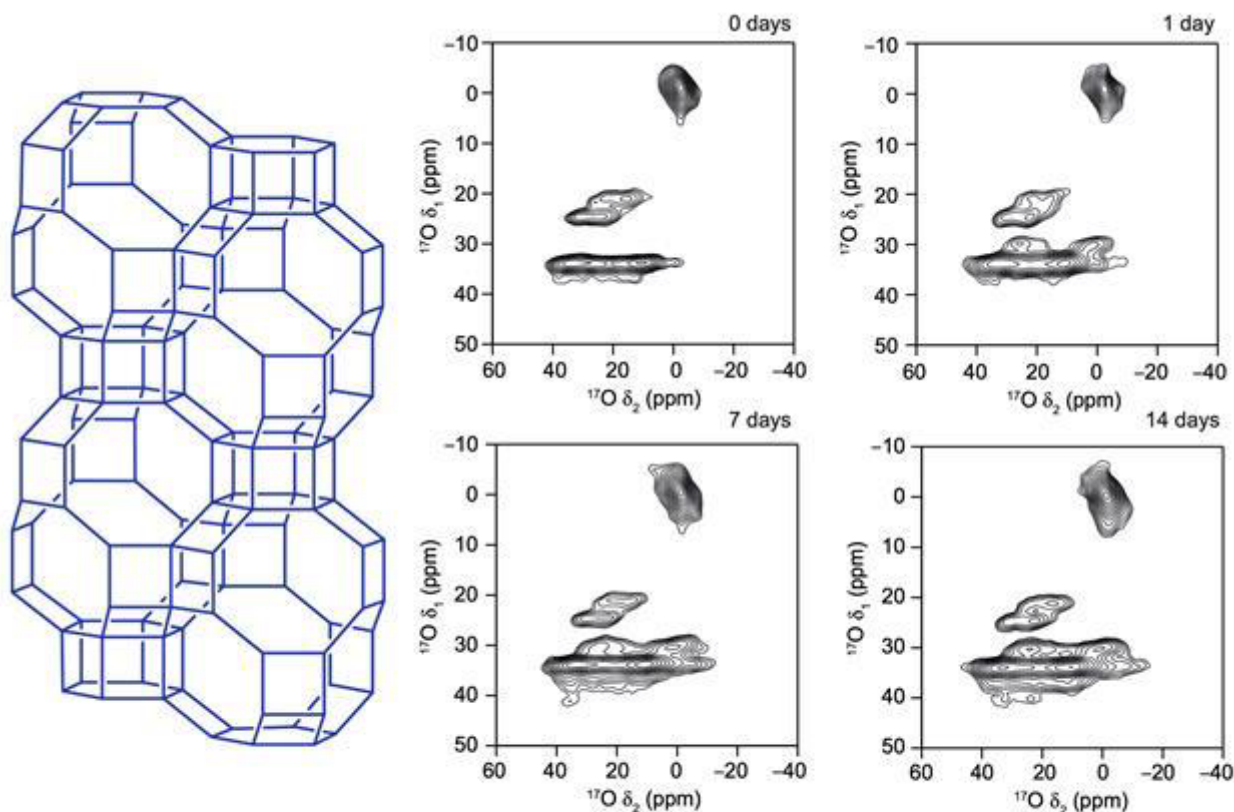


Figure 1 – Structure of SSZ-13 chabazite crystal and ^{17}O (14.1 T) MQMAS spectra taken over a 14 day period for a “slurry” of CHA zeolite and $\text{H}_2^{17}\text{O}_{(l)}$

P-037 - Structural basis of carrier protein specificity directed O-methylation from gladiolin polyketide synthase

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction: Modular polyketide synthases (PKSs) are responsible for the biosynthesis of gladiolin, a novel macrolide antibiotic with promising biological activity against *Mycobacterium tuberculosis* H37Rv. Also mosaic architecture of modular polyketide synthases often referred to as type I PKSs presents an attractive opportunity for biosynthetic engineering. O-methylating submodules have a conserved KS0-OMT-ACP tri-domain architecture with dedicated O-methyltransferase (OMT) domains are ubiquitous in trans-acyltransferase (trans-AT) modular PKSs. Non-elongating condensation incompetent ketosynthase (KS0) mediated transacylation overcomes the inability of certain types of catalytic domain to interact productively with the acyl carrier protein (ACP) domain during the polyketide chain assembly.

Aims: To establish the structural basis for the specific interaction of dedicated OMT domain with ACP domains in O-methylation submodule from gladiolin trans-AT polyketide synthases.

Methods & Significance: In this work, we report that specific protein-protein interactions of ACP domains for OMT domain directs the incorporation of non-elongating KS0 domain within O-methylation submodule. Integrated multi-disciplinary structural biology approach combining the solution NMR assignment based structural models of ACPs, structure predictions of ACPs//OMT protein-protein complexes by open-source AlphaFold2, solution NMR based titrations of ACPs//OMT, MD simulations of Apo and Acyl substrate docked ACP//OMT complexes with carbene footprinting mass spectrometry (MS) and high resolution intact protein MS based functional assay with rationally designed site-directed mutants have afforded the detailed residue level insights of protein-protein interface to elucidate the recognition mechanism between OMT and ACP domains in the trans-AT PKSs that assemble the antibiotic gladiolin. Extensive structural insights gained into binding and catalysis in multi-domain enzymatic trans-AT PKS assembly lines paves the way for future rational reprogramming of O-methylation.

P-223 - Metal-free catalysts for post-consumer PET recycling characterized by solid-state NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Polyethylene terephthalate (PET) is commonly used to produce fibres, films, and bottles due to its water resistance, safety, and widespread availability. As a result, it is one of the largest components of post-consumer plastic waste. Consequently, finding an effective recycling strategy for this polymer is crucial. Glycolysis is one of the most effective methods for PET recycling as it is low-cost and involves mild reaction conditions. In the presence of a catalyst, PET is converted into its bis-(hydroxyethyl) terephthalate (BHET) monomer. BHET can be repolymerized to PET and used to synthesize other fine chemicals. Although several catalysts have been developed for PET depolymerization, they still have shortcomings such as high costs, possible contamination by metals, low yield or selectivity.

This work seeks to investigate the structures of new amine-functionalized supported catalysts achieving up to 70% PET conversion. These SiO₂ supported amines were investigated by a combination of ¹³C and ²⁹Si solid-state NMR. ¹³C CPMAS experiments allow the identification of the main alkyl and aromatic groups on the silica surface confirming the successful grafting of the amine groups onto SiO₂. ²⁹Si experiments show the distinct types of silicon environments confirming that the SiO₂ surface remains intact after successful functionalization. Quantitative ²⁹Si experiments are used to determine the degree of surface functionalization, finding surface coverages between 40-70%. However, no clear correlation between surface coverage and PET conversion was found. These results indicate that the surface chemistry is more complex than previously thought and that the catalytic activity may be the result of an interplay between the different species on the surface. The results demonstrate the potential of catalysts without active metal species for PET glycolysis.

P-301 - A rationally designed glycan hairpin

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

- Introduction

The primary sequence of a biopolymer encodes the essential information for folding, permitting to carry out sophisticated functions. Inspired by natural biopolymers, peptide and nucleic acid sequences have been designed to adopt particular 3-D shapes (i.e. foldamers) and programmed to exert specific functions (A). In contrast, synthetic glycans capable of autonomously folding into defined 3-D conformations have never been explored due to their structural complexity and lack of design rules.

- Aims

Here we present the rational design and synthesis of a glycan adopting a stable secondary structure, challenging the common belief that glycans are not capable of folding due to their flexibility.

- Methods

By combining natural glycan motifs (B), stabilized by a non-conventional hydrogen bond and hydrophobic interactions, we have designed a glycan hairpin, a secondary structure not present in nature (C,D). Automated glycan assembly enabled rapid access to synthetic analogs, including site-specific ¹³C-labelled ones, for NMR conformational analysis.

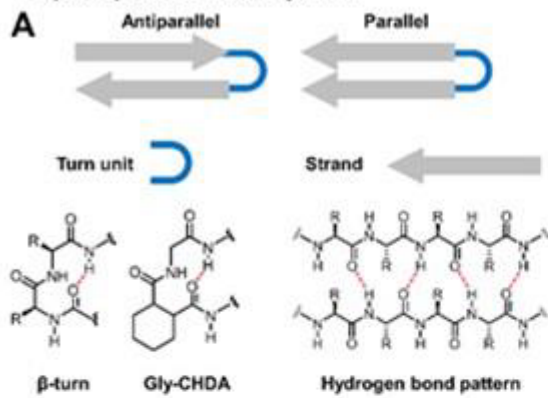
- Results

Long-range inter-residue nuclear Overhauser effects (NOEs) unequivocally confirmed the folded conformation of the synthetic glycan hairpin.

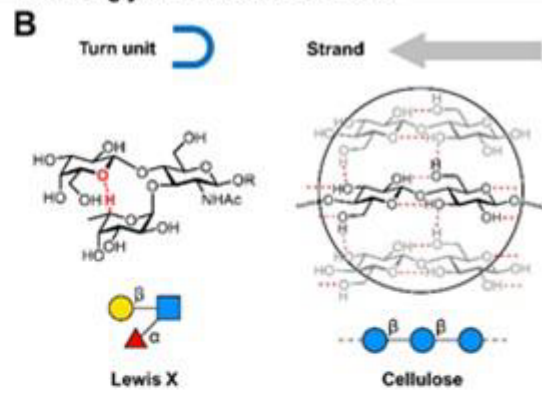
- Conclusions

The vast pool of monosaccharides available, combined with the control over the 3-D shape, has the potential to create a new class of foldamer scaffolds with programmable properties and functions.

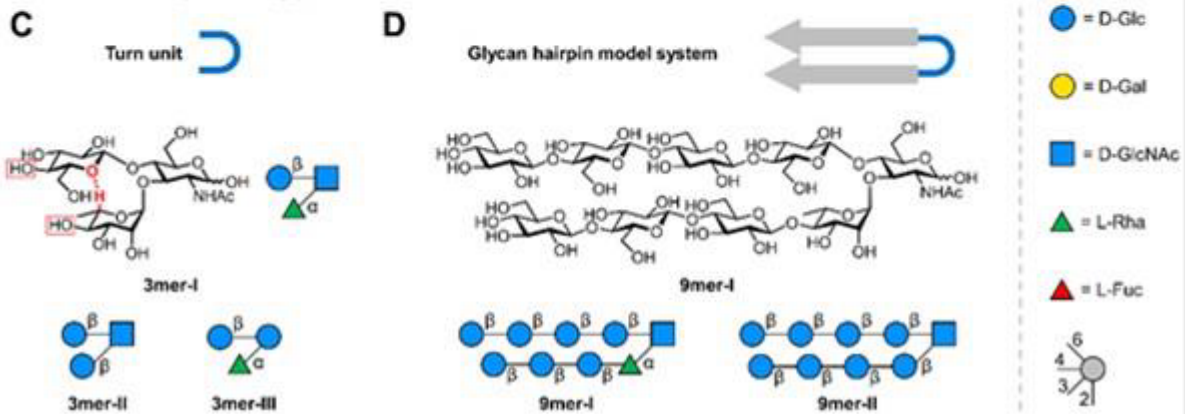
Peptide β -sheet model systems



Natural glycan structural elements



This work: a synthetic glycan hairpin



P-133 - Neural network assisted backbone resonance assignment for large proteins with single HNCA

Mr Uluk Rasulov¹, Dr Scott Robson³, Dr Thibault Viennet², Mr Maxim Droemer², Mr Srdan Matosin², Mr Harrison Wang², Prof Haribabu Arthanari², Professor Ilya Kuprov¹

¹School of Chemistry University Of Southampton , Southampton, United Kingdom, ²Dana-Farber Cancer Institute and Harvard Medical School, Harvard, United States of America, ³Indiana University, Bloomington, United States of America

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The assignment of protein backbone resonances is an essential step in protein NMR spectroscopy. This is often done using sets of triple-resonance NMR experiments. The HNCA sequence provides sequential correlations, but becomes hard to use when chemical shifts overlap.

A new strategy of using 2-¹³C and 3-¹³C pyruvate labelling in bacterial feedstock was recently developed. This isotope labelling strategy suppresses CA-CB J-coupling and yields better resolution on CA. Isotopomer patterns that emerge during biological synthesis of the amino acids from labelled pyruvate also yield distinctive peak shapes. This allows for near complete – but laboriously manual – backbone assignment from a single HNCA experiment. There is a need for automation in the peak shape identification process.

In this communication, we report neural network assisted assignment which integrates with the CCPN framework and reduces assignment times from days to hours. Neural networks were trained on millions of synthetic HNCA signals generated using parameters (shifts, line widths, CB isotope fractions, etc.) drawn from experimentally determined probability distributions. Networks are given the signal itself and its chemical shift location information. For each HNCA signal pair, the networks return probabilities of the signal belonging to each of the 20 amino acids; these probabilities are then combined with prior and posterior assignment information.

With only the HNCA and the primary sequence, the new method achieves the accuracy of over 70% for a 42 kDa protein. This is far greater than using chemical shift predictions alone. If the assignment is done by human eye an assignment of 85% can be achieved but would take weeks to months of processing the data visually

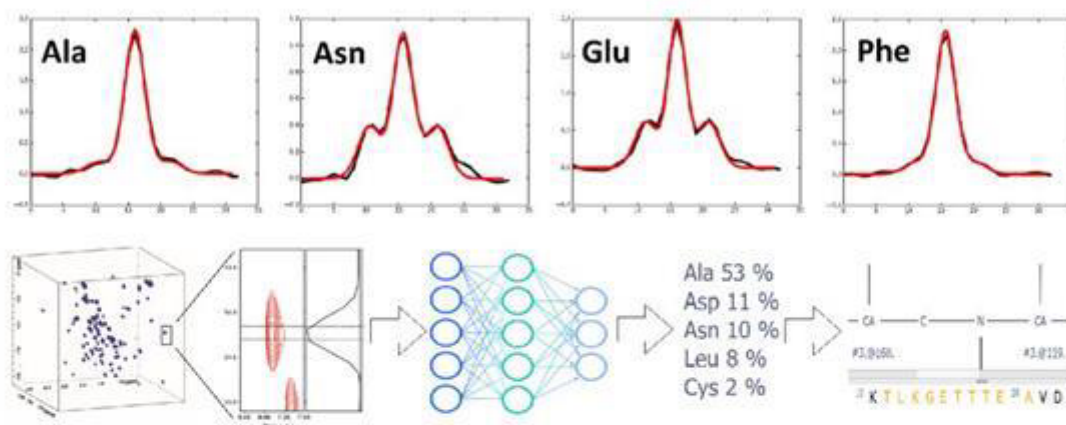


Figure 1. A schematic diagram of the neural network processing flowchart. **Top row:** an illustration of the fact that pyruvate labelling described in yields distinctive multiplets for each of the 20 amino acids (except Gly, which is missing CB). **Bottom row:** J-coupling and CA-CB fraction distributions supplied with chemical shift data are used to generate of synthetic 3D spectra. From the spectra each image of the residue is isolated and supplied with the truth vector containing the probability of the residue belonging to each amino acid type. A deep neural network is trained on millions of these residues. The output of the neural network are the predicted probabilities for the residue image. Inside CCPN standard assignment procedure follows, but the predictions of residues are based on the neural network instead of the internal chemical shift predictions. When sequential assignments are made the correct parts of the chain light up.

P-063 - Nanobody Binding to the Major Birch Pollen Allergen

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

IgE-associated allergies represent a serious and potentially life-threatening health problem for about 30% of the population, with symptoms ranging from a simple itching of the throat to severe anaphylaxis. The most common IgE-related allergy in the northern hemisphere is the birch-pollen allergy. A PR-10 protein that is highly abundant in birch pollen, Bet v 1, is considered to be the primary sensitizer for birch pollinosis and numerous related food allergies. Bet v 1-specific IgE antibodies exhibit immunologic cross-reactivity to PR-10 proteins that are contained in plant food, due to the homologous structure and similar IgE binding epitopes. Recently, clinical trials for passive immunization with allergen-specific IgG antibodies showed success in inhibiting patients' IgE binding to major allergens. However, the generation of monoclonal IgG is cumbersome and expensive, whereas recombinant antibody fragments, e.g. nanobodies, are much easier to obtain. Furthermore, conventional antibodies have been inconvenient to study by solution NMR spectroscopy due to their large size. Nanobodies exhibit the same binding affinity and specificity as their conventional counterparts, while being easier to produce in the laboratory. Additionally, due to their small size thorough investigation of the antigen-antibody complex by NMR spectroscopy is feasible.

Aims

The aims of this work were to gain more insight into the properties of the proposed IgE-binding epitope of PR-10 proteins and to better understand the dynamics of antigen-antibody interactions.

Methods

Recombinant protein expression was used to prepare milligram amounts of a recently described Bet v 1 binding nanobody. We subsequently used a variety of 2D and 3D NMR experiments along with protein isotope-labelling (antigen and antibody) to characterize the interactions between this low-molecular-weight antibody and antigen in detail.

P-331 - Diffusion and magnetization transfer experiments provide an NMR lens into invisible biomolecular condensates

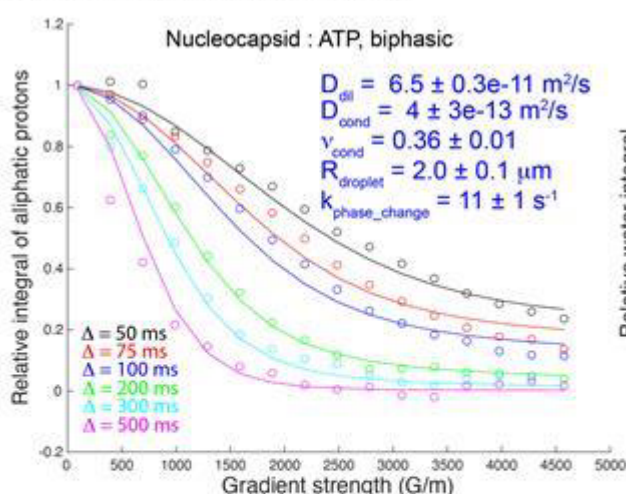
Dr Mihajlo Novakovic¹, Dr Leonidas Emmanouilidis¹, Nina Han¹, Yinan Ni¹, Prof. Dr. Frédéric Allain¹

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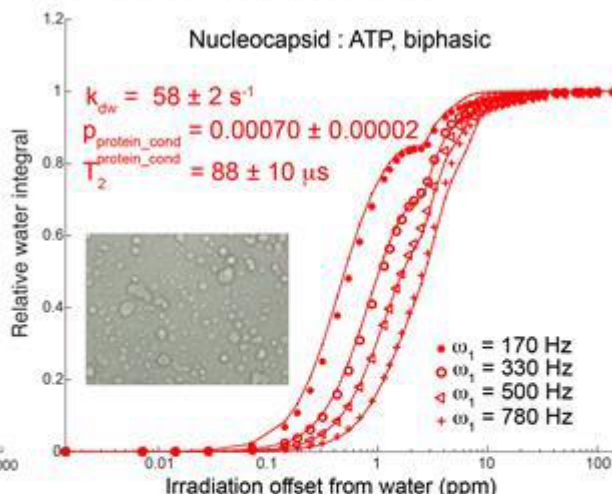
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Liquid-liquid phase separation (LLPS) phenomena play a vital role in multiple cell biology processes, providing a mechanism to locally concentrate proteins and nucleic acids and promote cellular reactions. Proteins containing IDRs and RBDs that can engage in multivalent biomolecular interactions, often induce such biomolecular condensates. Especially intriguing is that upon LLPS, condensates can adopt a continuum of material properties (liquids-gels-amyloids-solids). One of many examples is the SARS-CoV-2 Nucleocapsid protein that undergoes phase separation with RNA, a process critical for virion assembly. However, depending on RNA, Nucleocapsid can form in-vitro droplet-like condensates, but also semisolid gels and irregular assemblies, all awaiting different fates. Despite its significance, many questions about LLPS remain unanswered because most conventional approaches fail when tackling these aspects in the condensed phase. Here, we introduce a new, sensitive, and label-free approach to indirectly probe biphasic condensates relying on NMR diffusion and magnetization transfer (MT) experiments. Dynamic equilibrium between two phases imparts valuable information about invisible condensed phase to the diffusion decays of the NMR-observable protein in dilute phase. The diffusion curves can be fitted to restricted diffusion models with exchange which enabled us to characterize Nucleocapsid:ATP droplets (Figure1A) and extract diffusion coefficients ($D_{\text{Nucleocapsid_condensed}} \approx 4 \times 10^{-13} \text{ m}^2/\text{s}$), volume fraction ($\approx 35\%$), average droplet radius ($\approx 2 \mu\text{m}$) and protein phase transition rate ($\approx 11 \text{ s}^{-1}$) akin to DLS and FRAP techniques. Furthermore, protein in condensed phase creates a sizeable MT effect in water upon long off-resonance irradiation. Fitting the water-detected MT curves to a multiple-pool Bloch-McConnell equations (Figure1B) provides additional relaxation parameters of the Nucleocapsid in droplets ($T_{2\text{condensed}} \approx 88 \mu\text{s}$) and its exposure to bulk water. Acquired in under 90 minutes, these experiments allow us to probe the material properties of the biomolecular condensates and, upon proper stabilization in agarose, to follow their maturation. Full theoretical models and applications with other condensates including FUS and PTB will be presented.

A) Diffusion NMR experiments



B) CEST/MT NMR experiments



A) Diffusion NMR curves acquired on biphasic ATP-bound Nucleocapsid. Diffusion decays for free and dilute phase Nucleocapsid:ATP can be fitted to mono-exponential function and are used as a reference to fit the data for Nucleocapsid condensates using a two-phase restricted diffusion model with exchange. This provides the diffusion coefficient of invisible protein in condensed phase, volume fraction and average radius of the droplets as well as the rate of protein phase change characterizing the droplet permeability. B) While soluble Nucleocapsid in dilute phase produces distinct CEST dip on the water signal, the Nucleocapsid in condensed phase produces a considerable MT effect far off resonance from water. This MT baseline broadenings can be fitted to a Bloch-McConnell model to extract the parameters such as condensed protein fraction, transverse relaxation as well as the chemical exchange to the bulk water describing how liquid-like the condensates are.

P-315 - Redefining Automatic 1D ^1H NMR Analysis: Progress in Scalar Coupling Constant Determination via Deconvolution Techniques

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Traditionally, the analysis of scalar coupling constants in 1D ^1H NMR spectroscopy has been restricted to first-order analysis, with little tolerance to the so-called roof-effect, reliant on the interpretation of previously detected peaks.

Methods

Efforts have been made to transition towards more sophisticated deconvolution techniques with our recent contribution being a novel algorithm designed to handle not only spins greater than 1/2 but also some second-order effects.

Results and conclusions

In this work, we present a series of illustrative examples showcasing the algorithm's capabilities, as well as a comprehensive statistical analysis of its performance against a large dataset consisting of both experimental and simulated data. Furthermore, we highlight the latest advancements and improvements made to this algorithm, marking a significant progression in the field of automated scalar coupling analysis of NMR spectra.

P-053 - A direct interaction between Paxillin's LIM2 and LIM3 domains and beta-integrins mediates focal adhesion targeting and healthy cell adhesion

Dr Heiko Möller¹, Dr. Timo Baade², Dr. Marcus Michaelis¹, Mr Nikolai Klishin¹, Dr. Andreas Prestel³, Dr. Christoph Paone², Laura Scheinost⁴, Dr Ruslan Nediakov¹, Dr. Christof Hauck²

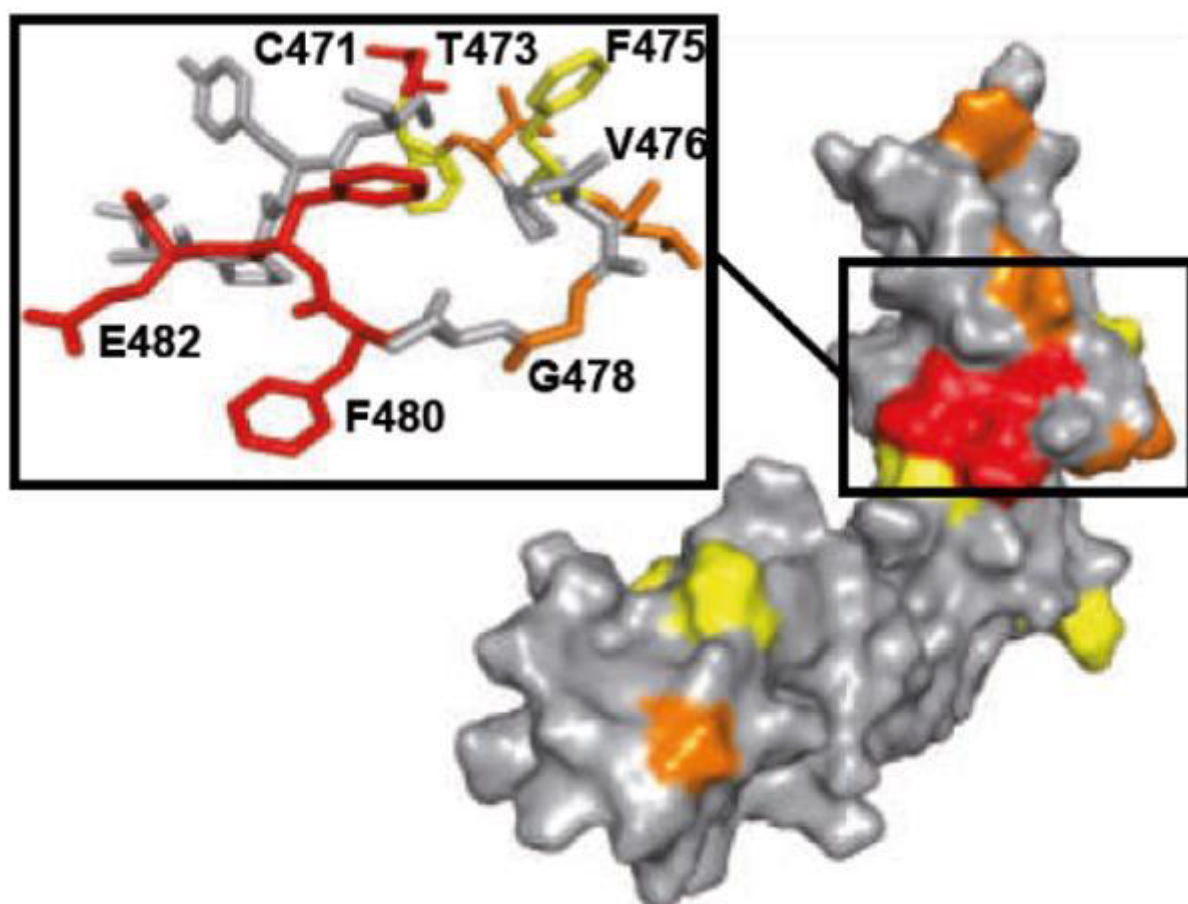
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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Integrin-mediated cell migration is of key importance for fundamental physiological processes like embryonic development, tissue homeostasis or leucocyte trafficking.[1] Integrin clustering triggered by interactions with the extracellular matrix induces the formation of focal adhesions (FAs), complex assemblies of cytoskeleton and scaffold proteins that physically link integrins to the actin cytoskeleton.[2] Paxillin is a major component present in early FAs [3] and, as a transducer of integrin signaling, it is important for embryonic development and linked to cancer progression.[4] By combining NMR spectroscopy, cell biology and fluorescence microscopy techniques, we could recently show that paxillin directly binds to the cytoplasmic tail (ct) of β -integrins. The 3D structure of paxillin's LIM2 and LIM3 domains was determined, and the binding sites involved in its interaction with β 1- and β 3-integrin were characterized. Mutations within Paxillin's LIM domains or truncations of the β -Integrin CTs impair binding and lead to defective cell adhesion. [5] Taken together our results provide a structural basis for focal adhesion targeting of paxillin. Furthermore, our characterization of how paxillin recognizes the cytoplasmic tails of β 1- and β 3-integrin will contribute to understanding integrin activation, which is important for a multitude of physiological and pathophysiological processes.

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P-177 - Towards analytical applications using hyperpolarised ^1H and ^{19}F benchtop NMR

Mrs Ana Silva Terra

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Benchtop NMR spectrometers offer a portable and more economical alternative to high-field instruments ($\geq 7\text{T}$). However, the intrinsic sensitivity limitation of NMR is exacerbated in the lower magnetic fields ($1 - 2.4\text{ T}$) of benchtop NMR spectrometers. An additional challenge is reduced chemical shift dispersion, which leads to peak overlap and limits the identification and isolation of target signals.

The hyperpolarisation technique SABRE (Signal Amplification By Reversible Exchange) can be used to overcome limitations in sensitivity in a relatively cheap and easy way. SABRE harnesses the high spin order of parahydrogen to enhance the detected signal of target molecules through reversible binding to an iridium complex in solution. [1] While the combination of SABRE and benchtop NMR can increase sensitivity by several orders of magnitude, the development of analytical applications, particularly quantification, remains a significant challenge. A promising strategy to reduce limits of detection and promote quantification is the use of a co-substrate to stabilise the SABRE catalysis. [2]

In this work we explore the limits of detection and quantification potential of SABRE-enhanced benchtop (1 T) NMR spectroscopy. In particular, we highlight the benefits of ^{19}F measurements, which are similar in sensitivity to ^1H but less prone to peak overlap due to the broader chemical shift range and minimal background signals. Through the use of dimethylsulfoxide (DMSO) as a co-substrate, the single-scan limit of detection of 3,5-difluoropyridine (DFP) using SABRE-enhanced ^1H and ^{19}F NMR at 1 T was found to be $14\text{ }\mu\text{M}$, an improvement by a factor of ~ 5000 over non-hyperpolarised measurements. Additionally, we report the accurate quantification of a $220\text{ }\mu\text{M}$ test sample of DFP using SABRE-hyperpolarised benchtop (Figure 1).

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[2] N. Eshuis et al., *Angew. Chem., Int. Ed.*, 2015, 127, 1501–1504.

P-333 - Diffusion or exchange - MGSE study of sucrose solution

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Knowledge of molecular translational dynamics and the dynamics of molecular bond formation is instrumental in understanding of multitude of processes in chemistry, biology, or physics. In NMR high temperature studies of liquid self-diffusion rapid molecular motion usually cancels spin dipole-dipole and first-order quadrupole interactions, if not considering their effect on the spin relaxation. However, in liquids the effects of molecular and chemical dynamics causing fluctuations in chemical shift and J-coupling can attenuate echo similar to attenuation caused by diffusion in inhomogeneous magnetic field.

Aims

The aim of the study was to test feasibility of the MGSE method to simultaneously measure the diffusion and chemical exchange spectrum.

Methods

CPMG is a method for the study of correlation fluctuation functions with the use of successive echo attenuation in a train of π RF pulses. Attenuation can be augmented by diffusion in inhomogeneous magnetic field as employed in MGSE method. The echo attenuation factor is a sum of a term proportional to diffusion spectrum multiplied by gradient strength squared and a term proportional to the spectrum of chemical exchange rate at experimentally controlled frequency given as reciprocal of time interval between the π pulses.

Results

Spectra of chemical exchange rate and diffusion of sucrose/water solution were measured by changing the repetition time π pulses and gradient strength. Chemical exchange rate spectrum exhibits previously reported features and the diffusion spectrum could be determined showing features that indicate clustering at low frequencies.

Conclusions

MGSE is suitable for measuring relaxation rate spectra as well as diffusion spectra and can even discern the effect of diffusion in internal gradients from the chemical exchange fluctuations.

P-029 - Role of disorder of proteins regulating bacterial transcription revealed by advanced NMR approaches

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Bacterial RNA polymerase is controlled by species-specific partially disordered proteins, including the subunit delta, unique for Gram-positive Firmicutes, and sigma factors.

Methods

Non-uniform sampling, relaxation and relaxation dispersion analysis, high-resolution relaxometry, computational approaches were applied

Aim

Our aim is to reveal the relationship between structural features, dynamics, and function of disordered proteins regulating bacterial transcription

Results

We investigated of the delta subunit of *Bacillus subtilis* by a quantitative conformational analysis based on NMR and SAXS data [1], revealing importance of a lysine cluster balancing electrostatic repulsion in the acidic C-terminal domain. We verified the role of the lysines by mutagenesis and characterized dynamics of the delta subunit by combining high-field relaxation data with high-resolution relaxometry and MD simulations [2]. We obtained insight into so-far inaccessible time scales of molecular motions and revealed the specific dynamics of the lysine cluster. As the cluster is missing in *Staphylococcus aureus*, we continued with structural and functional characterization of the delta subunits of this important pathogen. In parallel, we studied the role of disorder in vegetative sigma factors of *B. subtilis* and *Mycobacterium smegmatis* differing in the N-terminal domains. In *Mycobacteria*, the N-terminal domain is disordered and the distribution of charged residues resembles delta subunits. Our recent data suggest a novel regulatory role of the charged amino acids. The N-terminal domain of the *B. subtilis* sigma A factor is well ordered at room temperature, but relaxation dispersion revealed a less ordered state dominating at elevated temperatures optimal for the bacterium and facilitating transcription.

Conclusion

The conformational dynamics precisely defined by charge distribution specifically regulates transcription in bacterial genera including serious pathogens.

The work was supported by the Czech Science Foundation, Grant 22-12023S.

[1] Kuban et al., J. Am. Chem. Soc. 2019, 141, 16817-16828.

[2] Salvi et al., Biophys. J. 2022, 121, 3785-3794.

P-209 - Overhauser DNP Solvent Dynamics Measurements of Binary Mixtures

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Overhauser dynamic nuclear polarization (ODNP) is a technique capable of enhancing the nuclear spin polarization by on-resonance microwave irradiation of a paramagnetic polarizing agent. This technique requires the hyperfine coupling between an electron and nuclear spin to be modulated at frequencies close to the electron spin Larmor frequency. By measuring the enhancement of the NMR signal, one can probe the molecular dynamics at frequencies relevant to the rotational and translational diffusion of molecules.

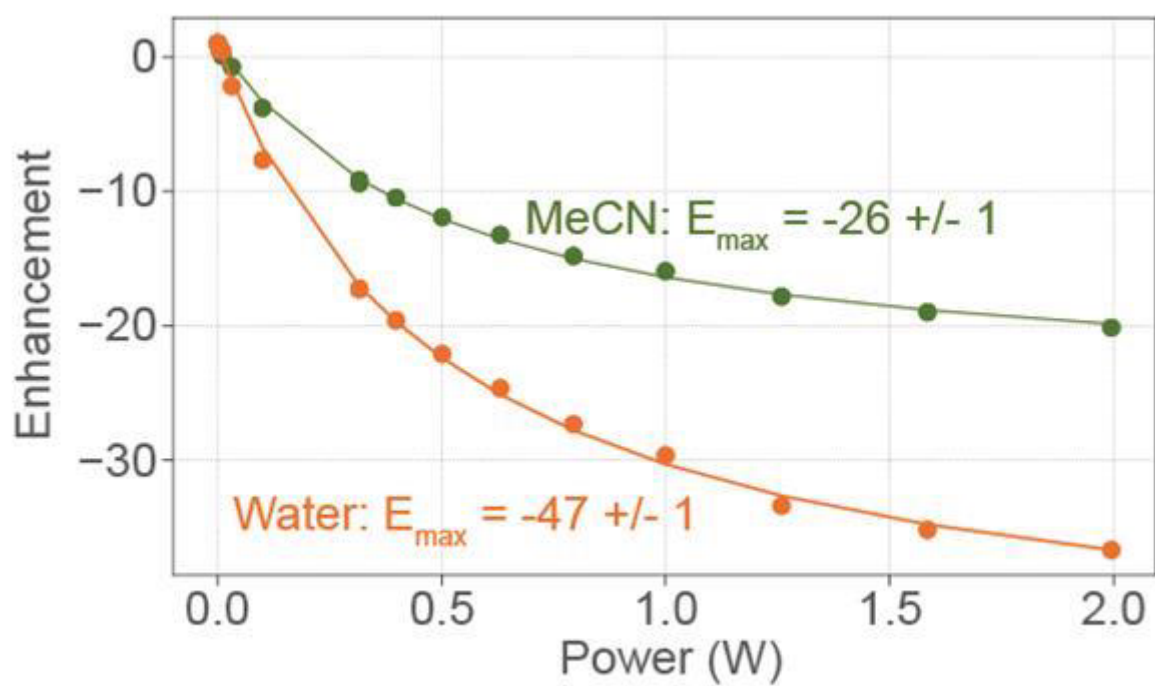
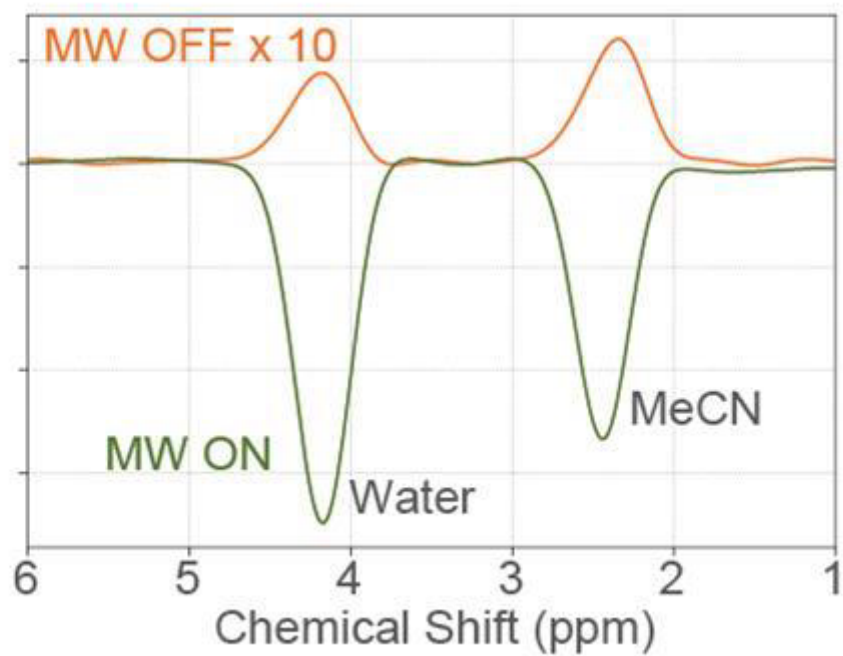
Recent instrumentation developments have enabled high-resolution ODNP measurements at low magnetic field. [1,2] At a magnetic field of 0.35 T (15 MHz ¹H), we achieve linewidths of < 2.3 Hz (0.16 ppm) for a water sample with 200 μ M TEMPOL. The improvements in resolution introduce the possibility of performing site specific solvent dynamics measurements on mixtures to extract a more detailed understanding of molecular dynamics in these mixtures.

As a model system, we use binary mixtures of water and acetonitrile. It has long been known that acetonitrile acts as a water structure “enhancer” that promotes water hydrogen bonds. [3] Although water and acetonitrile are completely miscible on a macroscopic level, at the molecular level water prefers to hydrogen bond with water molecules. In Fig. 1, we show the enhancement of water and acetonitrile. Water consistently exhibits larger enhancements. We find that the molecular dynamics of water becomes slower as the amount of acetonitrile is increased. This is contrary to the bulk viscosity which tends to decrease with increasing acetonitrile content. Our results are interpreted as acetonitrile acting as a water structure enhancer.

[1] T.J. Keller, et al. J. Magn. Reson. 313 (2020): 106719.

[2] T.J. Keller, et al. Magn. Reson. 2 (2021): 117–28.

[3] H. Kovacs, et al. J. Am. Chem. Soc. 113 (July 1991): 5596–5605.



P-293 - When does Computer-Assisted Structure Elucidation (CASE) spare you from recording (IN)ADEQUATE?

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The INADEQUATE and 1,1-ADEQUATE experiments were described in the literature in 1980 and 1996 respectively, and have long been considered as the “holy grail” experiments for the elucidation of structures of organic compounds using NMR.

During the same period, computer-assisted structure elucidation (CASE) has evolved quite significantly allowing scientists to elucidate large and complex structures using NMR data. Modern CASE systems are extremely efficient and can generate millions of isomeric structures within minutes. After the structures are generated, they are ranked, usually based on the agreement between the predicted ¹³C chemical shifts and those observed experimentally.

In this poster, we investigate how relevant experiments like INADEQUATE and ADEQUATE truly are, given the existence of powerful tools like CASE. To do this, we analysed a series of published examples in which (IN)ADEQUATE information was stated as being vitally necessary for unambiguous structure elucidation. We looked to determine whether using HMBC and COSY data within a CASE system could elucidate the structure(s) in a reasonable amount of time without using (IN)ADEQUATE spectra. We found that using only HMBC and COSY allowed us to get the correct solution in a reasonable time without utilizing time-consuming experiments in a series of examples. However, we also found that in most cases of large hydrogen deficient molecules, structure elucidation requires (IN)ADEQUATE spectra, and CASE only facilitates the structure elucidation.

We will be presenting different examples to illustrate the observations. Moreover, we will be showing examples where even though the correct structure has been generated, it was not possible to clearly identify it as the correct structure because others existed that were ranked similarly. We will be discussing methods to resolve these ambiguities and get the correct result without necessarily involving low-sensitivity experiments.

P-145 - An EPR Point of View on Structural Defects in Mo-based MXenes

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

MXenes is a recently discovered family of two-dimensional (2D) early transition metal carbides, nitrides and carbonitrides. MXenes combine strong covalent bonds and metallic conductivity with the hydrophilicity of their surfaces (owing to O, OH and F surface terminating groups, denoted Tx), resulting in high activity in electro- and thermal catalysis.

Here, we report structural characterization of Mo-based MXenes, Mo₂CTx:M, where M is a transition metal such as Co or Fe. EPR studies reveal that while Co and Fe substitute Mo positions and remain in the lattice of Mo₂CTx:M, for M = Cu, leaching and deposition of Cu on the surface is observed.

Mo₂CTx:M materials were characterized by XRD, XAS (Mo- and dopant edges), XPS, solid-state NMR, among other methods. However, unambiguous identification of the local environment remains challenging, especially for M = Cu. We used EPR to study the paramagnetic centers in Mo₂CTx:M and uncover thereby their local structures.

CW and pulsed EPR techniques (HYSCORE and ENDOR) were used. The experimental results were rationalized using crystal field theory and validated by DFT calculations.

Mo(IV) and Mo(V) sites were identified in the undoped Mo₂CTx. Mo(V) centers feature local geometry distinct from Mo sites in the Mo₂CTx model, consistent with distortion of sites through rotation of Tx groups. Aided by the crystal field theory arguments, analysis of EPR spectra of Mo₂CTx:M allowed to distinguish materials with dopant substituting Mo in the lattice (Fe, Co) from the surface-coordinated sites (Cu).

This study shows the potential of EPR to provide detailed element-specific information about the local structure of paramagnetic sites in MXenes, their location (lattice or on-surface) and identify defects and distortions in these emerging 2D structures.

P-221 - ^{207}Pb chemical shift of CsPbX_3 over the thermal and compositional range

Mr Sebastian Sabisch

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Semiconductive cesium lead halide perovskites (CsPbX_3) have emerged as promising materials for their compelling electronic and optical characteristics. While recent efforts mostly focused on improving the stability and efficiency of perovskite-based devices, the relationship between structural and optoelectronic properties remains poorly understood. In this study, we employ a combined approach of ^{207}Pb ssNMR, X-ray diffraction methods, and DFT-computational modeling to link the structural parameters to key optical properties. This study focuses on the anomalous behavior of the bandgap with changes in temperature which increases upon heating and the tunability of the bandgap with the halide composition. Both parameters are reflected in the ^{207}Pb chemical shift and $^1\text{J}(\text{Pb-X})$ coupling. Variable temperature ssNMR experiments between 100 K and room temperature show a linear correlation of the bandgap and the ^{207}Pb chemical shift and a close relation of the $^1\text{J}(\text{Pb-X})$ coupling constant to the octahedral tilting in the crystal structure. Static CPMG was employed to acquire the inhomogeneously broadened spectra of mixed halide perovskites obtained from Brigman growth. The local environments and compositions of the materials could be obtained from these broad spectra over a wide thermal and compositional range. From these experiments, we can conclude that while the spectroscopic changes with regard to composition are dominated by the altered bandgap, the temperature correlation of the ^{207}Pb chemical shift is a result of altered Pb-X orbital overlap. This is supported by the reduced $^1\text{J}(\text{Pb-X})$ coupling when heating, as well as by DFT calculations on structures obtained from variable temperature single crystal X-ray diffraction. This study highlights the combined application of NMR spectroscopy, X-ray diffraction, and computational methods for local structure determination of complex systems and their effect on tunable material properties. The obtained information will help further improve the control over the optoelectronic properties of this next generation of semiconductors.

P-261 - CcpNmr Metabolomics Database

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Metabolomics is the system-wide study of all the metabolites (small molecules with < 1.5KDa) in a biological sample. Nuclear Magnetic Resonance (NMR) based metabolomics experiments generate dense spectra consisting of hundreds to thousands of peaks that collectively describe the sample. In order to ascertain biologically relevant results from these spectra, peaks must be annotated and assigned to the metabolite/s they represent. As such, proper annotation of NMR spectra requires high quality reference spectra from previously measured pure metabolite standards.

Current publicly available databases for NMR metabolomics standards are built around extensive metabolite collections but do not typically provide a broad range of conditions for each metabolite and are not designed for spectrum manipulation to account for sample condition differences. This can dramatically limit reference value as metabolite NMR spectra will vary depending on sample conditions.

We examined experimentally supported metabolite entries from the Human Metabolome Data Base (HMDB), Biological Magnetic Resonance data Bank (BMRB) and Guided Ideographic Spin System Model Optimization (GISSMO) libraries. Experimental spectra were compared to simulated spectra from annotation data, i.e. peak-lists, and automatically assessed for similarity, accounting for moderate global misalignment and solvent/reference artefact interference. Simulated spectra with a similarity score of < 0.9 were visually inspected and modified where appropriate.

Our analysis revealed issues across all three databases, most commonly missing files and peak-list imprecision but also including improbable peak-widths, missing peaks, low-quality of the experimental data, and interference from experimental artefacts. I will report on an integrated, remediated and freely available database of annotation data from the three public databases as a primary source.

P-033 - Dynamics of the Alzheimer's disease-related intrinsically disordered protein Tau modulated by its liquid-liquid phase separation

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Intrinsically disordered proteins (IDPs) are abundant in the human proteome and also are often found in the biomolecular condensates that form through liquid-liquid phase separation. IDPs are highly dynamic molecules, and their conformational plasticity is a key element of mechanisms describing interactions with their partners. Inside biomolecular condensates, increased viscosity and the presence of intermolecular contacts are expected to modulate the dynamics of IDPs and their interactions with other molecules(1). The 441-residue human Tau protein (htau40) is involved in the formation and stabilization of microtubules in neurons. Phase separation of htau40 is believed to be important part of its functioning. Its malfunction and aggregation are linked to multiple neurodegenerative diseases(2). We used ¹⁵N NMR relaxation to study conformational dynamics of the htau40 in the picosecond-to-nanosecond timescale range both in monomeric and in the condensed phase. We observe an overall slowing down of htau40 dynamics in the condensate on multiple timescales. However, the changes in htau40 dynamics across the sequence and at different timescales can only in part be explained by increased condensate viscosity. Remaining changes in dynamics observed in the condensate are likely to be associated with intermolecular contacts and possible conformational rearrangement of htau40. Based on obtained results, we propose the possible nature of these contacts and the timescales of their formation. In the end, we discuss how relevant could be the observed htau40 dynamics in the condensate to its functional and aggregation properties.

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(2) Rai, S. K., Savastano, A., Singh, P., Mukhopadhyay, S., & Zweckstetter, M. (2021) Liquid- liquid phase separation of tau: From molecular biophysics to physiology and disease. *Protein Sci.*, 30, 7, 1294–1314

P-237 - Local structure and dynamics in emerging tellurium-based optoelectronic materials from high-temperature ^{125}Te MAS NMR at 20 T

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Halotellurates represent an emerging class of materials that hold great promise for advancing optoelectronics. While their fascinating chemistry can be studied using diffraction techniques, the complementary picture of their local structure has been missing.

Aims

We set out to develop ^{125}Te MAS NMR of A_2TeX_6 compounds, where A is Cs, MA, and FA, and X is I, Br, and Cl. In addition to exploring the chemical shift range, we were curious to see what microscopic mechanisms drive ^{125}Te relaxation and how they could be used to study dynamic processes.

Methods

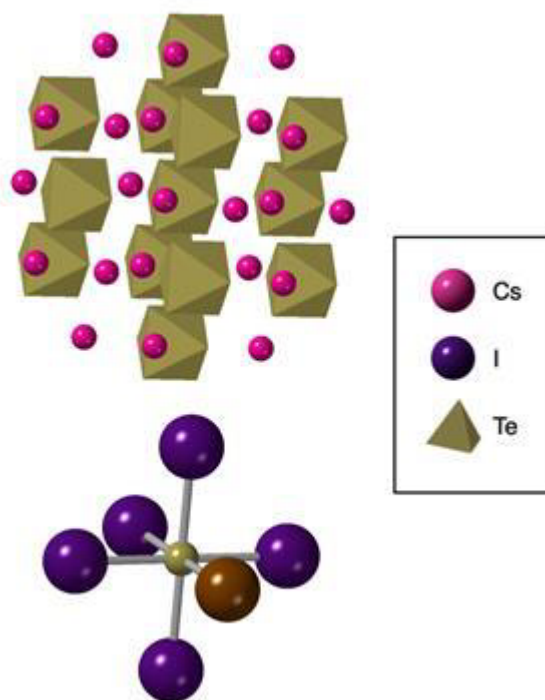
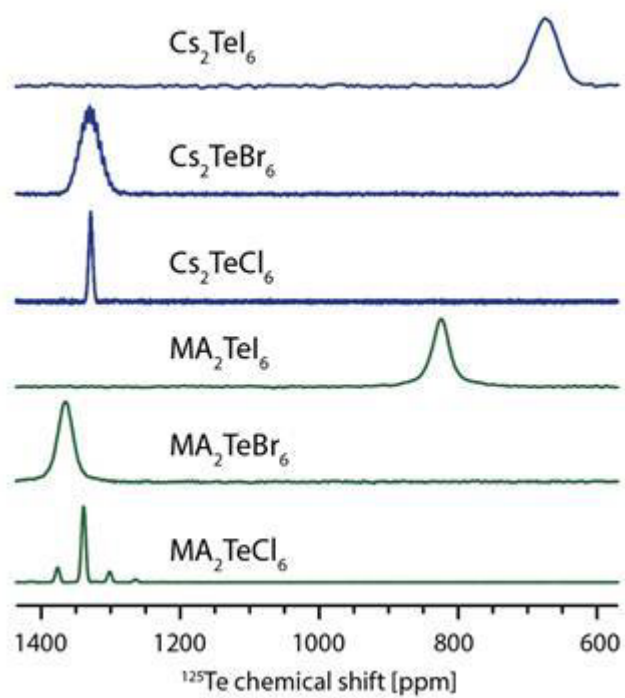
We used materials synthesized in aqueous solutions as polycrystalline powders and studied their ^{125}Te NMR under MAS at 20 T between room temperature and 300°C. We also explored ^{127}Te NMR.

Results

We discovered that ^{125}Te NMR chemical shifts and T_1 relaxation in halotellurates are highly sensitive to the type of halide and temperature. By considering various contributions to T_1 relaxation (dipolar, CSA, Raman, scalar, MAS-induced heteronuclear polarization exchange), we were able to conclude that in chlorides, T_1 relaxation is driven by phonons, while in bromides and iodides, it is driven by halide diffusion. Therefore, it can be used to determine the corresponding halide diffusion activation energies, which are on the order of 40-80 kJ/mol, depending on the composition. Using high-temperature MAS experiments, we were able to study halide mixing in situ with excellent spectral resolution and found that it occurs on the timescale of seconds to minutes at elevated temperatures. In one material, we observed fascinating interplay between ^{125}Te - $^{35/37}\text{Cl}$ self-decoupling and phonon softening as a phase transition is approached.

Conclusions

^{125}Te NMR is highly sensitive to the halide composition and provides a unique insight into tellurium coordination environments. By studying ^{125}Te NMR relaxation and its underlying physics, we were able to quantify halide diffusion and determine its activation energy.



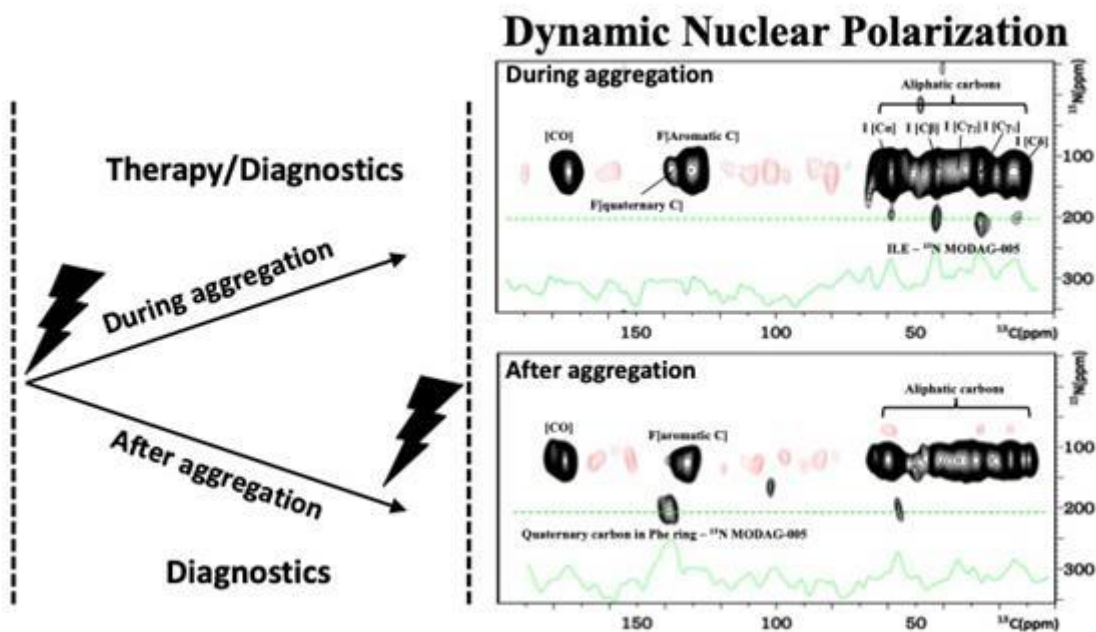
P-307 - Exploring binding site of MODAG-005 on α -Synuclein aggregates as a novel PET tracer

Mr Myeongkyu Kim¹, Mr Benedikt Frieg², Mr Christian Dienemann¹, Mr Sergey Ryazanov¹, Mr Andrei Leonov¹, Mr Armin Giese³, Dr. Loren Andreas¹, Prof. Dr. Christian Griesinger¹

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Neurodegenerative diseases such as Parkinson's disease (PD) are manifested by deposition of misfolded α -synuclein (α SYN) aggregates in human brain. Early detection of these aggregates and observing the pathological process has been a challenge. Positron emission tomography (PET) is a non-invasive in vivo imaging technique useful for early diagnosis of aggregates in the human brain. However, a target-specific tracer to detect pathological aggregates of α SYN is missing. Here, we report the development of MODAG-005 (PET tracer) based on MODAG-001 (2), which in turn is a derivative of anle138b, a compound shown to have therapeutic activity in animal models of neurodegenerative diseases. MODAG-005 interacts with α SYN aggregates grown in the presence of phospholipid, a protocol that was developed for anle138b (1). The structure of the fibril was determined by cryo-EM (3) and the binding sites (internal and external) of MODAG-005 were identified through solid-state nuclear magnetic resonance (NMR) spectroscopy, specifically NHHC experiments (4) combined with dynamic nuclear polarization (DNP), a technique that allowed the determination of the binding site of anle138b in fibrils (5). Interestingly, two binding sites are found depending on the preparation protocol. The internal binding site is similar to the one found for anle138b (5). The external one had not been detected previously.



P-295 - ^{19}F transverse nuclear spin-relaxation reveals small-molecule binding to intrinsically disordered proteins

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Intrinsically disordered proteins are highly dynamic biomolecules that rapidly interconvert between many structural conformations. Traditionally, these proteins have been considered 'undruggable' because of their lack of classical long-lived binding pockets for small-molecule drugs. Recent evidence suggests that intrinsically disordered proteins can indeed interact with small, drug-like molecules, however, there are limited approaches to characterize these interactions experimentally.

Aims

The aim of this work was to establish experimental NMR-based techniques to quantitatively detect and characterize small-molecule interactions with disordered proteins.

Methods

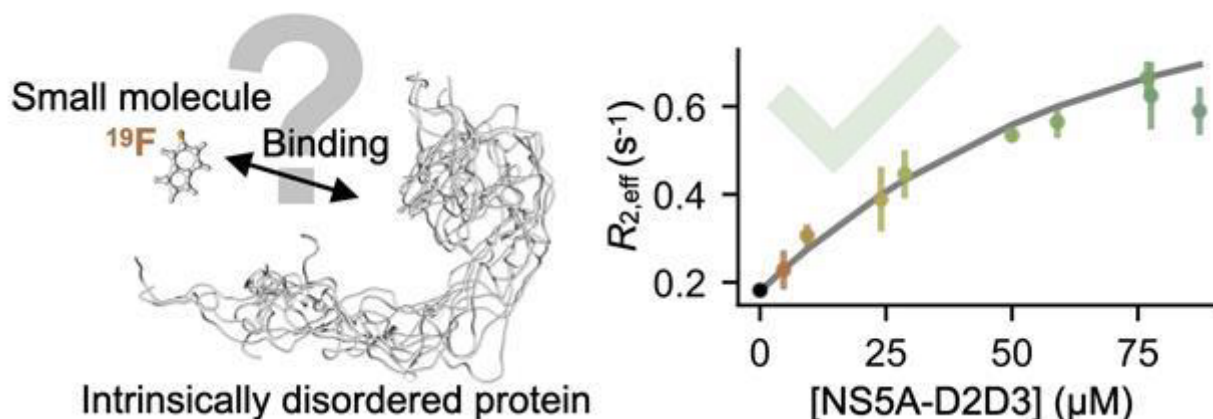
We investigated several solution-state NMR observables for their ability to report on interactions between small molecules and IDPs, including protein- and ligand-detected chemical shifts, translational diffusion, and longitudinal and transverse spin-relaxation rates. As a model system for a disordered protein, we employed the disordered domains 2 and 3 (NS5A-D2D3) from the non-structural protein 5A from hepatitis C virus.

Results

We demonstrate that small-molecule ligand-detected ^{19}F transverse relaxation (R_2) rates are highly sensitive to the interaction of small-molecules with disordered proteins. Notably, for the scenarios that we investigated, both ligand- and protein-chemical shifts as well as translational diffusion measurements were relatively insensitive to binding. Using these approaches, and primarily ^{19}F R_2 rates, we discovered that 5-fluoroindole interacts with a micromolar affinity with NS5A-D2D3 (380 μM). We also show that 5-fluoroindole remains highly dynamic (free-like rotational diffusion time) while interacting with the disordered protein.

Conclusions

Given the high prevalence of disordered proteins in many diseases including cancer, cardiovascular disease, diabetes, and neurodegeneration, we anticipate that ^{19}F ligand-detected spin-relaxation measurements offer a promising medium-throughput screening strategy to identify small molecules that bind these dynamic biomolecules.



P-151 - Concentration and Loading Dependencies of Copper Coordination Compounds Studied with Magnetic Resonance in Solution and Solid

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Copper coordination compounds exhibit a wide range of coordination motifs. Consequently, a single ligand can be found coordinating in different manners to copper, depending on subtle changes in the experimental conditions.¹ The heterogenization of the molecular coordination compounds in metal-organic frameworks (MOFs) allows to spatially separate ligands, preventing e.g. dimerization.² Selective techniques were employed to characterize the local copper site.

Aims

NMR and EPR complete each other to probe the concentration- and temperature-dependent aggregation (molecular complexes) and the loading dependencies (solid materials) of Cu(I) and Cu(II) materials.

Results

Molecular copper complexes with ligands bearing N-heterocycles were synthesized. Their aggregation behaviour was studied with a combination of ¹H VT-NMR (Figure 1, top) and DOSY. ¹⁵N NMR coordination shifts were determined for the molecular compounds. In one case, a set of shifts was obtained for both monomer and dimer, highlighting the changes in the coordination environment upon dimerization.³

These compounds were subsequently incorporated into a MOF. The synthetic heterogenization route affected the loading scalability of the synthesis. The comparison of the CW X-band EPR spectra (Figure 1, bottom) of two different copper(II)-functionalised Zr MOFs (isorecticular frameworks, different ligands, and therefore different incorporation approaches) revealed that while missing linker defect-coordinating ligands showed a change in the nature of the copper moiety with the ligand loading, the derivatised linker system displayed no such effect.

Conclusions

The aggregation behaviour of nitrogen-ligated copper(I) complexes was studied through a combination of NMR techniques. Additionally, the heterogenization of small model complexes into MOFs was monitored with CW X-band EPR, unveiling the effect of ligand loading.

References

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- 2 Nash et al., J. Am. Chem. Soc., 2021, 143, 2194–2199.
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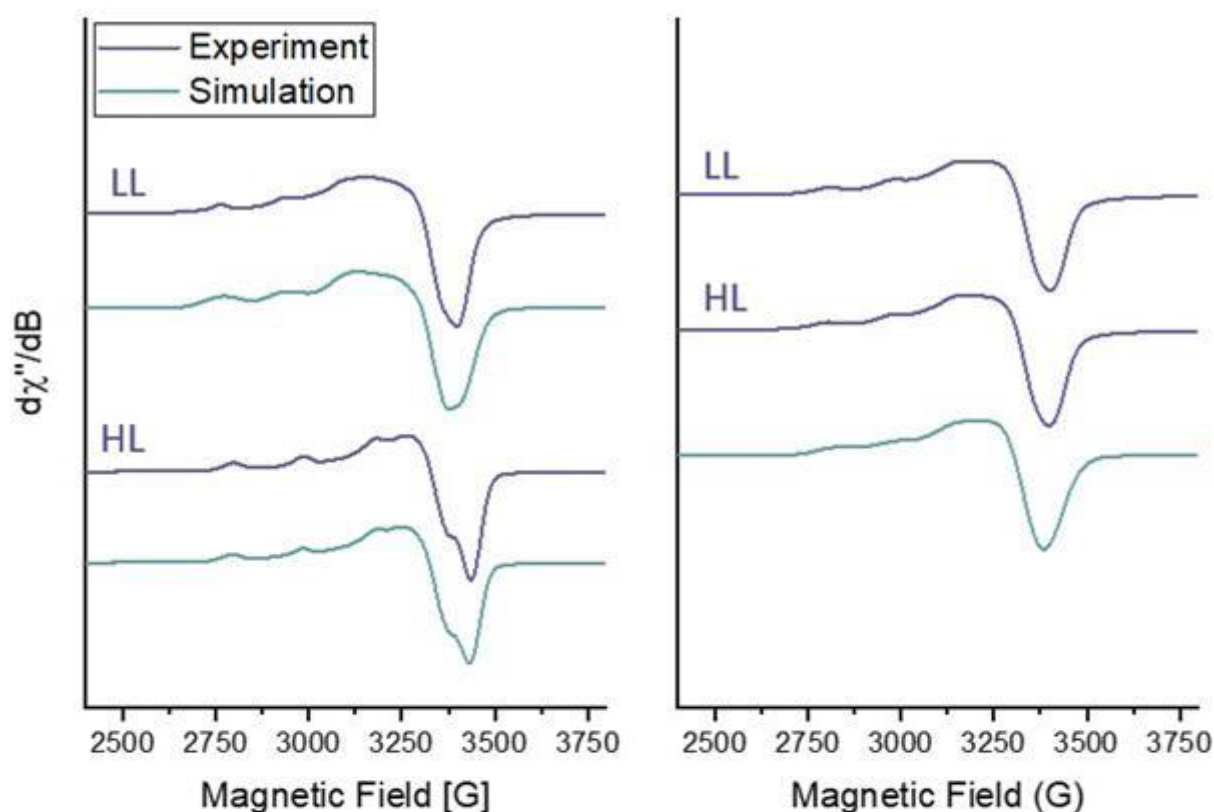
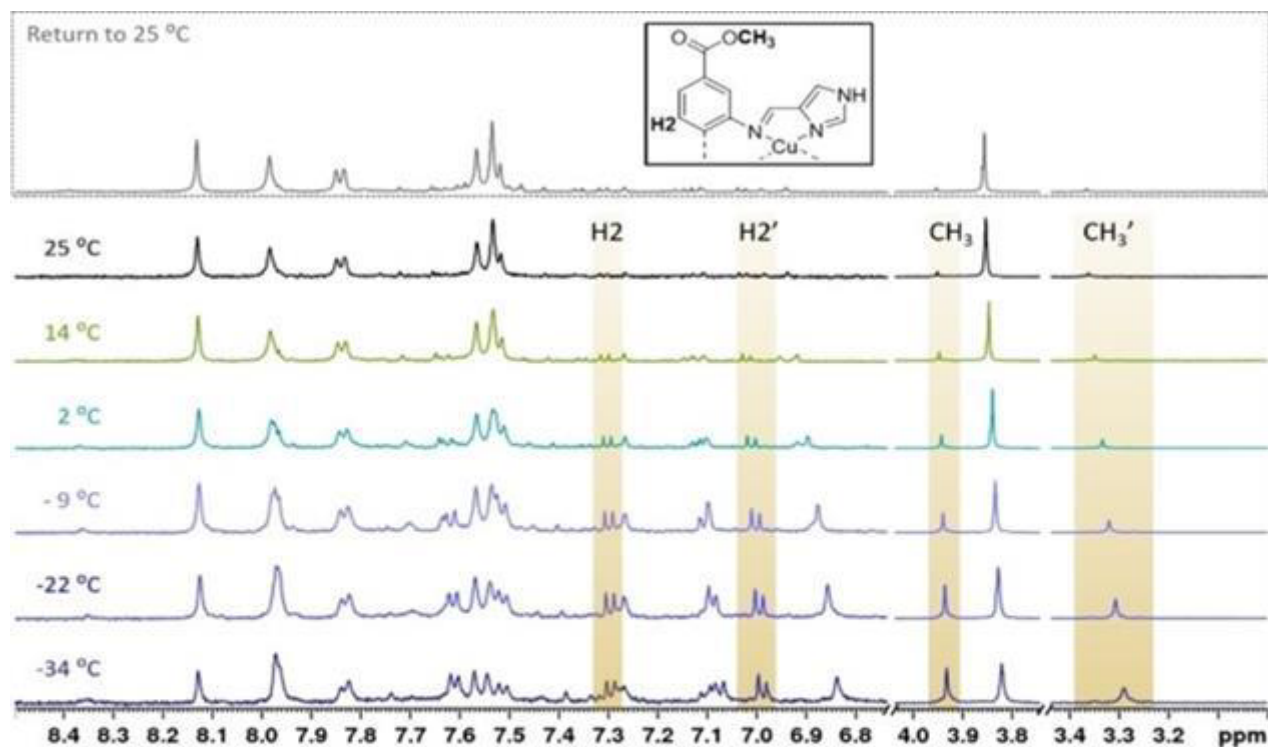


Figure 1. Top: Partial ^1H NMR spectra of a molecular copper(I) complex at different temperatures (500 MHz, CD_3CN).³ Bottom: EPR spectra (30K, CW X-band, neat) of Zr-MOFs with incorporated copper(II) coordination compounds. The ligand loading (LL= low loading; HL= high loading) affects the nature of the copper site for the compounds to the left, while it remains unchanged for the material to the right.

P-305 - Towards Detecting and Characterising Ligand Binding by Bullet-DNP – The Example of Prolyl Hydroxylase Domains

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Bullet-DNP is a powerful dissolution DNP technique. It is especially promising for biological and pharmaceutical applications due to low sample dilution, room-temperature dissolution and fast sample transfer.

Still, bullet-DNP suffers from strong relaxation during transfer and only allows one experiment per hyperpolarised sample. For many NMR drug screening approaches, however, at least two experiments are required in order to obtain a reference and a read-out spectrum.

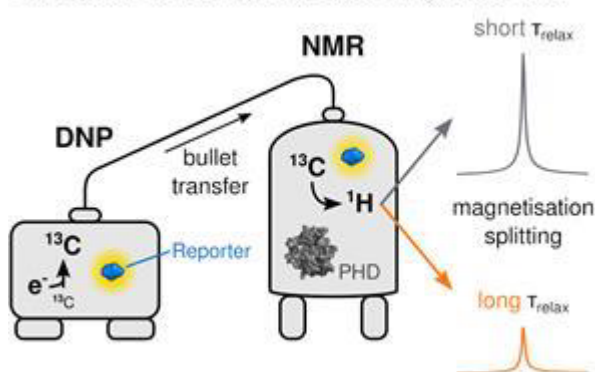
For ligand-observed T_2 -measurements to detect protein binding, these challenges can be overcome by magnetisation splitting and multiple acquisitions in one experiment. This can be combined with magnetisation storage on slow-relaxing ^{13}C nuclei during sample transfer and dissolution, to further increase the polarisation levels in solution. Just prior detection, magnetisation is transferred to ^1H for highest sensitivity and contrast in the recorded spectrum.

Here, we demonstrate the applicability of this approach to identify binders and to determine dissociation constants (K_d) for pharmaceutically relevant prolyl hydroxylase domains (PHDs). PHDs play a crucial role in the oxygen sensing mechanism of cells and are therefore potential drug targets for example in oncology. By introducing ^{13}C labelled pyruvate as reporter ligand for competition experiments, a qualitative and quantitative characterisation of ligand binding with hyperpolarised NMR becomes possible.

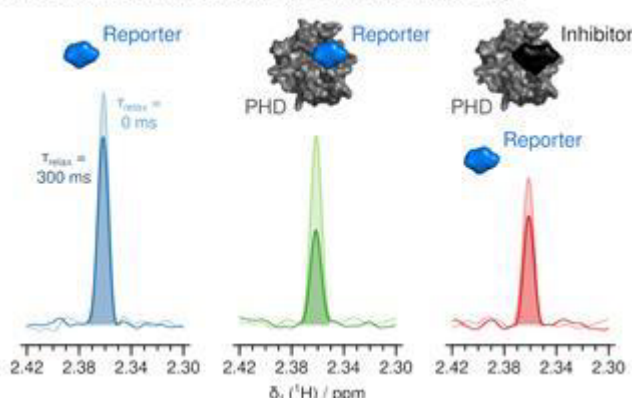
As an alternative, reporter-free approach for K_d -determination, the repeated addition of hyperpolarised ligand could offer a direct, ligand-observed method for the quantification of binding. We assessed the feasibility of this approach, predestined for the application of bullet-DNP, by means of the K_d -determination for pyruvate.

Our results are a first step towards dissolution DNP-enhanced drug discovery, which promises high-throughput screening with low protein concentrations and could thus make new drug targets accessible for NMR-based screening techniques.

Small molecule reporter is polarised by bullet DNP:



Reporter relaxation indicates inhibitor binding:



P-291 - Practical aspects of ^{13}C qNMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Quantitative NMR is one of the most important tools for the quantification of chemical species in samples. It is most commonly run through the measurement of ^1H detected single pulse experiments[1], but severe spectral overlap makes the quantification of analytes a challenging task. ^{13}C detected NMR alleviates the spectral overlap problem, and with modern instruments achieving higher sensitivity, ^{13}C qNMR becomes a suitable alternative to ^1H qNMR. For example, the resolution achieved in ^{13}C data enables the determination of long-chain branching in polymers[2]. Several aspects such as the acquisition of data with low signal-to-noise ratio and the larger bandwidth required to excite the ^{13}C spectrum lead to a number of considerations that must be taken into account when acquiring ^{13}C qNMR. Here we describe some of the practical aspects and consideration to take into account to acquire accurate ^{13}C qNMR data[3].

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P-067 - Investigating the influences of RNA abasic sites on RNA/RNA and RNA/DNA duplexes

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Every day, we are confronted with numerous endogenous and exogenous factors that can compromise our genomic integrity. To function properly, our bodies must perform extraordinarily to prevent and repair potentially harmful disruptions. Failure to do so results in mutations in our DNA that can ultimately lead to cancer. Depurination/depyrimidination of DNA as part of the base excision repair mechanism is the most common approach to counteract these. In this process, an apurinic/apyrimidinic site is created at the "defective" nucleotide site, excised from the double strand, and replaced with the correct nucleotide.

However, this mechanism is not limited to DNA. The peptide toxin ricin and its effect on ribosomal RNA is just one example of abasic sites in RNA. Since abasic sites (AS) are also present in nascent transcripts, it is reasonable to assume that they have another function.

The aim of this work is to investigate the influences of abasic sites on the structures and dynamics of RNA/RNA and RNA/DNA duplexes using NMR spectroscopy. We have successfully incorporated an "abasic" building block into several RNA sequences using solid phase synthesis. Using SI-labeled sugar units, we were able to observe the structural changes and dynamics following photolytic generation of the abasic site. In addition, we synthesized site-specifically labeled RNA/RNA and RNA/DNA duplexes to obtain complete assignment of all aromatic ¹³CH as well as 1-¹⁵N-purine and 3-¹⁵N-pyrimidine signals.

Laser-assisted deprotection of a photolytic NPE protecting group in the NMR spectrometer allowed us to follow the equilibrium of SI-labeled ribose in RNA at the moment of release. X-ray structures and cleavage experiments of AS-containing RNAs with the AS-cleaving enzyme APE 1 complemented our studies.

Yaojuan Liu et al., RNA abasic sites in yeast and human cells

Pascal A. Küpfer et al, The chemical stability of abasic RNA compared to abasic DNA

P-213 - An accessible para-hydrogen-based route to hyperpolarized nuclear magnetic resonance from precursors to injectable solutions

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Nuclear Magnetic Resonance (NMR) applications could benefit from an increase in sensitivity. Hyperpolarization methods can enhance NMR signals significantly, allowing the detection and monitoring of low-concentration species in (bio-)chemical reactions. Para-hydrogen-based methods provide an attractive approach to hyperpolarization.^{1,2}

Aims

Since the ingenious introduction of the side-arm approach (SAH)³ has expanded the molecular systems amenable to additive para-hydrogen-induced polarization (PHIP), considerable efforts have been directed toward designing precursors and methods with the aim to achieve high polarizations in substrates of biological interest.

Methods-Results

We report an easy-to-scale approach for synthesizing gram quantities of ¹³C-labeled precursors for field-independent PHIP-SAHP methods from commonly available reagents.^{4,5} We polarized pyruvate and acetate precursors at 18% and alanine precursors at 6% in situ at 21 mT in a portable custom-built system. Using a fast purification procedure, we obtained [1-¹³C] pyruvate in neat biocompatible water solutions with 6.5% polarizations at 40 mM concentrations. Our findings demonstrate that it is possible to perform time-resolved ¹³C MRI of HP pyruvate at millimetric resolutions in preclinical scanners. We used the method to follow the metabolism of pyruvate in vivo. Thanks to the fast experimental turnover, we were able to monitor metabolism in the brain and the liver in the same mouse in the same study, demonstrating the feasibility of PHIP-based multiorgan real-time metabolic studies in preclinical settings.⁶

Conclusions

In this work, we presented an accessible PHIP-based strategy that facilitates access to hyperpolarized NMR in preclinical metabolic studies.

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P-313 - Fast ^{19}F Fragment Screening as a Tool for Ligandability of Drug Targets

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

^{19}F NMR fragment screening was proposed in 2005. The methodology has many benefits:

- 1) Fast acquisition
- 2) Quick analysis
- 3) Low protein and fragment consumption
- 4) Most buffers are tolerated

There is a question whether it is a compromise due to the availability of fluorinated fragments, i.e. is the chemical space sampled as well as for the traditional fragment libraries that use ^1H NMR ligand observed experiments? A comparison is shown of the physico-chemical properties of our well curated ^1H fragment library and a fluorinated library.

Aims

The improvement in physico chemical properties of available ^{19}F fragment libraries. To exemplify the speed and ease of carrying out ^{19}F fragment screening. The correlation of ^{19}F fragment hits resulting in crystal structures is a strong indicator of the ligandability of a target.

Methods

Computational analysis of fragment libraries. ^{19}F NMR CPMG and T2 experiments; ^1H STD and waterLOGSY. SPR and X-Ray crystallography.

Results

^{19}F fragment libraries are improving in terms of physico-chemical properties as well as their chemical diversity. The ligandability of different targets is demonstrated showing that ^{19}F screening is a very convenient tool for finding hit material and for assessing the suitability of a target as a small molecule drug discovery project. The process of going from screen to orthogonal validation and then to crystal structure in <4 weeks shows the possibilities for fast progression from the initial ^{19}F NMR ligand observed screen.

P-159 - Shedding Light on Photochemical Reactivity with LED-NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

NMR is well-established as a particularly powerful tool for reaction monitoring and mechanistic study [1]. In recent years, photochemistry has become an increasingly important tool for synthetic chemistry, with many new reactions being developed and implemented in both industrial and academic settings. However, the mechanisms of many of these reactions remain poorly understood and few mechanistic studies have been conducted; in part because of the intrinsic challenges of gathering robust kinetic data on photochemical systems.

Methods and aims:

Here we present the results of a detailed NMR kinetic study conducted on the silane-mediated cross-electrophile coupling developed by the MacMillan group (figure 1.a) [2]. This was conducted via a bespoke in-situ illumination NMR spectroscopy system (LED-NMR) that we constructed in-house (figure 1.b). Important considerations for implementation of this type of system are also discussed.

Results:

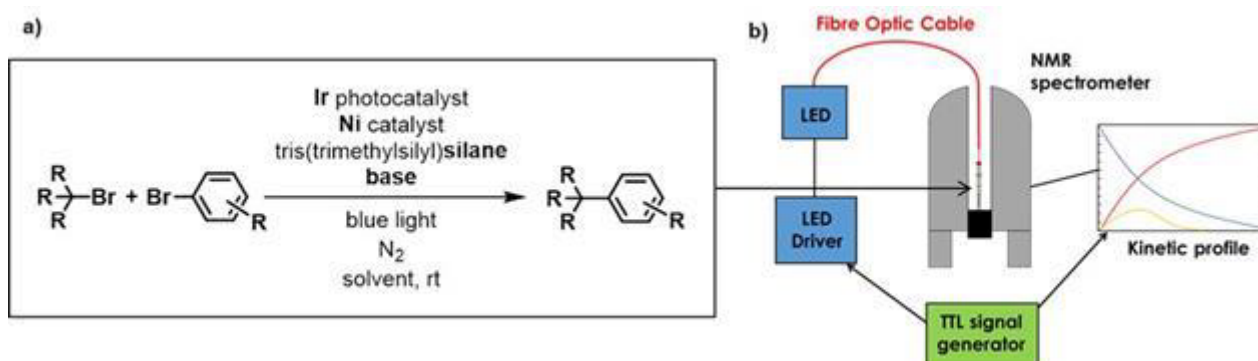
The simultaneous direct monitoring of a large number of components in the reaction solution by ¹⁹F LED-NMR has enabled several important mechanistic observations. One particularly important outcome of this monitoring was the direct observation of a key intermediate that is the major resting state of the Ni catalyst throughout the cycle. The role of this intermediate species in the reaction was further elucidated through sophisticated isotope labelling studies, taking advantage of the unique properties of NMR. A subsequent combination of control experiments, systematic variation of the reaction conditions and kinetic modelling has enabled mechanistic conclusions to be drawn [3].

Conclusions:

This case study demonstrates how LED-NMR is a powerful tool for enabling understanding of photochemical reactivity. Further innovations in hardware for NMR reaction monitoring may also be discussed.

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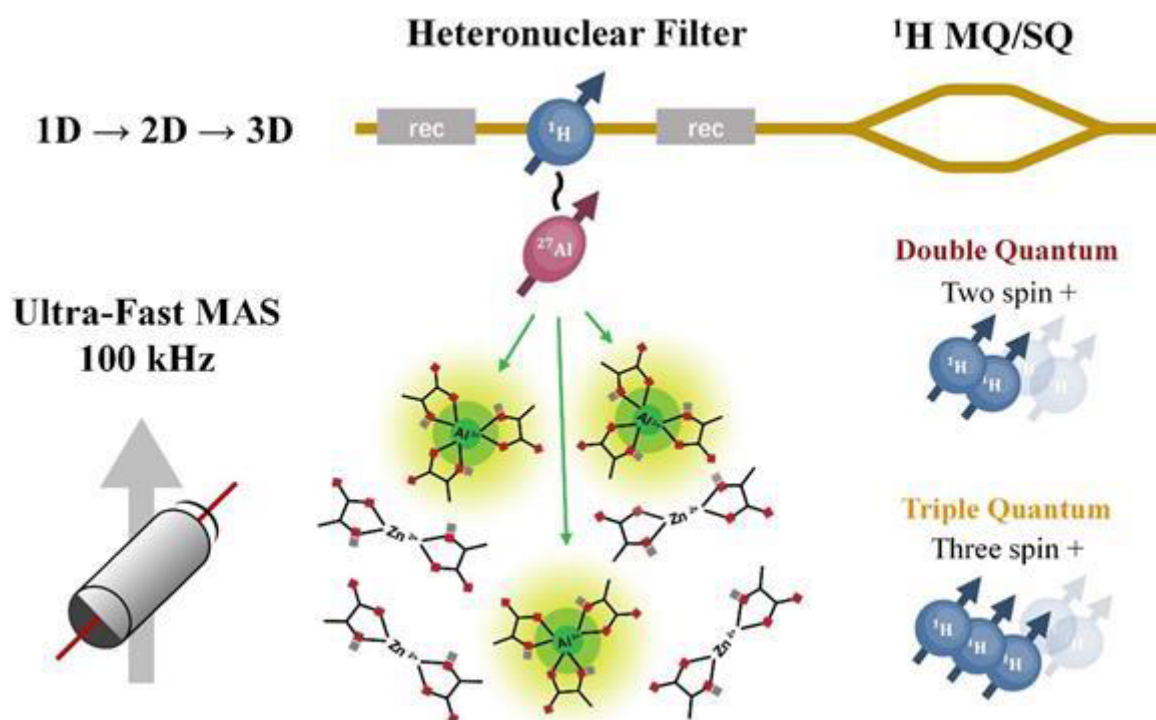
P-383 - Heteronuclear-filtered ^1H homonuclear multi-quantum correlation experiment at 100 kHz magic-angle spinning

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Fast magic angle spinning (MAS) (≥ 70 kHz) technique and homonuclear multiple-quantum correlation experiments have proven to be useful approaches to improve the resolution of ^1H solid-state NMR spectroscopy. Here, we introduce a heteronuclear filtered ^1H homonuclear multiple-quantum correlation strategy available at a MAS speed of 100 kHz, by combining the $^1\text{H}\{\text{X}\}$ heteronuclear-filtered methods and ^1H homonuclear multi-quantum correlation experiments. For a mixture of aluminum lactate (Al-Lac) and zinc lactate (Zn-Lac), ^1H signals of Al-Lac can be selectively extracted via ^{27}Al -filtered methods (i.e. $^1\text{H}\{^{27}\text{Al}\}$ Heteronuclear Multiple Quantum Correlation (HMQC) or $^1\text{H}\{^{27}\text{Al}\}$ Symmetry-based Resonance-Echo Saturation-Pulse DOuble-Resonance (S-RESPDOR)). The incorporation of these ^{27}Al -filtered methods into two-dimensional (2D) ^1H - ^1H double-quantum (DQ)/single-quantum (SQ), Triple-quantum (TQ)/SQ, and even three-dimensional (3D) $^{27}\text{Al}/^1\text{H}(\text{DQ})/^1\text{H}(\text{SQ})$ experiments allows for the acquisition of easy-to-interpret spectra without signal-overlapping. Moreover, this approach facilitates the targeted characterization of the featured micro-environments of ^1H species surrounding ^{27}Al sites, thereby providing a means of extracting key structural information from complicated spin systems.



P-057 - Cost-Efficient Stable Isotope Labeling of Proteins Overexpressed in Mammalian Cells

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¹Silantes GmbH, Munich, Germany

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Protein structure and dynamics determination by nuclear magnetic resonance (NMR) spectroscopy has long relied on uniform ¹³C and ¹⁵N stable isotopic labeling. The expression in eukaryotic cells ensures the protein's proper folding and relevant post-translational modifications needed for its function. However, for a wide range of proteins that are of great interest, the major obstacle for NMR studies is the lack of an affordable eukaryotic expression system for isotope labeling. We have developed culture media containing stable isotope-labeled amino acids (¹⁵N and ¹³C¹⁵N) for mammalian HEK293T and insect Sf9 cells. The culture media are based on stable isotope-labeled protein hydrolysates from fermented *Cupriavidus necator*. The media were optimized to achieve high isotope incorporation and protein yields. For this purpose, the cells were adapted to low serum culture media. In addition, labeled biomass-derived hydrolysates, labeled yeast autolysates and lipid extracts were explored as media ingredients. After five passages, ¹⁵N incorporation rates of 78 % were achieved for enhanced Green Fluorescent Protein (eGFP) overexpressed in HEK293T cells. Overexpressed eGFP yields in the new media were comparable with cells cultured in standard DMEM/F12 media. Based on pH, osmolality, glucose concentration and turbidity the media are stable for at least 8 weeks when stored at 4°C. The 'Silantes Isotope-labeled Growth Medium for Mammalian Cells' enables protein structural investigations in a cost-efficient manner.

P-325 - Mechanistic features underlying the phototransformation properties of fluorescent proteins revealed by solution NMR coupled with in-situ sample illumination

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Fluorescent proteins (FPs) that undergo reversible or irreversible photo-transformations when exposed to light at specific wavelengths are of crucial importance for a wide range of applications in advanced fluorescence microscopy and biotechnology. The exact mechanisms behind these photo-induced conformational changes remain poorly understood, which makes engineering of improved variants based on rational protein design a difficult or even impossible task. At present, mechanistic information on FPs has been derived from crystallographic structures, complemented by data from optical spectroscopy and quantum-chemical calculations. Solution NMR spectroscopy, combined with in-situ sample illumination provides an unique tool to investigate at atomic resolution the conformational and dynamic properties of FPs in their different photo-stationary states. It also allows to access the interconversion dynamics of various conformational states and to derive kinetic models underlying the observed photophysical properties.

We have set up a portable NMR in-situ illumination device that is compatible with high-field NMR spectrometers, and currently permits sample illumination at 3 wavelengths (405, 488, and 561 nm). Recently, we have added light detection capabilities to our setup in order to record simultaneously NMR and fluorescence emission data. This allows to correlate changes in the populations of conformational states (NMR) with their fluorescent properties (emitted light). Here, we present a recent application of this setup where solution NMR has provided information on conformational dynamics and structural heterogeneity in the chromophore pocket of FPs from the mEos family. In particular, we could establish that the 2 NMR-observed ground states correspond to different local hydrogen-bond networks caused by a change in the protonation state of amino-acid side chains in the chromophore vicinity. Finally, combining NMR and fluorescence data we could show how these 2 states differ in terms of their photoswitching and photoconversion behavior, and shed some new light on the underlying mechanisms.

P-073 - Assessing the Behaviour of Lipopolysaccharide in Formulations via Novel Fluorination and ^{19}F NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Lipopolysaccharide (LPS), otherwise known as endotoxin, is a cell-wall component of Gram-negative bacteria that contributes to bacterial toxicity. During processes such as cell division, shedding of outer membrane vesicles or bacterial cell death, LPS is released into the surrounding media. If such contamination got into the bloodstream, it would induce pro-inflammatory immune responses which can result in sepsis and death. Therefore, detection of LPS is essential in the pharmaceutical and food industries to prevent exposure of LPS to patients. The Limulus Amebocyte Lysate (LAL) assay is the current major assay used by industry to detect and quantify LPS contamination. However, in recent years the phenomenon of Low Endotoxin Recovery (LER) has gained significant scientific attention. The phenomenon describes the inability of LAL assays, in some cases, to detect LPS due to a masking effect caused by interaction between LPS and particular formulation excipients (chelating agents and surfactants). Although the mechanism of LER has not been fully determined, it is widely thought that the origin of the effect is associated with these interactions perturbing the supramolecular formation of LPS aggregates. As such, it is imperative to understand the interplay between the supramolecular structure of LPS and the interactions with the formulation excipients.

Our work demonstrates the successful fluorination of LPS molecules to assess their complex aggregation behaviour upon interaction with formulation excipients via ^{19}F NMR spectroscopy. Utilising ^{19}F NMR allows simplified data interpretation compared to the intricate deciphering of ^1H and ^{13}C NMR spectra that would otherwise be required, due to the complex carbohydrate structure of LPS and very large size. We used ^{19}F experiments, including DOSY, to explore how the content of formulations, including the presence of chelating agents ($\leq 10\text{ mM}$) and surfactants ($\leq 0.05\%$), affects LPS assemblies.

P-113 - FapA is an Intrinsically Disordered Chaperone for Pseudomonas Functional Amyloid FapC

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Bacterial amyloid fibrils act as scaffolds of biofilms, which provide chemical and antibiotic resistance, making them a prevalent issue in infections. *Pseudomonas aeruginosa* has a functional amyloid system [Fap] which contains FapC, a small intrinsically disordered protein that is a main component of the amyloid fibrils, and FapB, thought to be a minor component. FapA is another IDP whose role has until now been unknown. Due to their intrinsic disorder, NMR is best suited for their biophysical analysis.

Aims

We aimed to use NMR to calculate the structure and observe the interactions between the Fap subunits.

Methods

CD, SAXS, and ThT assays were used for preliminary biophysical analysis of the subunits. NMR triple resonance and titrations were used to corroborate and further explore the data in detail.

Results

CD and SAXS data revealed that FapA, FapB, and FapC are IDPs. Using the chemical shifts obtained from NMR triple resonance experiments, d2D confirmed this and calculated residue specific secondary structure propensities. ThT assays showed that FapA inhibits FapC by preventing nucleation, while FapB is inhibited at the elongation step. NMR titration of unlabeled FapA into labeled FapC showed no peak shifts, while the reverse did. Titration experiments between FapA and FapB showed no changes in the spectrum.

Conclusion

We have concluded that FapA is an intrinsically disordered chaperone of FapC that inhibits the formation of FapC fibrils in the periplasm. While the mechanism of action is unclear, it seems that monomeric FapA interacts with oligomeric FapC. This finding may serve as an inspiration toward new approaches to tackling the biofilm problem.

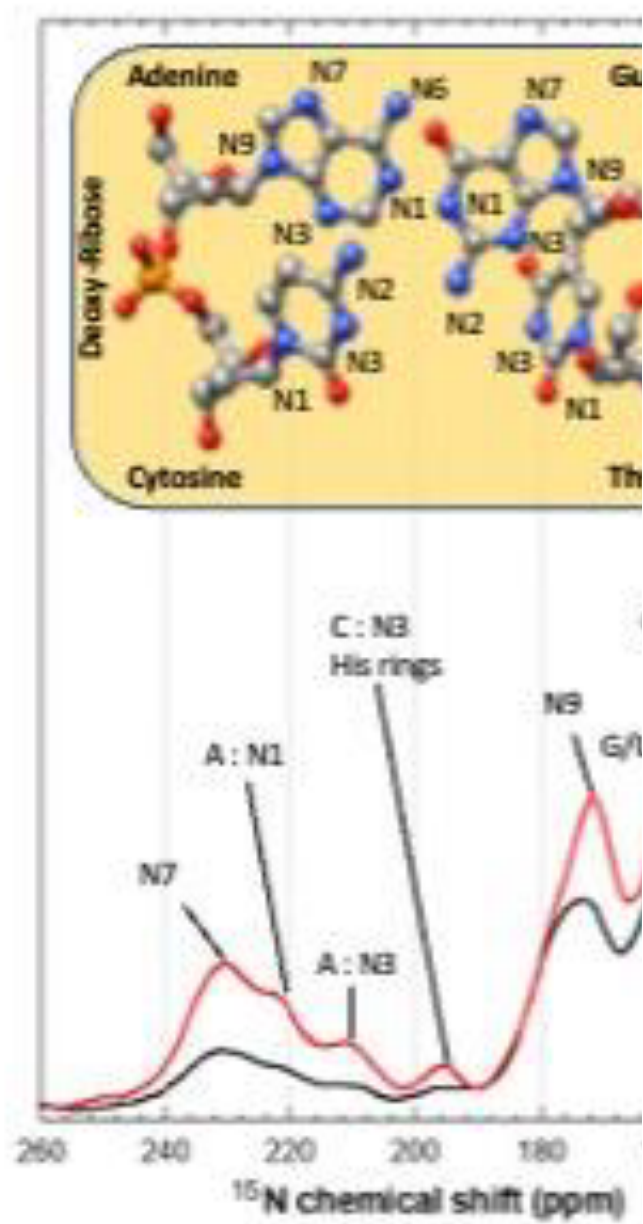
P-371 - Untargeted in-cell DNP NMR for Monitoring global perturbations and trafficking of antimicrobial peptides

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¹University of Melbourne, Parkville, Australia

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The use of dynamic nuclear polarization (DNP) NMR to investigate biologically active molecules in native cell environments is rapidly offering unprecedented levels of structural and functional details. Efforts to characterise the radical lifetime and its cellular location, the cell integrity and survival rate, together with engineering of in-cell protein production have generated multiple methods to perform in-cell DNP NMR. Thus, we previously have shown the benefit of using spin labelled peptides for targeted DNP NMR in *E. coli* systems. The protocol led to localized enhancement of the NMR signals at the membrane interface, and improved prospects for understanding the mechanism of action of membrane-active peptides, such as antimicrobial peptides (AMPs). While information at the membrane interface is of particular interest for AMP studies, other internal structures have been shown to be perturbed and to contribute to the death of bacteria. Thus, a more global monitoring of the impact of AMPs on bacteria could provide new insights in their mode of action. We will show that untargeted DNP NMR is a useful tool to globally monitor the effects of AMPs on bacteria by using ¹⁵N labelled *E. coli* (Fig. 1). The non-specific ¹⁵N labelling allows simultaneous observation of nucleic acids, proteins and lipids in-cell. We will show how several AMPs have different impacts on these structures. For instance, some AMPs disrupted the lipid packing while some also perturbed the nucleic acids. Overall, the ability to monitor the action of antimicrobial peptides in situ provides greater insight into their multi-impact mode of action.



P-393 - Pseudo-6D assignment strategies for streamlined chemical shift assignment of small and large microcrystalline proteins

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Over the last 15 years, ¹H-detected solid-state NMR spectroscopy has emerged as a powerful tool in structural biology, suitable for various applications ranging from structure calculation to protein dynamics covering time scales of multiple orders of magnitude. Despite continuous efforts to improve solid-state NMR hardware and optimized labeling schemes, the necessity for comprehensive chemical shift assignments remains the major bottleneck.

While for small to medium-sized proteins 3D spectra and derived assignment strategies yield sufficient chemical shift assignments for downstream applications such as relaxation experiments, the assignment problem increases significantly for proteins exceeding a molecular weight of approximately 30 kDa especially when manual assignment protocols are applied. In recent times, new higher-dimensionality and interleaved experiments have been introduced to ameliorate this problem, by increasing chemical shift redundancy and therefore facilitating access to a molecular weight regime of proteins that are of great biological interest, e.g., for drug development. Despite these improvements, most assignment strategies still rely heavily on resolved peaks in a 2D source experiment, as well as C β chemical shifts for residue type identification.

Here we introduce a new 4D hCAconCANH experiment in combination with a previously published 5D HNcoCANH experiment that facilitates a pseudo-6D HNCA-HNCA assignment approach. This combination consequently continues the shift from dispersion-limited and overlap-prone amide-to-amide experiments towards 3D-source, i.e., HNCA-based, assignment protocols. We show that within competitive measurement times, full backbone assignment is obtained from only these two experiments without any residue-type specific information for the 7 kDa model protein, the SH3 domain of chicken α -spectrin. Additional preliminary data will be shown for the assignment of the 42 kDa protein kinase p38 α for which the assignment process is eased by the reduced number of required spectra compared to the previously introduced strategies requiring six or often more experiments, the user must oversee.

P-281 - The Development of Phantoms to Explore New CEST Contrast Agents

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

Chemical exchange saturation transfer (CEST) is a technique offering enhanced contrast in MRI.¹ It is based on the selective saturation of exchangeable protons, and unlike other MRI contrast methods, it offers the ability to turn contrast on and off within a system. This makes CEST a particularly useful technique as it provides a method for distinguishing the presence of a contrast agent (CA) in heterogeneous environments with exchangeable protons.^{2 3 4}

A new class of CEST CAs are to be investigated. To evaluate the effectiveness of these new CEST agents, phantoms are generated to act as in vivo models. By developing phantoms which have a CEST effect, the ability to switch on and off contrast will allow the spatial localisation of the CEST agents in the phantom.

Aims:

The goal of this project is to develop a suitable phantom by which new CEST CAs can be investigated.

Method:

¹H MRI images were acquired for alginate beads (2% w/w) in 0.2 M CaCl₂ solution in the absence of CEST agents. CEST images were produced from a series of 41 images acquired with pre-saturation rf pulses over a frequency offset range 1200-1800 Hz.

Results:

Figure 1a) shows a spin density image of alginate beads in CaCl₂ solution, showing no contrast whereas 1b) shows a CEST image with contrast between the alginate beads and solution.

Conclusion:

Alginate is shown to produce a CEST effect, which will need to be considered when assessing the behaviour of new CEST CAs in this phantom.

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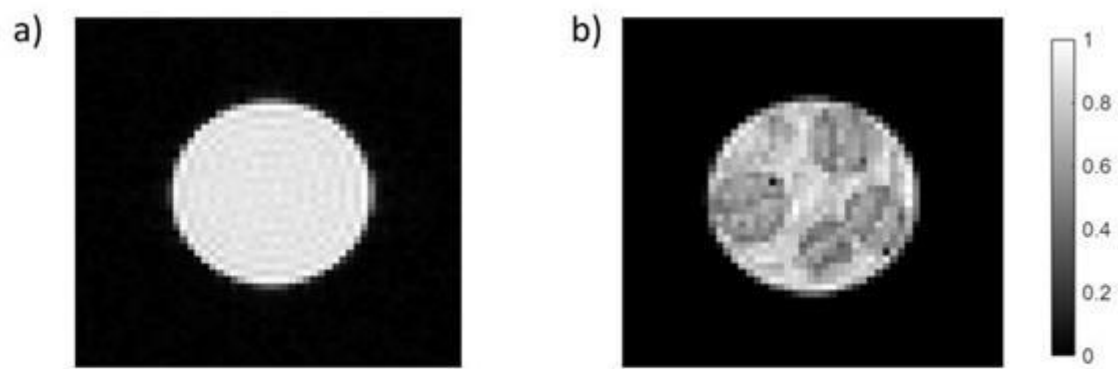


Figure 1: a) ^1H MRI spin density and b) CEST map of alginate beads (axial) in 0.2 M CaCl_2 solution in the absence of CAs in a 10 mm NMR tube.

P-011 - TD-NMR Analysis of the Structure and Composition of Soft Candy Confectionery During Processing

Mr Erdem MERCAN¹, Mr Umur Tuna İsmail⁴, Prof. Robert Morris³, Dr. Nicasio Geraldi³, Professor Mecit Oztop², Dr. Edward Breeds³, Dr. Michael Newton³

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Soft candy confectionery is a popular treat consumed worldwide, and maintaining consistent quality during processing is a critical aspect of product development. Portable devices offer several advantages over traditional TD-NMR instruments, such as ease of use, lower cost, and the ability to perform on-site analysis. Moreover, these devices are also suitable for monitoring product quality during transportation and storage, allowing for timely quality control measures [1].

This study employed TD-NMR measurements to analyze the structure and composition of soft candy confectionery during processing using a custom-built magnetic resonance system based on a Halbach arrangement. Samples were subjected to a series of processing steps, including boiling, cooling, and aging, and were analyzed.

The results showed that TD-NMR was a highly effective technique for characterizing the changes in the structure and composition of the confectionery during processing, and for measuring key physical properties such as viscosity, moisture content, and Brix values. Moreover, the effectiveness of measuring T2eff and T1 in complex soft candy processing was demonstrated to optimize the process to obtain desired texture and moisture content [2]. As can be seen from Figure 1a, the T1 values were found to provide information on the moisture content and brix values of the prepared sample. Figure 1b shows the potential of TD-NMR a quality control method in a continuous flow. As a valuable tool to maintain consistent output, this system will be integrated into the production line of a confectionery factory.

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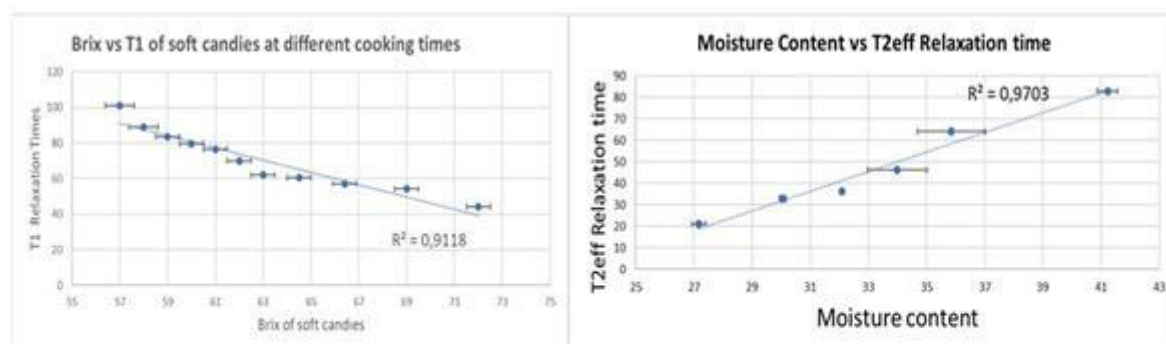


Figure 1a. Brix vs Longitudinal Relaxation times of soft candies at different cooking times

Figure 1b. T_2^{eff} vs Moisture content of confectionery by-product syrup (measurements done at continuous flow)

P-285 - Towards Quantum Sensing of Chiral-Induced Spin Selectivity: Probing Spin-Polarized Radical Pairs with NV Centers in Diamond

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¹ETH Zürich, Zürich, Switzerland

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Chiral-induced spin selectivity (CISS) refers to spin-dependent interactions between electrons and inversion asymmetric materials.¹ Charge transfer in photoexcited donor-chiral bridge-acceptor (D-B-A) molecules is an ideal system to probe the influence of chirality in these electron transfer reactions, with implications in biology for the radical pair mechanism and for quantum information science using spin qubit pairs.^{2 3 4} However, the extent to which CISS influences spin polarization or spin coherence in the initial state of spin-correlated radical pairs following charge transfer through a chiral bridge remains an open question.

Here, we introduce a quantum sensing scheme to measure directly the hypothesized spin polarization in radical pairs using shallow nitrogen–vacancy (NV) centers in diamond at the single- to few-molecule level.⁵ Using explicit spin dynamics simulations, we demonstrate how frequency-switched Lee-Goldberg decoupling, which is a common technique to mitigate homonuclear interactions in solid state NMR spectroscopy,⁶ can preserve spin polarization in D–B–A molecules for enantioselective detection by a single NV center. Furthermore, we establish critical measurement design rules to overcome geometrical limitations of dipolar sensing with NV centers, and evaluate the detection sensitivity for spin polarization resulting from CISS in experimentally relevant systems.

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P-101 - Design of a polyglutamine-based helical scaffold to target globular proteins

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Polyglutamine (polyQ) expansion beyond a pathological threshold is associated with a number of neurodegenerative diseases. The polyQ tract of several proteins, such as androgen receptor (AR) [1], huntingtin [2] and CBP [3], can adopt α -helix conformations propagated and stabilized by unusual bifurcated hydrogen bonds, in which the side and main chains of glutamine residues simultaneously donate a hydrogen to the backbone carbonyl of residue $i-4$. [1]

Using a combination of solution NMR and molecular dynamics we have studied in detail how the sequence context influences the helical content of the polyQ tract of AR and expanded the analysis to the tract of the TBP protein. We have exploited our observations to present rules to design linear peptides that fold into short single α -helices by concatenating glutamine side chain to main chain hydrogen bonds. The resulting peptides are highly soluble, uncharged and contain only natural amino acids. An important feature of these peptides is their versatility: several hydrophobic residues can act as efficient H-bond acceptors and the design can also incorporate a pH-sensitive switch or can be complemented by electrostatic interactions between charged side chains. Remarkably, our scaffold design defines the identity of only a fraction of the peptide residues and the rest can be chosen or optimized for specific applications. As a proof of concept, we have designed two helical peptides that successfully bind to the globular target RAP74-CTD. [4]

P-193- Liquid-state two-dimensional ^{13}C - ^{13}C correlation NMR enhanced by Overhauser dynamic nuclear polarization at 9.4 Tesla

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Liquid-state high-field (≥ 9.4 Tesla) two-dimensional (2D) ^{13}C - ^{13}C correlation NMR spectroscopy is a powerful method in the structural determination of natural products and biologically-active molecules by directly probing the carbon connectivity. However, these methods require the presence of two neighboring ^{13}C nuclei, leading to low sensitivity and limited practical application. One promising strategy to improve the sensitivity of liquid-state ^{13}C NMR is through Overhauser dynamic nuclear polarization (ODNP), whereby the polarization of a radical-centered electron spin is transferred to ^{13}C nuclei of the target molecule through cross-relaxation during microwave irradiation. To date, several high-field liquid-state ODNP-NMR setups have been reported, allowing 10^1 - 10^2 -fold enhancements of the signal intensity in one-dimensional pulse-acquire ^{13}C experiments for small molecules compared with spectra acquired under thermal Boltzmann condition. However, no report has yet studied the application of ODNP in high-field liquid-state 2D NMR. Herein, we report the first example of ODNP-enhanced liquid-state 2D ^{13}C - ^{13}C correlation NMR measurements at 9.4 T, enabled by a liquid-state double-resonance ODNP-NMR setup developed in our group and optimized towards 2D NMR. ODNP-enhanced ^{13}C - ^{13}C COSY, TOCSY, and NOESY can be performed using standard pulse sequences on ^{13}C -enriched compounds upon applying continuous microwave irradiation to the sample. Compared to spectra recorded under Boltzmann conditions, the same set of diagonal and cross peaks are observed, with up to 30-fold enhancements in favorable cases. This corresponds to $\sim 10^3$ -fold reduction in experiment time for achieving a similar signal-to-noise. Furthermore, ODNP-enhanced ^{13}C INADEQUATE was performed on ~ 20 mg of a natural-abundant compound, allowing assignment of one-bond scalar coupling constants and carbon connectivity. Lastly, we observed a correlation between the ODNP ^{13}C enhancement and the chemical functionalities of the target molecules. Combined, these results present ODNP-enhanced high-field liquid-state 2D ^{13}C - ^{13}C correlation experiments as promising tools for NMR-based structural elucidation of small molecules.

P-175 - Quantitative analysis of cross-talk in partly deuterated samples during dynamic nuclear polarization (DNP) by thermal mixing

Mr Bogdan Rodin

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Dynamical Nuclear Polarization (DNP) is a powerful method that allows one to polarize virtually any spin-bearing nucleus by transferring electron polarization by microwave irradiation of the electron Zeeman transitions. Under certain conditions, the DNP process can be described in thermodynamical terms using the thermal mixing (TM) model.

Different nuclear species can exchange energy indirectly through their interactions with the electron spins and reach a common spin temperature. Such "cross-talk" effects can occur between proton (H) and deuterium (D) nuclei in de- and re-polarization experiments. In this work, we investigate such effects experimentally, using either protonated or deuterated TEMPOL radicals as polarizing agents. An analysis of these experiments based on Provotorov's equations allows one to extract the relevant kinetic parameters, such as the rates of energy transfer between the different reservoirs, and the heat capacity of the non-Zeeman (NZ) electron reservoir. In contrast, the heat capacities of the proton and deuterium reservoirs can be estimated based on their usual expressions. Surprisingly, it was found that D plays an essential role in polarization transfer, and it has to be included in the model to describe the visible effects correctly, which was avoided in the previous works exploiting the TM approach.

These parameters allow one to make predictions of the behaviour of heteronuclei such as carbon-13 or phosphorous-31, provided that their heat capacities are negligible. Finally, we present an experimental study of the dependence of Provotorov's kinetic parameters on the TEMPOL concentration and the H/D ratio, thus providing insight into the nature of "hidden" spins that are not observable directly because of their proximity to the radicals, where protons on H-TEMPOL seems to suite perfectly on this role.

P-071 - 1-¹⁵N-labeled 8-oxo-2'-deoxyguanosine to study DNA lesions and DNA quadruplexes by solution NMR spectroscopy

Mr Alessandro Marotto¹

¹University Of Innsbruck, Innsbruck, Austria

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction and Aims

Reactive oxygen species (ROS), resulting from the aerobic metabolism, are able to oxidize biomolecules including RNA and DNA. If their concentration in the cell rises over the range, in which it is normally kept by homeostatic mechanisms, oxidative stress occurs, a redox state which is related, among others, to tumorigenesis. Oxidative damage is the most common DNA altering mechanism. The nucleobase, which is most prone to oxidation, is guanine.[1] Its oxidation product, 8-oxo-guanine, can affect the structure and thermal stability of G-quadruplexes, a four-stranded structural motif found in the guanine rich repeats of promoter regions and telomeres.[2] This secondary structure element is stabilised by Hoogsteen hydrogen bonds between 4 guanines, it plays an active role in gene expression and seems to be involved in the inhibition of cell proliferation.[2,3] This study, aims to investigate the relation between the presence of 8-oxo-guanine in G-quadruplexes and their structure and thermal stability, employing NMR spectroscopic techniques capitalizing on a 1-¹⁵N-labeled 8-oxo-2'-deoxyguanosine building block.

Methods

The synthesis of an [1-¹⁵N]-deoxyguanosine and an 8-oxo-[1-¹⁵N]-deoxyguanosine phosphoramidites, enables the solid phase synthesis of guanine rich DNA quadruplex sequence with position specific ¹⁵N-labelling. The secondary structure and also dynamics are investigated by solution state NMR spectroscopy.

Results and Conclusion

Atom-specific ¹⁵N labeling of 8-oxo-deoxyguanosine allowed a detailed NMR spectroscopic investigation of a G quadruplex sequence. The dynamics of folding was probed by relaxation dispersion and real-time NMR experiments.

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P-399 - Probing Paramagnetism in Actinide Materials with $^{35}/^{37}\text{Cl}$ Solid-State NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Solid-state NMR (SSNMR) can be a powerful technique for studying actinide chemistry but has been significantly limited due to the complex redox chemistry, paramagnetism, and radiological hazards presented by these materials. Lanthanide and actinide salts often feature magnetic ordering and can be paramagnetic, ferromagnetic, or antiferromagnetic depending on temperature, pressure, etc. Paramagnetic interactions can manifest in NMR both as secular spectral shifts and/or couplings as well as non-secular relaxation contributions. Both effects can be directly measured with NMR and used to extrapolate rich chemical information such as coordination environments, bonding characteristics, local molecular dynamics, correlation times, etc.¹ Typically, these studies are carried out on high- γ and highly abundant NMR-active isotopes (e.g., ^1H , $^6/^7\text{Li}$, ^{19}F , ^{23}Na , etc.) or on enriched rare isotopes (e.g., ^2H and ^{17}O).²

Herein, we demonstrate facile ^{35}Cl SSNMR measurements as a probe for the structural and magnetic properties of several lanthanide and actinide salts. These salts generally feature one counterion to chlorine, as XCl_n , where $\text{X} = \text{La}^{3+}$, Nd^{3+} , U^{3+} , U^{4+} , and UO_2^{2+} all at natural abundance. The relative magnetic susceptibilities of these counterions are well known,³ and in each case cause unique manifestations of paramagnetic shift anisotropies and paramagnetic relaxation enhancements as observed by ^{35}Cl SSNMR at 9.4 T; these effects can be measured independently of the manifestation of the second-order quadrupolar interaction. Results are corroborated by ^{37}Cl SSNMR and measurements at a second magnetic field. These results demonstrate the feasibility of low- γ , quadrupolar, non-spinning, and natural abundance SSNMR to measure paramagnetic interactions and can enable new strategies in actinide chemistry.

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- (2) Martel, L.; Magnani, N.; Vigier, J. F.; et al.. *Inorg. Chem.* 2014, 53, 6928–6933.
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P-075 - Measuring ^{15}N T_1 and T_2 relaxation with pure shift resolution HSQC experiments

Mr Jorge Moreira

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The measurement of longitudinal and transverse relaxation time constants, T_1 and T_2 , of backbone ^{15}N nuclear spins provides an important tool to characterise the structural and motional features of proteins in solution¹. Conventional relaxation-encoded ^1H - ^{15}N HSQC experiments allow for backbone ^{15}N relaxation time constants to be measured from high-resolution 2D spectra, with the combined benefits of ^{15}N chemical shift dispersion in the indirect dimension and amide ^1H sensitivity in the direct dimension¹. However, when signal overlap is prevalent in the direct dimension, it can limit the unambiguous extraction of the cross-peak signal intensities needed to measure relaxation times. Pure shift NMR methods yield spectra in which the effects of J coupling have been suppressed and multiplet structure collapsed to singlets². For proteins, band selective decoupling (BASHD) pure shift methods are well suited to suppressing the homonuclear coupling between vicinal amide and alpha protons, $^3\text{J}(\text{HN-H}\alpha)$, due to their well-defined, separated chemical shift regions². Using real-time (i.e., single-scan) BASHD, ^1H - ^{15}N HSQC spectra can be recorded with increased spectral resolution, removing the contribution of multiplet structure to signal overlap². Here, new relaxation-encoded real-time BASHD pure shift ^1H - ^{15}N HSQC experiments have been developed and used to measure relaxation times for ^{15}N -ubiquitin. Comparison with results obtained from the corresponding conventional experiments shows that ultra-high resolution 2D spectra can be acquired at no extra cost in experiment time and with no detriment to the measured T_1 and T_2 values. Increases in signal intensity, particularly for peaks with higher multiplicity and/or large $^3\text{J}(\text{HN-H}\alpha)$ values, are seen. One added advantage is that pure shift spectra eliminate the need to mask multiplet structure with heavy line broadening, and indeed allow the use of resolution enhancement.

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P-093 - Characterisation of invisible conformation of domain 1.1 of σ A factor of RNA polymerase from *Bacillus subtilis*

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¹National Centre for Biomolecular Research (NCBR), Faculty of Science, Masaryk University, Brno, Czech Republic, ²Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic, ³Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

σ factors are essential components of bacterial RNA polymerase (RNAP) as they allow to recognize promotor sequences and initiate transcription. Domain 1.1 of vegetative σ factors occupies the primary channel of RNAP and also prevents binding of the σ factor to promoter DNA alone. Here, we show that domain 1.1 of *Bacillus subtilis* σ A exists in two structurally distinct variants in dynamic equilibrium.

Aims:

To elucidate the structure and dynamics of minor conformation and to discover how does minor conformation affect transcription.

Methods:

Relaxation dispersion analysis, chemical exchange saturation transfer analysis, in vitro transcription

Results:

The major conformation at room temperature is represented by a previously reported well-folded structure solved by nuclear magnetic resonance, but 4 % of the protein molecules are present in a less thermodynamically favorable state. We show that this population increases with temperature and we predict its significant elevation at higher but still biologically relevant temperatures. We found that, in contrast to the major state, the detected minor state is partially unfolded. Its propensity to form secondary structure elements is especially decreased for the first and third α helices, while the second α helix and β strand close to the C-terminus are more stable. Functional experiments with full length σ A and its shortened version lacking domain 1.1 (σ A_ Δ 1.1) then revealed that while full length σ A increases transcription activity of RNAP with increasing temperature, transcription with σ A_ Δ 1.1 remains constant.

Conclusions:

In conclusion, this study reveals conformational dynamics of domain 1.1 and provides a basis for studies of its interaction with RNAP and effects on transcription regulation.

P-311 - Multimodal Techniques for Detecting Alien Life using Assembly Theory with NMR and other Spectroscopic Techniques.

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¹University Of Glasgow, Glasgow, United Kingdom, ²University of Girona, Girona, Spain, ³ICREA, Barcelona, Spain

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Detecting alien life is a difficult task because it's hard to find signs of life that could apply to any life form. However, complex molecules could be a promising indicator of life and evolution. Currently, it's not possible to experimentally determine how complex a molecule is and how that correlates with information-theoretic approaches that estimate molecular complexity. Assembly Theory has been developed to quantify the complexity of a molecule by finding the shortest path to construct the molecule from simple parts, revealing its molecular assembly index (MA). (1)

Aims

This study aim was to develop an approach to rapidly and exhaustively calculate molecular assembly and explore the MA of over 10,000 molecules using analytical methods such as NMR.(2)

Methods

Using three independent techniques: nuclear magnetic resonance(NMR), tandem mass spectrometry(MS) and infrared spectroscopy (IR) we can show how to experimentally measure molecular complexity (MA) and obtain consistent results with good correlation. By identifying and counting the number of carbon resonances in NMR, absorbances in IR spectra, or molecular fragments in tandem MS, the molecular assembly index of an unknown molecule can be reliably estimated from experimental data.

Results

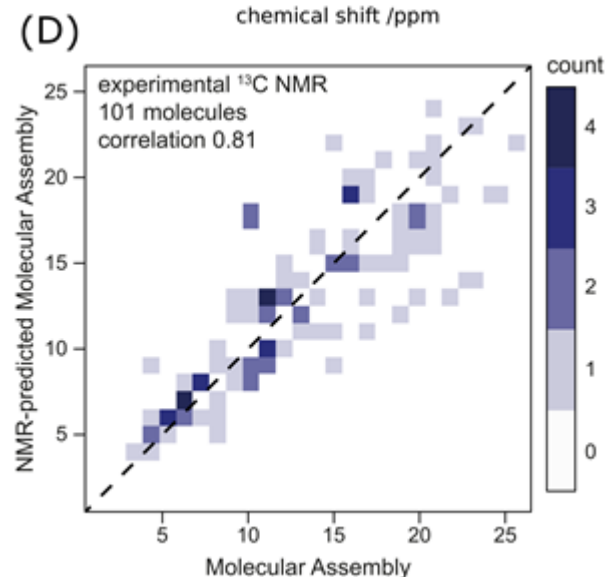
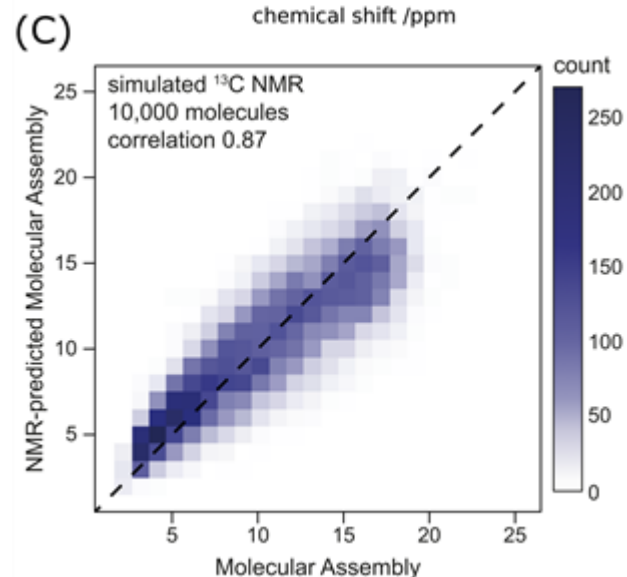
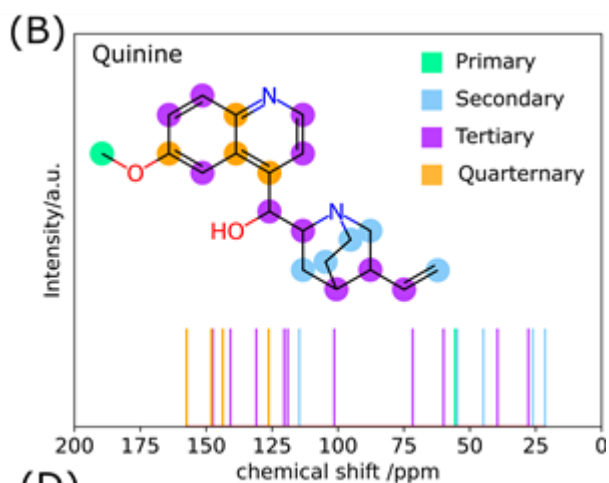
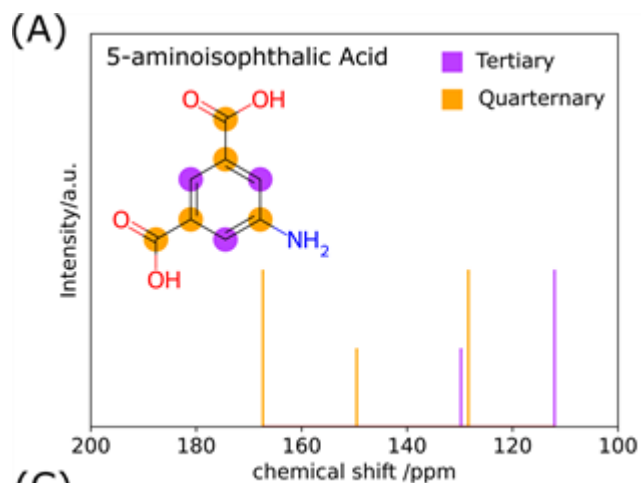
This analytical approach is the first experimentally quantifiable method to defining molecular assembly, a reliable metric for complexity, as an intrinsic property of all molecules and can also be performed on complex mixtures.

Conclusions

With these results, there is now a methodology of spectroscopic techniques to unambiguously detect alien life in the solar system, and beyond on exoplanets.

Reference

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2. Bame, J., Jirasek, M., Sharma, A. et. al. Multimodal Techniques for Detecting Alien Life using Assembly Theory and Spectroscopy Feb. 2023 arXiv:2302.13753[q-bio.QM]



P-329 - Unexpected functional plasticity in a constrained toxin domain: converging insights from NMR and MD simulations

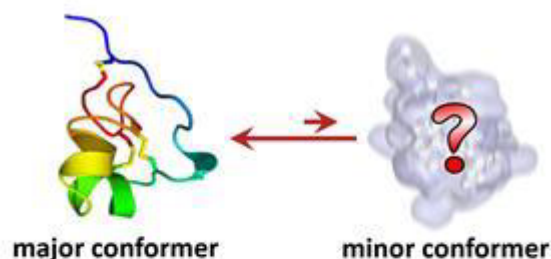
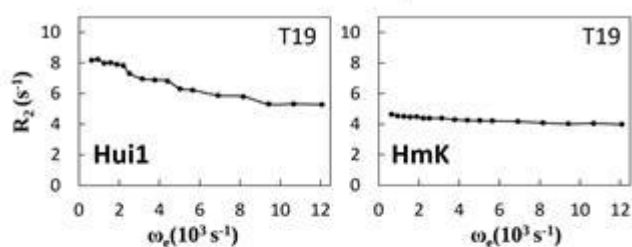
Ms. Chen Timsit¹, Dr. Inbal Sher¹, Dr. Chaitanya Chiliveri², Dr. Adriaan Bax², Prof. Dan-Thomas Major¹, Professor Jordan Chill¹

¹Bar Ilan University, Ramat Gan, Israel, ²NIDDK, National Institutes of Health, Bethesda, USA

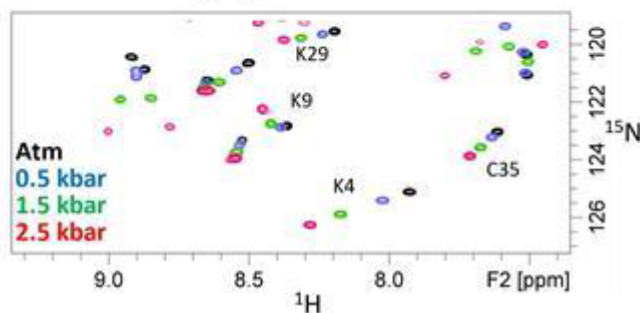
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The first decades of research in structural biology were guided by the structure-function dogma, emphasizing the importance of the static protein conformation. Gradually it has been recognized that protein dynamics are also instrumental in modulating their biological function, raising interest in disordered protein segments, scarcely populated conformations, and 'invisible' states. This paradigm shift propels NMR methods, capable of following biomacromolecular dynamics at multiple molecular sites and under native conditions, to the forefront of mechanistic studies. Here we employ a range of solution NMR applications to investigate functional dynamics in toxins of marine origin and their binding to the bacterial potassium channel KcsA. SAK-I toxins bind to the turret region and inhibit ion conduction in K⁺-channels, with subtle variations in sequence determining their affinity and selectivity. Despite their compact appearance (35 amino acids, 3 disulfide bonds) they present a lowly populated minor conformation, most likely involving 'switching' around disulfide bridge dihedral angles, with an estimated lifetime of 0.2-0.5 ms. We probe the nature of these 'invisible' conformations using two-field relaxation dispersion measurements and NMR under hydrostatic pressure. A comparison between two KcsA inhibitors, the naturally occurring HmK and the de novo Hui1, is particularly interesting in that they exhibit very different dispersion and pressure profiles despite obvious structural similarities. These observations are complemented by analysis of molecular trajectories of toxin domains created using replica-exchange metainference/metadynamics simulations that provide structural insight into the nature of minor conformations and how they may influence channel recognition. This is an excellent example of how seemingly structured domains harbor latent dynamics with functional implications, also demonstrating unique and newly emerging abilities of NMR to address protein dynamics.

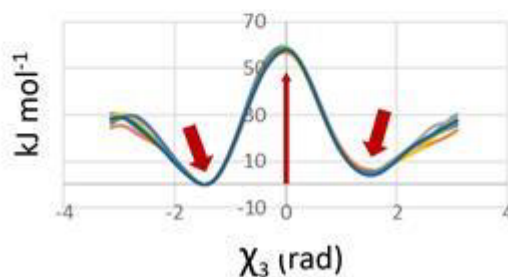
Relaxation dispersion



High-pressure NMR



Computational: metadynamics



P-283 - Understanding the Membrane-Bound Structure of CLIC1 and Developing Selective CLIC1 Inhibitors with Antiproliferative Activity

Miss Victoria Cheung¹

¹University of Kent, Canterbury, United Kingdom

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Glioblastoma multiforme (GBM) is the most aggressive and prevalent form of primary brain cancer and its poor prognosis makes GBM a public health concern. Increasingly, evidence points towards Chloride Intracellular Channel 1 promoting oncogenic development with its high level of activity and expression during tumorigenesis. CLIC1's unique 'moonlighting' abilities means that it may serve separate functions at both the cytoplasm and membrane. Intriguingly, the metamorphic nature of CLIC1 serves as a biological switch for malignant transformation in which only the membrane-bound form is carcinogenic. This distinct feature could pave way for a new selective, conformation-specific cancer therapy which would potentially spare normal cells making CLIC1 a highly promising pharmacological target.

Our research focuses on understanding the membrane-bound structure of CLIC1 as this is currently unknown and developing selective CLIC1 inhibitors with antiproliferative activity for the treatment of glioblastoma. To explore the structure of the membrane-bound form, we have used solid-state NMR and TEM/CryoEM with CLIC1 inserted into vesicles/nanodiscs. To detect drug binding, we have performed solution-state NMR and X-ray crystallography of the soluble form of CLIC1 to a panel of FDA-approved compounds derived from an in silico drug screen to probe drug interactions. To test inhibition, we performed viability assays in U87 human glioblastoma cells in the presence and absence of the drugs.

P-015 - NMR Depth Profiling of Painted Walls: Ostia Antica

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The mortar-layer stratigraphy of Roman wall paintings at Ostia Antica, the historic port of ancient Rome, was studied at two occasions with nuclear magnetic resonance (NMR) depth profiling [1] to explore manufacturing details in the context of the use and history of the buildings. In the first campaign in 2019 the NMR signature of covered wall paintings was studied [2,3], while in the second campaign in 2022, depth profiles were compared to answer the question if different painted walls were prepared for painting at the same time or by the same workshop [4]. The experience gained in handling the portable NMR hardware for onsite measurements has stimulated the development of an improved NMR depth-profiling instrument [5,6]. The information provided by NMR depth profiling is illustrated with experimental data, and progress in hardware development is reported.

P-303 - Assessing different ways of determining affinity by NMR in theory and practice

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Affinities of biomolecular interactions are important in biochemical research and drug discovery. With NMR spectroscopy even weak interactions can be studied in solution close to physiological conditions and without the need for chemical labeling of the molecules. However, the established NMR methods still lack the possibility of determining high affinity binding with Kds in the nM range, which are a necessity for clinical drugs. Furthermore, imprudently chosen parameter for titration experiments can severely limit the reliability of the results. In order to choose optimal parameters, simulations for different approaches were done, including:

- Protein-observed experiments in the fast and slow exchange regime
- Ligand-observed experiments in the fast exchange regime and CSAR experiments
- Competition titration experiments in the fast and slow exchange regime

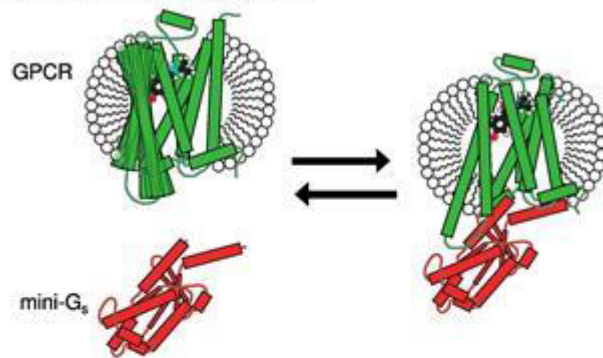
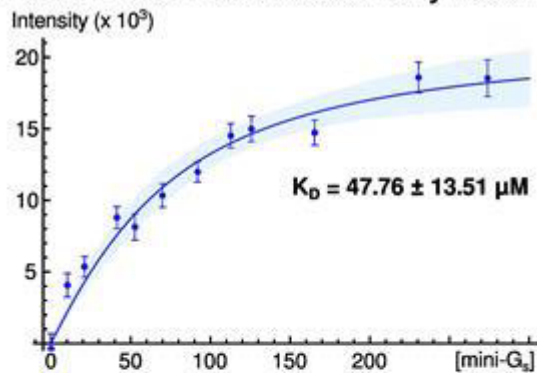
Based on these simulations, the influence of different error sources was investigated, such as concentrations and NMR readouts. Titration patterns were optimized for high accuracy

Using this knowledge, affinities of various ligands for different drug targets can be determined. For FKBP, a key enzyme in the human immune system, protein observed experiments were used for potential immune suppressants with affinities in the medium micromolar range. For Ablason kinase, the central protein in CM-Leukemia, and human β_1 adrenergic receptor, the pivotal protein of human cardiovascular regulation, ligands were examined using competition titration in the slow exchange regime.

Conclusion:

Several approaches have emerged for affinity determination by NMR. Our theoretical analysis helps in experimental design of different types of titrations and assessing their range of validity. Based on this knowledge, we show experimental approaches for important drug targets, which can serve as models for general Kd measurements with a large variety of targets and affinities.

Part I: Protein observed affinity determination on human GPCR

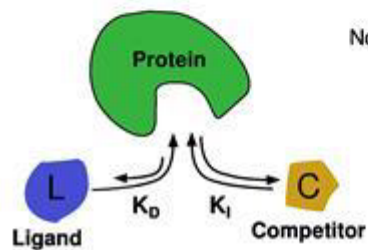


Part II: Analysis of NMR methods for affinity determination

Observed compound

Titrated compound

Exchange regime



No Competition

Competition

			mM	K_D range μM	nM
		either			
		either			
		fast			
		slow			

P-105 - NMR titration studies of the integrin binding to paxillin lim2/3 domain mutants

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Integrins are a family of heterodimeric transmembrane proteins that link the extra- and intracellular environments, mediating signal transduction as well as transmission of mechanic forces. [1] It was recently shown that the focal adhesion-associated adaptor protein paxillin [2] binds to the cytoplasmic tail (ct) of $\beta 3$ -integrin. The 3D structure of paxillin's LIM2 and LIM3 domains was determined, and the binding sites involved in this interaction were characterized. [3] $\beta 3$ -integrin is involved in $\alpha V\beta 3$ and $\alpha IIb\beta 3$ heterodimers formation, which are essential for platelet functions, making $\beta 3$ -integrin an attractive model for studying integrins. At the same time, $\beta 1$ -integrin is much more promiscuous and forms at least 12 different types of heterodimers with α -integrins. [1] In light of the foregoing, we focused on the identification and characterization of $\beta 1$ integrin ct binding sites on the LIM3 domain of the focal adhesion-associated protein paxillin. NMR titration studies of paxillin LIM2/3 wt with $\beta 1$ -integrin indicated a similar binding motif as for $\beta 3$ -integrin, but with approximately one order higher affinity.

To confirm the putative integrin-binding motif in the paxillin LIM3 domain revealed in earlier studies, the alanine scanning mutational analysis was applied. A series of alanine mutants were prepared and characterized. Subsequently, a four-alanine mutant was prepared. NMR titration studies of paxillin's 4A mutant with β -integrin ct showed a substantial difference in observed affinity compared to the wild-type protein.

Characterization of how peptides derived from the cytoplasmic tail of $\beta 1$ -integrin and $\beta 3$ -integrin can be recognized by the adaptor protein paxillin could provide detailed insight into integrin activation, which is important for a multitude of physiological and pathophysiological processes.

Literature:

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P-045 - Auto-Inhibition in the Signal Transducer CIN85 Modulates Activation-Induced Response in B cells

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The successful activation of the B cell receptor (BCR) signaling pathway is crucially dependent on the organization of signaling intermediates within phase-separated condensates before receptor stimulation. Past research has focused on the formation of these liquid-like condensates, but there are still open questions about how the proteins facilitate their signaling state after BCR stimulation. This study introduces a novel intramolecular interaction between the third SH3 domain (SH3C) of CIN85 and a proline-rich motif (CIN85-PRM1) located in the adjacent linker region. We used high-resolution nuclear magnetic resonance (NMR) experiments to assign the intrinsically disordered linker via ¹³C-direct-detection. The determination of residue-specific effective correlation times using TRACT yielded the effective molar fraction of the intramolecular complex of CIN85-PRM1 bound to SH3C. Moreover, we observed that phosphorylation of a nearby serine residue likely regulates this interaction in a context-sensitive manner, which could allow for a dynamic modulation of CIN85's valency towards SLP65 by the cell. The comparison of in vitro phase separation assays with computer simulations of SLP65/CIN85 condensate formation using LASSI supported this mechanism of tuning CIN85 SH3 domain availability. In vitro cell culture experiments further revealed that the CIN85-PRM1/SH3C interaction is crucial for maintaining the physiological level of SLP65/CIN85 condensate formation, activation-induced membrane recruitment of CIN85, and subsequent mobilization of calcium ions. Furthermore, using label-free mass spectrometry, we found evidence supporting the involvement of this interaction in the regulation of steady-state and activation-induced cytoskeletal reorganization. Therefore, the CIN85-PRM1 interaction may be a means for the cell to allow for a dynamic and contextual response, depending on the cell's activation state.

P-165 - A compact short deadtime stray-field NMR sensor

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With conventional NMR and MRI instruments, samples are inserted into a magnet enclosing the sensitive volume, thus limiting sample size and accessibility. Single-sided NMR makes use of a permanent magnet placed close to the sample surface, enabling nondestructive analysis of arbitrarily sized objects for materials testing and in-situ studies, for example, at archaeological and cultural heritage sites by means of NMR depth profiles.

This work reports progress in constructing a single-sided NMR system with 1) short echo times capable of sampling rapidly decaying echo trains of solid materials and bound water at larger than 15 mm depth access and 2) a sensor system that simplifies rapid alignment of the sensitive slice parallel to the surface of the object.

A novel analog frontend was designed for a conventional PM25 NMR-MOUSE sensor and a smaller, custom-built magnet for 5 mm depth of access following a modified magnet design. The electronics combine a current-mode switching power amplifier which drives the excitation coil, a fast Q modified switch, and a newly proposed readout scheme to achieve both the required excitation currents at the operating frequency and a low noise figure in the receiver. Ring-down times below 5 μ s can be achieved with the new front end.

An array of four high-precision ultrasonic distance sensors for rugged industrial environments was mounted on the magnet to support the operator in aligning the sensor with the object's surface. The alignment system was calibrated and tested with a silicon rubber layer confined between two glass slides by recording the width of the signal-amplitude step in the depth profile. A resolution of 200 μ m was achieved with 6 μ s long RF pulses. Once calibrated the stray-field sensor can be aligned with the object surface in less than one minute.

Supported by DFG grant DFG AN 984/24-1

P-231 - Insights into the coating effects on NMR relaxation properties of iron-oxide based nanoparticles.

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Introduction

Magnetic nanoparticles (MNPs) can be used as MRI contrast agents, with the aim to improve the detectability of certain tissues/structures. The spin-lattice and spin-spin interactions - which describe the NMR relaxation process of the hydrogens' nuclear spins - depend on several factors, e.g. the chemical environment of the nuclei, the molecular motion, the magnetic field, the temperature, and so on. The MNPs coating could have a role in tuning the magnetic properties and the spin dynamics, consequently affecting the nuclear relaxation times which characterize the process.

Aims

We attempted to detect possible effects of the MNPs core-surface spin disorder on the ¹H-NMR nuclear relaxation times in iron ferrite core-shell NPs.

Methods

We investigated: (1) spherical γ -Fe₂O₃ based MNPs (core diameter $d \sim 4.4 \pm 0.7$ nm), coated with PAA or DMSA; (2) spherical γ -Fe₂O₃ based MNPs ($d \sim 8.9 \pm 0.9$ nm), coated with APPA or DMSA. The ¹H-NMR T₁ and T₂ measurements (from which r_1 and r_2 relaxivity profiles were estimated), were performed in the range 10 kHz÷298 MHz for the first set and 10 kHz÷60 MHz for the second one.

Results

Within the experimental error: (i) both r_1 and r_2 show no difference for 8.9 nm samples with different coatings; (ii) the smallest set displays NMRD profiles with significant differences in the r_1 intensity and frequency behavior between 4.4@DMSA and 4.4@PAA.

From the longitudinal ¹H-NMR relaxation data fitting by the Roch-Müller-Gillis model, we obtained further information about the minimum approach distance, the magnetic anisotropy and the Néel relaxation time.

Conclusions

In the smallest MNPs, due to the higher surface-to-volume ratio, the surface spin dynamics and/or the spin topology are affected by the polymeric layer, whose "interaction" with the core surface spins might influence the magnetic dynamics of the MNPs.

P-379 - Structure and dynamics of photochromic rare-earth oxyhydrides

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Oxyhydrides of rare earth metals such as Yttrium, Scandium and Gadolinium, are mixed-anion compounds showing reversible darkening under UV irradiation. The underlying mechanism of this intriguing phenomenon is not well understood. The empirical formula of these systems is $\text{ReOxH}(3-2x)$. It has been observed that the arrangement and composition of the anions, particularly the hydrides, dictate the efficiency of the photochromic behavior in these materials. Nevertheless, the exact arrangement of the anions in the FCC cation sublattice is not known in detail. A better insight into the local environment of the ions is key to understanding the underlying mechanism of the colour change. We use ^1H , ^{89}Y and ^{45}Sc and ^{17}O solid-state NMR spectroscopy and DFT calculations to unravel different hydride, yttrium and scandium and oxygen local environments in YHO and ScHO films. The limited sample amounts (1 μm films) is challenging for NMR, but nonetheless the hydrogen content of the samples can be determined. The lattice structure, oxidation state of the anions and cations and mobility of hydrogen species are investigated in detail. Preference for formation of hydride rich and hydride poor domains over complete mixing of the oxide and hydrides was noted in the anionic sub-lattice. Presence of a small percentage of hydroxyl groups and trapped molecular hydrogen was proved by ^1H and ^{17}O NMR and DFT modelling for the first time. We are exploring in-situ illumination in view of a better understanding of the structure-function relationship. This can lead to applications in smart windows. Moreover, compounds with mobile hydrides form an interesting class of novel electrolytes.

P-197 - Hyperpolarization of biomolecules in eutectic crystals at room temperature using photo-excited electron

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Dynamic nuclear polarization (DNP) has been a powerful technique that utilize polarization of electron spins to dramatically increase the NMR signal intensities. DNP using the photo-excited triplet state (Triplet-DNP) have potential to provide highly sensitive NMR spectra at room temperature for various molecules; however, applicable molecules were very limited due to poor solubility of pentacene. Here, we focus on Triplet-DNP of eutectic crystals that are mixtures composed of two types of molecules.

P-035 - Conformational dynamics dictate Histone de-acetylase activity and inhibitor potency

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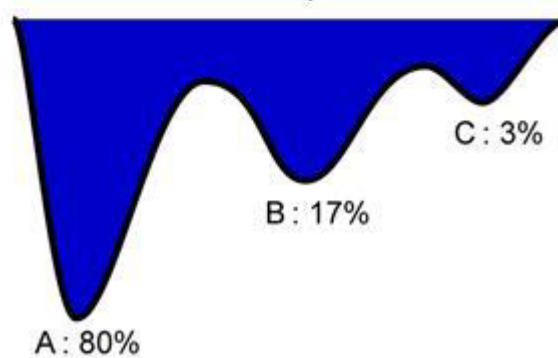
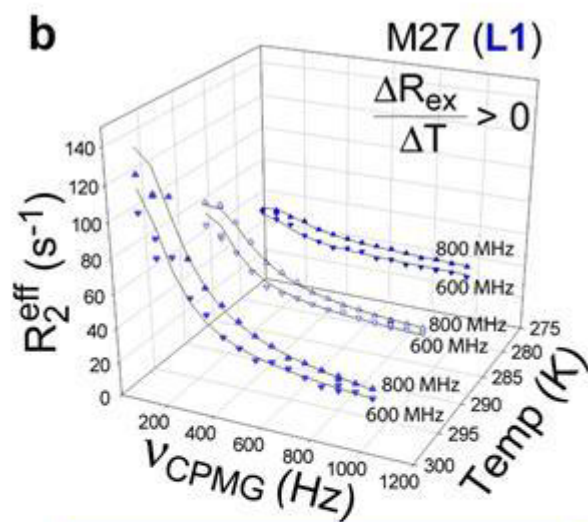
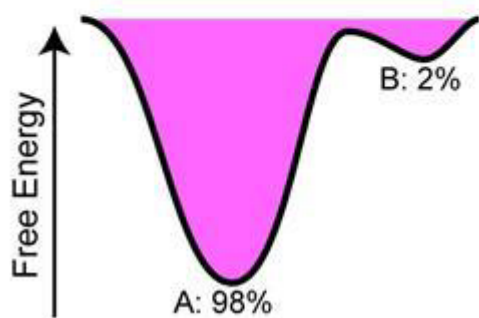
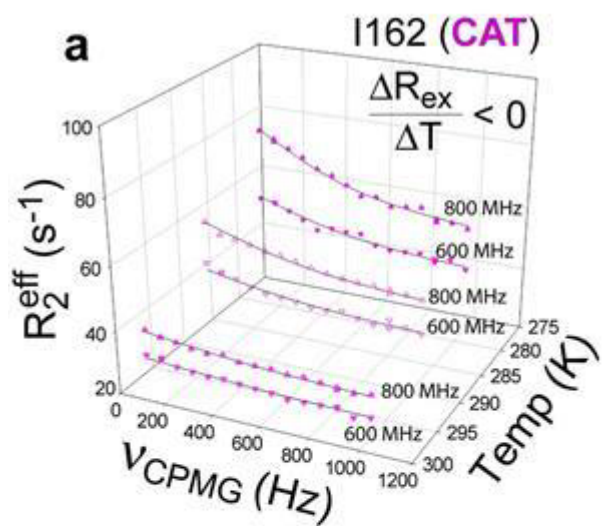
Introduction: Structural visualisations of enzymes predominantly focus on majorly-populated conformations, and single-point mutations that affect enzymatic activity often produce structures that are similar or even indistinguishable from the structure of the wild-type/active enzyme. Likewise, in the histone deacetylase 8 (HDAC8) enzyme, which is a potential target of cancer, wild type and mutant HDAC8s show very similar structures when bound to substrate/inhibitors. Therefore, static structures have not been able to adequately explain observed changes in biochemical activity and substrate/inhibitor binding affinities for various HDAC8 mutants. This implies that a dynamic sampling of various conformations could be imperative for the function of HDAC8.

Aims: Enzymes sample different conformations, and these are often critical for biological function. Our aim here is to characterise the coupling between intrinsic structural dynamics and enzymatic activity for HDAC8 and for enzymes more broadly, to enhance our understanding about enzymatic regulation and to deduce the effect of missense mutants.

Methods: We used methyl-TROSY based CPMG relaxation dispersion experiments to characterise intrinsic higher energy conformational states of HDAC8 and relate the population and lifetimes of these states to enzymatic activity.

Results: We show that the dynamic sampling of different conformations of HDAC8 dictate the enzymatic activity, inhibitor affinity, and inhibitor residence time. Reduced enzymatic activity as well as reduced inhibitor affinities and residence time for two missense mutants of HDAC8 are all captured by the microkinetic rate constants between intrinsically sampled conformations obtained independently by NMR CPMG relaxation dispersion methods. Our analysis of HDAC8 also dissects the functional role of the conformations sampled, where specific conformations, distinct from those in available structures, are responsible for substrate/inhibitor binding, catalysis, and product dissociation.

Conclusions: Our findings assign the functional roles to conformations of HDAC8, but also have general implications for understanding the mechanisms of missense mutations, including those implicated in diseases.



P-355 - Protein engineering for Structural Biology

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Highly purified and isotopically labeled proteins are essential to modern NMR technology. Over the past decades, tremendous efforts and consequently significant advancements have been made throughout the structural biology community to improve quality and quantity of protein samples. We present here two improved protocols: first -segmentally labeling of protein subunits using the Sortase A mediated Ligation (SML); second - a simplified and highly effective protocol for perdeuteration of recombinant proteins.

Domain-specific isotopic labeling (segmental labeling), in which only selected domain is labeled within the context of the whole protein, can significantly simplify NMR spectra and reduce signal crowding. Here, we evaluated the use of different variants of *Staphylococcus aureus* sortase A for a range of ligation reactions and demonstrated that conditions can readily be adapted to meet specific goals. In addition to producing high purity, selectively isotope labeled multidomain proteins for NMR studies, we also explored a broader range of other SML applications, such as lipidation to mimic posttranslational modifications, and protein circularization to aid in the development of nanodisc membrane mimetics. We anticipate that our findings in SML may enable many applications in structural biology.

Highly deuterated protein samples provide contrast matching in neutron diffraction experiments and reduction of dipolar spin interactions from normally protonated proteins in magnetic resonance studies. In NMR applications, deuteration is often combined with other isotopic labeling patterns to expand the range of conventional NMR spectroscopy research in both solution and solid-state conditions. We report here a simple, effective, and user-friendly protocol to produce highly deuterated proteins in *Escherichia coli* cells. One liter expression typically yields 5 to 50 mg of highly (>96%) deuterated protein. The protocol will enable a broader utilization of deuterated proteins in a number of biophysical techniques.

P-317 - What's in a biomolecular NMR structure? – A detailed structural analysis of RNA tetraloops by NMR spectroscopy

Mr Andreas Oxenfarth

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Biomolecules like proteins and RNA adopt intricate three dimensional structures but their function is often linked to their inherent conformational dynamics. For RNAs that are often highly dynamic, NMR spectroscopy is the best method to determine the tertiary structure and dynamics. However, to date the NMR structure bundles derived mostly from NOE distance restraints do not reflect the structural dynamics of the RNAs completely.

To understand underlying dynamics, the microscopic description of conformational trajectories is often derived from MD-simulations. Although force-field developments for MD-simulations are constantly improved, accurate force fields that correctly predict both the RNA conformation and its dynamics are still missing.

To change that, high quality NMR data of a suitable reference system are needed. Until now, the UUCG RNA tetraloop was the most widely used reference system due to its wealth of experimental NMR data.

Our work presented here provides an additional dataset of high quality NMR data for the CUUG tetraloop to complement the currently existing one. It shows how multiple NMR methods can be used in combination with MD simulations to elucidate the dynamic structure of the CUUG tetraloop model hairpin. A near complete set of J-couplings, NOE contacts, RDCs and cross-correlated relaxation rates were measured. Evaluation of the CUUG tetraloop data shows that the conformation of the tetraloop cannot be described by a single static state as it was previously stated. This is due to the partial presence of a GC base pair between the first and the last loop residue. To get a better understanding of the structure, molecular dynamic simulations were performed. The resulting structures were further refined using reweighting with the experimental data. Multiple structural clusters are needed to adequately explain the experimentally measured data. In contrast, a single structure like the previously published 1RNG pdb of CUUG is not sufficient.

P-141 - Conformational Flexibility of the Lipopolysaccharide ABC Transporter Elucidated Using Pulsed Dipolar Electron Spin Resonance Spectroscopy

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The outer membrane (OM) of Gram-negative bacteria is an asymmetric bilayer made of phospholipids and lipopolysaccharides (LPS). The lipid A-core oligosaccharide and the O antigen are synthesized in the cytoplasm and are joined at the inner membrane (IM) to form the LPS molecule. In *Escherichia coli*, seven essential proteins, LptABCDEFG form the LPS transport (Lpt) system to export LPS from the IM to the OM.^{1 2} Using pulsed dipolar electron spin resonance spectroscopy (PELDOR or DEER),³ we characterized the conformational heterogeneity of LptB2FG complex, which is the ATP-binding cassette (ABC) exporter for LPS transport from the outer leaflet of IM to periplasmic domain of LptC. Our results are in agreement with previous observations that the nucleotide binding domains (NBDs) close upon ATP binding.⁴ This is coupled to the opening of the lateral gate formed by the transmembrane domain (TMD) helices TM1 of LptF (TM1 F) and TM5 of LptG (TM5-G). At the second other lateral gate formed by TM5-F and TM1-G, the short loops attached to these helices exhibits a dynamic behavior. However, a closed conformation yet unseen from structures is most favored. In detergent micelles and in the absence of LPS, the β jellyroll domains of LptF and LptG shows a rather limited flexibility. Overall, these observations deviate from the general response in which the TMDs undergo a synchronized transition between inward-open and outward-open states. Such an asymmetric behavior of the TMDs might have an important role for LPS transport towards LptC.

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P-211 - SABRE Hyperpolarization with up to 200 bar Parahydrogen in Standard and Quickly Removable Solvents

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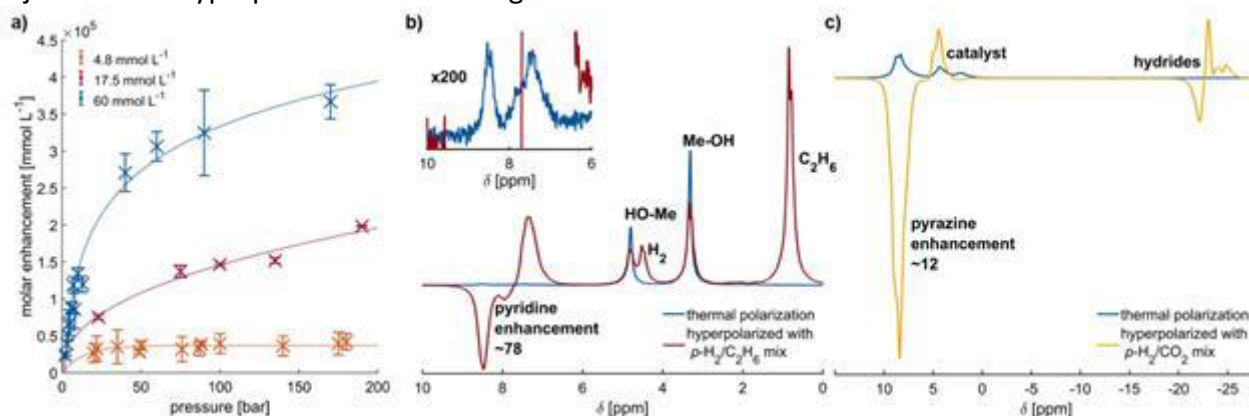
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Parahydrogen based techniques offer a way to drastically enhance NMR signals. Especially, the SABRE variant is very versatile and quick. The broad range of substrates and the opportunity to re-hyperpolarize molecules without altering the chemical structure allows for many different applications. However, the achieved polarization and amount of hyperpolarized material (concentration) are often smaller than in other hyperpolarization techniques or in hydrogenation reactions with parahydrogen. One reason for this can be a limited parahydrogen supply. Additionally in SABRE, to achieve high polarization levels, it is often necessary to use homogeneous catalysts, which need to be removed along with the used solvent for many applications.

In this work, we report on SABRE hyperpolarization up to 200 bar in standard and quickly removable solvents. For this, we employ a recently introduced low-cost, versatile high-pressure setup which enables spectroscopy measurements with a compact NMR magnet. With this setup, high molar polarizations, i.e. high polarization and concentration of the polarized material, can be directly accessed using SABRE. High molar polarizations are typically reached via hydrogenative PHIP or by concentrating SABRE solutions after polarization. We found that, especially at high concentrations, high hydrogen pressures allow for higher polarization: We achieved 2% polarization at a substrate concentration of 60 mmol/l, equal to a molar polarization of 1.2 mmol/l (see Fig. 1a).

Additionally, SABRE hyperpolarization in liquefied ethane and compressed CO₂ at 200 bar was demonstrated (Fig 1b,c). Polarization in liquefied gases could allow for rapid solvent removal by reducing the pressure. Eliminating standard SABRE organic solvents such as methanol in hyperpolarization techniques is a prerequisite for molecular medical research. In this way, a solution of hyperpolarized material in the solvent and concentration of choice can be generated, ready for injection as a hyperpolarized contrast agent.



P-139 - Understanding the Electronic Structures of Transition Metal Complexes using Paramagnetic NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

While acquisition and interpretation of NMR spectra is routine for diamagnetic systems, spectra of systems containing unpaired electrons remain difficult to interpret since i) the chemical shift no longer shows a characteristic response that can be assigned to specific functional groups, and ii) signal line widths can become so large as to obscure an entire spectrum.

Here we demonstrate that when coupled with Density Functional Theory (DFT) calculations, paramagnetic NMR experiments afford a wealth of information not only about the molecular structure, but also the electronic structure of the paramagnetic centre. We calculate the paramagnetic shift of a nuclear spin as the sum of psuedo-contact (dipolar electron-nucleus) and Fermi-contact (unpaired electron spin density at nucleus) contributions, using an electron-nucleus hyperfine coupling tensor (A) from DFT, and a magnetic susceptibility tensor (χ)

$$\delta = \delta^{fc} + \delta^{pc} = A^{iso} \cdot \chi^{iso} + 1/3 \text{Tr}(\Delta\chi \cdot A^{dip})$$

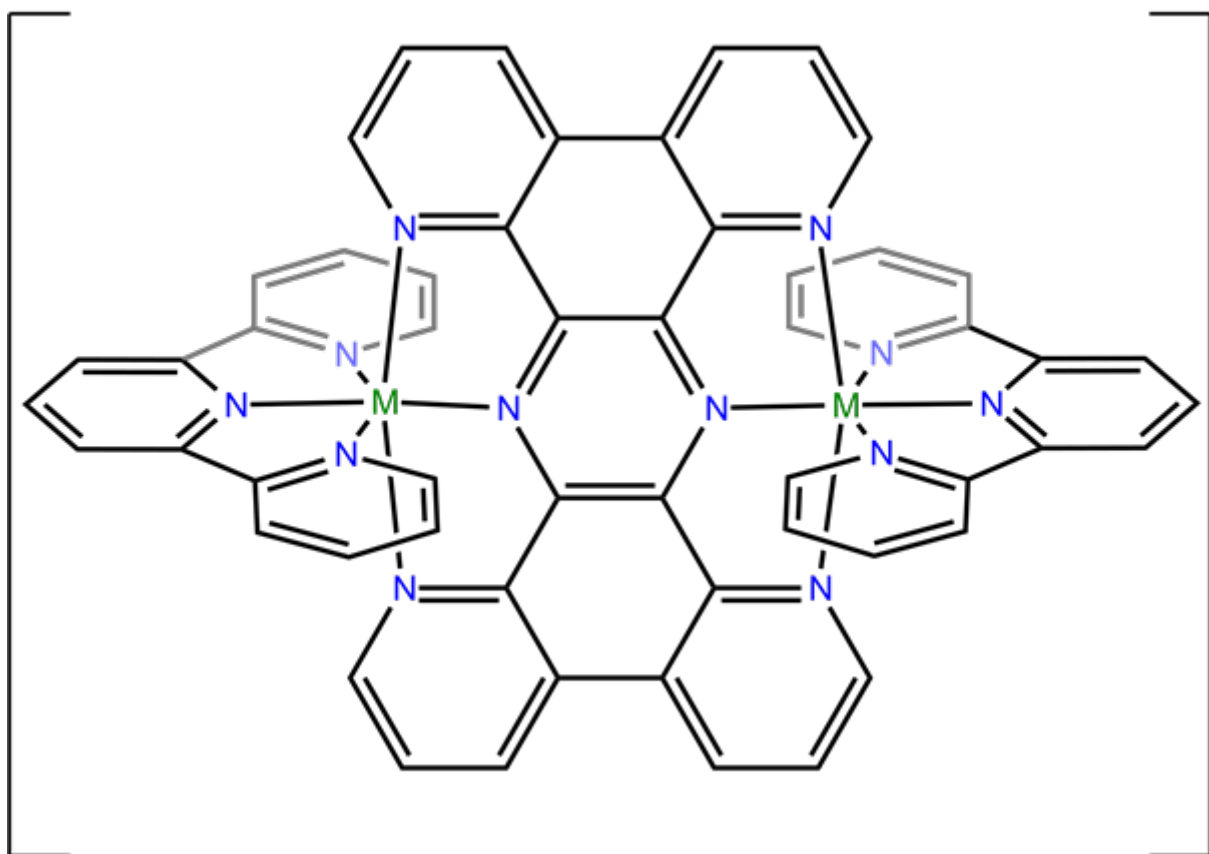
We fit χ such that the difference between calculated and experimental shifts is minimised.

Here, we focus on the dinuclear, bridged, complexes $[(\text{tpy})\text{M}(\text{tphz})\text{M}(\text{tpy})]^{n+}$ ($\text{M} = \text{Ni}, \text{Co}$; $n=4,3,2$; ; tphz= tetrapyridophenazine; tpy= terpyridine, Figure 1), which were first reported by Ma et al. Previously, these have been shown to possess considerably different magnetic properties depending on the oxidation state of the non-innocent tphz ligand. For example, the non-reduced Ni analogue shows anti-ferromagnetic exchange between two Ni(II) centres but, upon reduction, a tphz localised radical electron mediates a large ferromagnetic exchange coupling between the Ni(II) centres. While this interaction has been described using spin-Hamiltonian techniques and powder magnetic susceptibility measurements, here we show that this parameterisation can be vastly improved by fitting the entire susceptibility tensor of the complex from a solution pNMR experiment.

Ma, X. et al. J. Am. Chem. Soc. 141, 7721–7725, 2019

Ma, X. et al. Angew. Chem. Int. Ed. 57, 7841–7845, 2018

n^+



P-009 - Quantification of illicit cocaine samples using BT-qNMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Illicit cocaine samples vary widely in composition, reported to contain several potential harmful adulterants and diluents. Benchtop NMR (BT-NMR) spectroscopy provides a useful alternative to traditional techniques, such as gas chromatography – mass spectrometry (GC-MS) and infrared (FT-IR), for both qualitative and quantitative analysis, due to its low cost, non-destructive analysis. This study showcases a ¹H quantitative NMR (qNMR) method (6 mins), optimised for BT-NMR, with comparisons made to both high field qNMR and GC-MS, for cocaine in both its hydrochloride and freebase form, as well as seven common adulterants.

At least one analyte peak for each component was selected for binary mixture analysis. Calibration standards (0 – 25 mg/mL) were plotted for cocaine hydrochloride and freebase. Linearity was reported between 0.9977 – 0.9995 for the three peaks used for analysis. Limits of detection (LoD) and quantification (LoQ) ranged between 0.30 – 0.59 and 0.90 – 1.71 mg/mL respectively, with good agreement with QC samples ($\pm 2.8\%$) and good precision ($< 5.0\%$ RSD). 59 samples were analysed showing good agreement (0 – 10% deviation) between the three techniques. The methodology was also adapted for “no-D” qNMR, (to reduce cost further), with LoD and LoQ of 1.64 and 4.97 mg/mL respectively. Acceptable linearity (>0.98) was also obtained alongside good agreement with QC samples ($\pm 5.0\%$).

Samples ranged between 27.7 – 98.8% w/w and 20.1 – 94.5% w/w content for cocaine hydrochloride and freebase respectively with pharmaceutically active adulterants identified and quantified within 33.9% of samples.

The use of an accurate and robust method using BT-qNMR allows a rapid portable, and low-cost approach to traditional cocaine analysis for use in on-site harm reduction testing.

P-127 - Deep Learning-Based Multiplet Extraction and Parameter Determination with Transformers

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Introduction:

1D nuclear magnetic resonance (NMR) spectroscopy is a standard analytical technique in chemistry, biochemistry, and related fields. Despite the possibility of performing more complicated and complex NMR experiments, 1D NMR spectra are often preferred when time and simplicity are critical. However, manual evaluation of 1D NMR spectra can be time-consuming and error-prone, especially for complex spectra with numerous peaks. Deep learning has become an effective method for processing and interpreting NMR spectra in recent years. Transformer-based deep learning architectures are potent tools for machine learning tasks that involve processing sequential data, such as natural language and computer vision tasks, with state-of-the-art performance in the latter domains. However, they have yet to find their way into the processing of NMR spectra.

Aims:

Using transformers, we developed an automated algorithm for processing 1D ¹H NMR spectra. It focuses on extracting multiple types, e.g. singlet, doublet, etc., and their parameters, such as chemical shifts and coupling constants.

Methods:

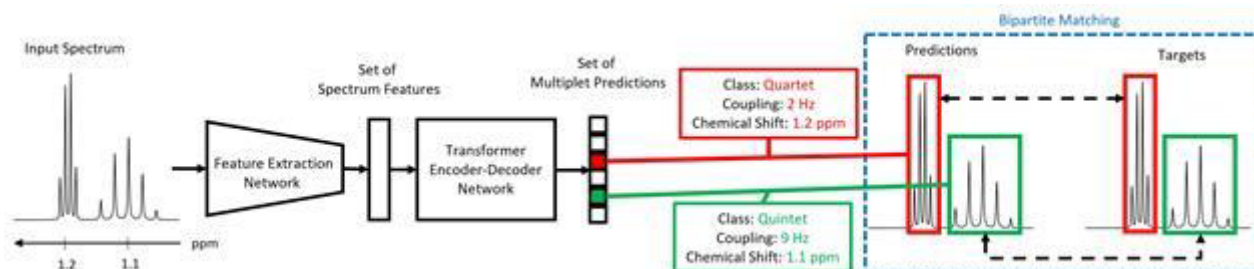
A feature extraction network backbone was combined with an encoder-decoder-based transformer with positional coding that relates these features with objects. The algorithm was trained on an extensive data set of 100,000 synthetic 1D ¹H NMR spectra samples.

Results:

The deep learning-based algorithm extracted multiplets and their parameters from 1D NMR spectra with high accuracy, nearly 100% on synthetic data, and speed. Furthermore, we demonstrate that transformer-based neural networks are valuable for processing experimental NMR spectra.

Conclusion:

Our research demonstrates the potential of transformer-based deep learning algorithms for automating 1D ¹H NMR spectral analysis of small molecules, especially for multiplet extraction and parameter estimation. Compared to manual analysis, the algorithm significantly improves speed and consistency. It can also help NMR specialists to work more efficiently and with fewer errors.



P-179 - Optimal sensitivity regime for proton detected relayed DNP

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Dynamic Nuclear Polarization (DNP) is a powerful hyperpolarization technique that can significantly increase the sensitivity of magic angle spinning (MAS) solid-state NMR. There has been tremendous interest in combining DNP with high magnetic field and fast MAS as these significantly improve ¹H resolution in DNP MAS NMR experiments and allow the implementation of ¹H detected 2D correlation schemes. Recently, we reported the first DNP MAS experiments at 21.2 T using a 0.7 mm MAS probe that enabled spinning rates of up to 65 kHz, at ~100 K [1]. Despite the high enhancements observed in the radical solution, in the context of relayed DNP (R-DNP), faster MAS rates have a detrimental effect on the DNP enhancement. Here we study the effect of faster MAS rates on the absolute sensitivity of the experiment recorded under μ w irradiation.

In MAS DNP, ¹H-¹H spin diffusion is a central component of the hyperpolarization mechanism [2] as it transfers hyperpolarization to the bulk of impregnated materials. Faster MAS rates, on the one hand, increase ¹H sensitivity and, on the other, reduce the level of hyperpolarization in the polarized materials by reducing the spin diffusion rate. We thus predict that there should be an optimal sensitivity regime.

Here we perform R-DNP experiments at fast MAS using 0.7 mm diameter rotors at 21.2 T to obtain a trend of the overall sensitivity as a function of MAS rate. We find that at faster MAS rates, the sensitivity gain due to ¹H detection, overcomes the loss of overall polarization in impregnated materials due to slower spin diffusion.

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P-205 - A flexible p-H₂ delivery system for in situ SABRE hyperpolarisation

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Conventional NMR spectrometers, which use strong magnetic fields to improve sensitivity, are complex and expensive to purchase and maintain. Hyperpolarisation techniques are an alternative route to improve the sensitivity of NMR by increasing the population difference between spin states, breaking the link between magnetic field and sensitivity. Of particular interest here is the Signal Amplification By Reversible Exchange (SABRE) technique, which uses an iridium catalyst to transfer the spin order of para-hydrogen (p-H₂) to a target substrate via a reversible exchange reaction in solution.[1]

Polarisation transfer from p-H₂ to the target molecule in SABRE is mediated by the active catalyst's J-coupling network and requires a polarisation transfer field (PTF) on the order of μT to mT, depending on the target nucleus.[2] Typically this means that the sample must be transported from the PTF to the NMR spectrometer for detection. By using ultra-low-field NMR detection, we can achieve optimal SABRE in situ, providing improved control over experimental parameters such as magnetic field, experiment delays, p-H₂ pressure and mixing. The resultant highly reproducible polarisation levels can be used to directly interrogate and optimise the SABRE process.[3]

We present here a new highly versatile platform for in situ SABRE that provides real-time monitoring and control of the p-H₂ gas flow throughout the SABRE experiment. In this work, SABRE signal is detected by a Magritek Terranova Earth's field NMR spectrometer with integrated field cycling to achieve the desired PTF. This system is used to explore and optimise p-H₂ mixing and the relaxation lifetime of p-H₂-derived spin order throughout the experiment, including imaging of the distribution of polarisation within the reaction cell.

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P-207 - Towards Direct Detection of Hyperpolarization via Parahydrogen Induced Polarization by Signal Amplification By Reversible Exchange at Ultra-Low Field

Mr John Myers

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

One of the biggest challenges of NMR is its lack of sensitivity. This historically has been compensated for by using stronger magnets. At low to ultra-low field (ULF), sensitivity, which is directly proportional to polarization, is worse than three orders of magnitude than that at clinical field strengths. Hyperpolarization techniques, such as parahydrogen (pH_2) induced polarization (PHIP), increase nuclear spin polarization into the range of percent, allowing for detection at ULF without pre-polarization. This opens up the opportunity to perform detection of hyperpolarized signal along the axis of the magnetic field.

Aims/Methods

The aim of this work is the direct detection of the hyperpolarization process along the B_0 field, as polarization occurs for the strongly field-dependent PHIP process. To this end, 99% pH_2 was bubbled into a sample inside of a magnetically shielded room, placed directly underneath a one channel, superconducting quantum interference device (SQUID) gradiometer detection system. Polarization transfer was performed in situ, using the signal amplification by reversible exchange in shield enables alignment transfer to heteronuclei (SABRE-SHEATH) technique with a static B_0 field on the order of 100s of nT, aligned along the sensitive axis of the gradiometer. The build-up of polarization via SABRE-SHEATH was then monitored directly without the use of field switching or rf tipping pulses.

Results/Conclusions

Here, we present the first results of direct detection, showing how vibrational noise can be suppressed to 100s fT/VHz and higher frequency noise (1-10 kHz) kept ~ 200 aT/VHz at fields typically used, when performing SABRE-SHEATH. We show that a minimum polarization of $\sim 0.1\%$ would be necessary to directly detect a nuclear magnetization signal of ~ 2 pT along the B_0 field, from a 3 ml sample of 50 mM $[1-^{13}C]$ pyruvate in our setup. This method can be used to further optimize the SABRE-SHEATH and other hyperpolarization methodologies.

P-147 - Probing methyl group tunneling in dimethylammonium-containing hybrid perovskites using different paramagnetic centres

Mr Gediminas Usevicius

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

At low temperature, methyl group behaves as a quantum rotor, exhibiting rotational quantum tunneling, which is highly sensitive to a local methyl group environment. Recently, we employed Mn^{2+} and Co^{2+} paramagnetic centres to probe the methyl group tunneling in dimethylammonium zinc formate $[(CH_3)_2NH_2][Zn(HCOO)_3]$ (DMAZn) hybrid perovskite using electron spin echo envelope modulation (ESEEM) spectroscopy [1,2]. These studies provided accurate measurements of the methyl group rotational barriers and resolved minute differences in their local environments upon introduction of Mn^{2+} and Co^{2+} ions

Here, we further study the feasibility of different paramagnetic centres to probe the methyl group quantum tunneling by incorporating other transition metal ions (e.g. Ni^{2+} , Cu^{2+}) into the DMAZn structure. To assess the effect of inorganic framework on the methyl group tunneling, we also study the tunneling ESEEM using Mn^{2+} and Co^{2+} ions in a related DMA-containing hybrid perovskite $DMACd(N_3)_3$.

Figure. 1. Low-temperature structure of DMAZn hybrid perovskite. Green lines indicate hyperfine interactions between the paramagnetic transition metal ions and protons of the nearest methyl group.

This project has been funded by the Research Council of Lithuania (LMTLT) (agreement No. S-MIP-22-73).

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P-185 - Hyperpolarized NMR of dilute suspensions – Real-time monitoring of solute-to-solid conversion provides insights into material formation mechanisms

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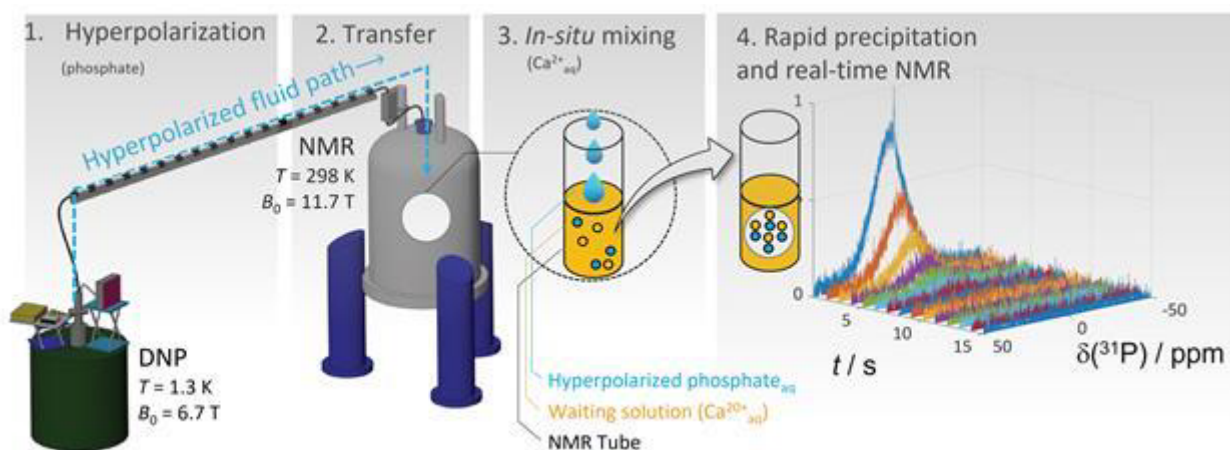
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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Nuclear magnetic resonance (NMR) spectroscopy is a key method for molecular structure determination. Due to its intrinsically high, i.e., atomistic resolution and versatility, it has found numerous applications in gases, liquids, and solids. However, NMR has found little to no application to dilute suspensions of solid particles, albeit their abundance and high academic as well as industrial importance. The resonances of such systems are prohibitively broadened, typically beyond the detection threshold. Herein, we show how to enhance the signals of particle suspensions >1.000-fold using dissolution dynamic nuclear polarization (d-DNP) coupled to rapid substrate solidification. For the proof-of-concept series of experiments, we employed calcium phosphate (CaP) as a model system, as it is essential in applications ranging from biomineralization to heterogeneous catalysis to artificial bone tissue. By d-DNP, we boosted the signals of inorganic phosphate immediately before rapid precipitation in-situ within the NMR spectrometer leading to the inclusion of the hyperpolarized phosphate within milliseconds into tens of micrometer-sized particles. With our approach, we could obtain spectra of dilute solid CaP particles within only 1 second of acquisition time and, thus, characterize the solid-liquid equilibrium in real-time. Integrating the hyperpolarized data with molecular dynamics simulations and electron microscopy enabled us to understand the CaP solidification mechanism in atomistic detail – from precursor formation to solidification kinetics to the precipitation mechanism. The presented methodology might be helpful for research programs aiming to characterize suspended solids, unravel material formation processes, and control the resulting solid-state morphologies.

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P-373 - Hyperpolarized and ultrafast MAS NMR at ultrahigh field for the study of performance polymers: HyperFast

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Solid-state nuclear magnetic resonance spectroscopy has played a crucial role for the investigation of structure and dynamics of polymers relating to their functional behavior. The unique advantage lies in the fact that it probes the local environment of nuclei without relying on their crystalline arrangement. Herein, we will present an example where conventional ¹³C CPMAS NMR present unique advantage over other techniques like FTIR and RAMAN, for understanding the oxidation chemistry of a complex natural carbohydrate polymer (Arabic Gum). The latter has a application in making environmentally friendly paints.

However, in case of complex synthetic polymers with industrial importance, conventional SSNMR is either too insensitive (for low gamma nuclei) or lacks resolution (for example, protons), which limits its use for the routine atomic scale structural studies at pilot scale. We will demonstrate that it is the case for studying the complex synthetic polymers like Polyurethanes and polyaramid (Kevlar) fibers. The complexity lies in their chemical structures possessing polycrystallinity and chain crosslinking, which influence their mechanical strength.

We will discuss two important methodological developments that can remedy shortcomings of NMR for studying these polymers, i.e., ultrafast magic angle spinning (MAS) NMR and Dynamic Nuclear Polarization (DNP). We will present the high resolution 1D ¹H NMR and 2D ¹H detected ¹³C and ¹⁵N NMR under ultrafast MAS at ultra-high magnetic field (>20 T). We further optimize and extend MAS-DNP methodology combined with ¹H ultrafast MAS NMR to provide the chemical and structural understandings of different polyaramid fibers possessing high mechanical strength supported by DFT calculations, and the crosslinking chemistry of three polyurethanes samples that were prepared with identical synthesis procedure, extracted at different time intervals, but found to differ in molecular weights. Finally, we will discuss the advantages and limitations of both the ultrafast MAS NMR and MAS-DNP for these systems.

P-271 - Magnetic resonance biomarkers of radiation dose-rate in glioblastoma cells

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Introduction

The origin of toxicity sparing effects in high dose-rate ('FLASH') radiotherapy at the molecular level is still under investigation. [1,2]

Aims

We detect by magnetic resonance the effects of radiation doses and dose-rates on cells at molecular level. The main aim is to quantify and understand the changes of concentrations of cell metabolites in order to propose magnetic resonance biomarkers of radiation in cells. [3]

Methods and Results

NMR metabolic profiles of irradiated glioblastoma and glia cells were detected within 1-2 hours after irradiation. U251 Glioblastoma cell in culture were irradiated using a Co-60 γ -source and an X-ray clinical irradiator and their metabolic profile was recorded by NMR at different doses and dose-rates. The spectra of non-irradiated and irradiated cells were compared and changes upon irradiation were observed in several metabolites ratios, e.g. [Cho]/[Cre] and [Lac]/[Ala]. The behavior follows prior observations in clinical studies by magnetic resonance spectroscopy in vivo [4].

Conclusions

Molecular biomarkers of radiation in cells are especially useful for short time-scale delivery of photons and particles, e.g. ns pulses of radiation accelerated by high-intensity lasers.[5] Molecular biomarkers detected by NMR can be translated from in vitro studies to in-vivo detection.[4]

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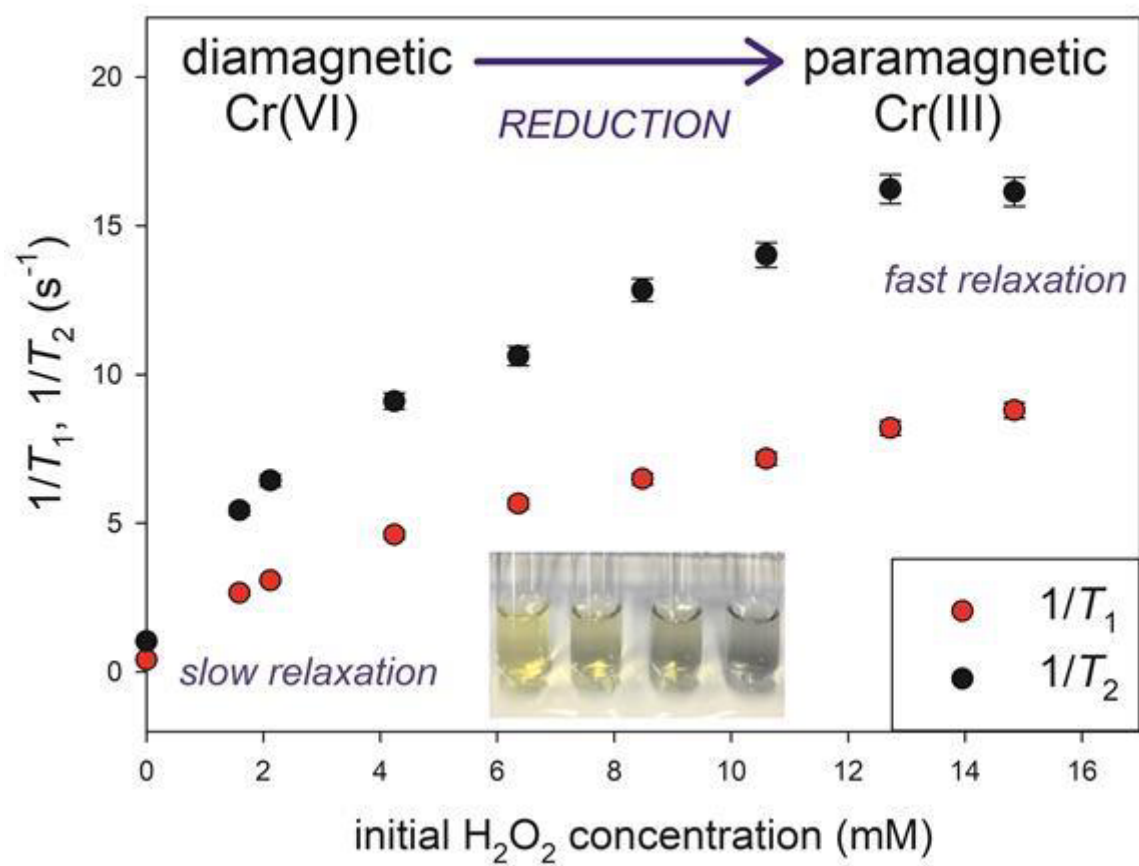
P-007 - NMR relaxometry to monitor chromium (VI) reduction by hydrogen peroxide, ascorbic acid and aluminum powder

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Chromium is released in the effluents of several industries. One strategy to decrease the toxicity of chromium in water is the reduction of Cr(VI) to Cr(III). The monitoring of the reduction is often carried out by measuring the Cr(VI) concentration using the carbazide test. This technique is sensitive but destructive for the sample and can only be used for clear solutions. Here we propose another method for the Cr(VI) reduction monitoring, using the magnetic properties of Cr(VI) and Cr(III): Cr(VI) is diamagnetic while Cr(III) is paramagnetic. From the proton NMR relaxation point of view, a solution of Cr(VI) ions is similar to pure water with long relaxation times (~ 2 s), while a solution of Cr(III) ions presents faster relaxation, because of the coupling of proton spin with the paramagnetic ion. This allows the use of NMR relaxometry for the follow-up of chromium reduction using only a small volume of sample, and even for turbid solutions. Low-resolution NMR devices were used for the measurement of T_1 and T_2 at 20 MHz and 29 MHz. UV-Vis spectroscopy was used to measure the remaining Cr(VI) concentration. The reduction of $K_2Cr_2O_7$ solutions by H_2O_2 was studied at different pHs. $1/T_1$ and $1/T_2$ increased during the reduction of Cr(VI) into paramagnetic Cr(III). The correlation between the relaxation rates and the remaining Cr(VI) concentration in the sample was excellent. Moreover, it was possible to study the reduction kinetics. The reduction of Cr(VI) by ascorbic acid was also successfully monitored by NMR relaxometry. The presence of complexing molecules in the reaction mixture drastically influenced the relaxation induced by Cr(III). Therefore, the comparison of results obtained in different reaction mixtures must be done carefully. Finally, as a proof of concept for a turbid solution, the kinetics of the reduction of a $K_2Cr_2O_7$ solution by aluminum powder was successfully monitored.



P-161 - Practicalities of correcting PFG NMR diffusion measurements for field gradient non-uniformity

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Diffusion NMR is an important technique in many scientific fields, both as a mixture analysis tool and for determining self-diffusion coefficients. Determining the latter with sufficient accuracy and precision is key to many applications. Pulsed field gradient (PFG) NMR experiments have multiple sources of error, including sample temperature gradients and field instability; the former can cause detrimental convection and care needs to be taken to minimise them. Another important source of systematic error, which is the focus of this work, is spatial non-uniformity of the PFGs used. Naturally, NMR manufacturers try to optimise their systems to minimize systematic errors, but uniform field gradients are technically challenging to achieve given the geometric restrictions imposed by probe construction. Non-uniform gradients (NUGs) result in different diffusional decay rates across the sample, producing a net signal attenuation that deviates from the expected exponential decay as a function of gradient squared, and in turn result in incorrect apparent diffusion coefficients. Fortunately, the effects of NUGs can be measured and accounted for. One possibility is to map the spatial variation of the gradient across the active sample volume, and use the information to construct a corrected decay function that more faithfully represents the experimental decay, e.g. by introducing additional parameters to the Stejskal-Tanner equation in the form of the exponential of a power series. The mapping method, although effective, is time-consuming. This work presents a more straightforward and user-friendly approach to finding the coefficients for a corrected Stejskal-Tanner equation, by careful acquisition of data from a sample of known diffusion coefficient. The data processing required is implemented in the open-source General NMR Analysis Toolbox (GNAT), allowing easy correction for the effects of gradient non-uniformity. This approach was successfully tested on a range of probes with different spatial non-uniformities.

P-091 - NMR-detected structural changes of oxidized nucleic acids

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Excessive formation of reactive oxygen species in the cell leads to oxidative damage of DNA, with guanine being most susceptible to oxidation among the four most common nucleobases. Guanines exhibit a lower redox potential when several concurrent guanine nucleobases are stacked in a nucleotide sequence, forming a G-tract. Guanine-enriched regions are predominantly found in promoter and telomere regions of the genome and can form tetrahelical structures called G-quadruplexes. The main building block of a G-quadruplex is the G-quartet, a planar arrangement of four guanines, which are hydrogen bonded in the Hoogsteen geometry. Since oxidized residues exhibit different hydrogen bonding capabilities compared to guanine residues, oxidative damage of guanine-rich DNA may lead to structural rearrangements and therefore affect cellular mechanisms, such as replication and transcription.[1,2]

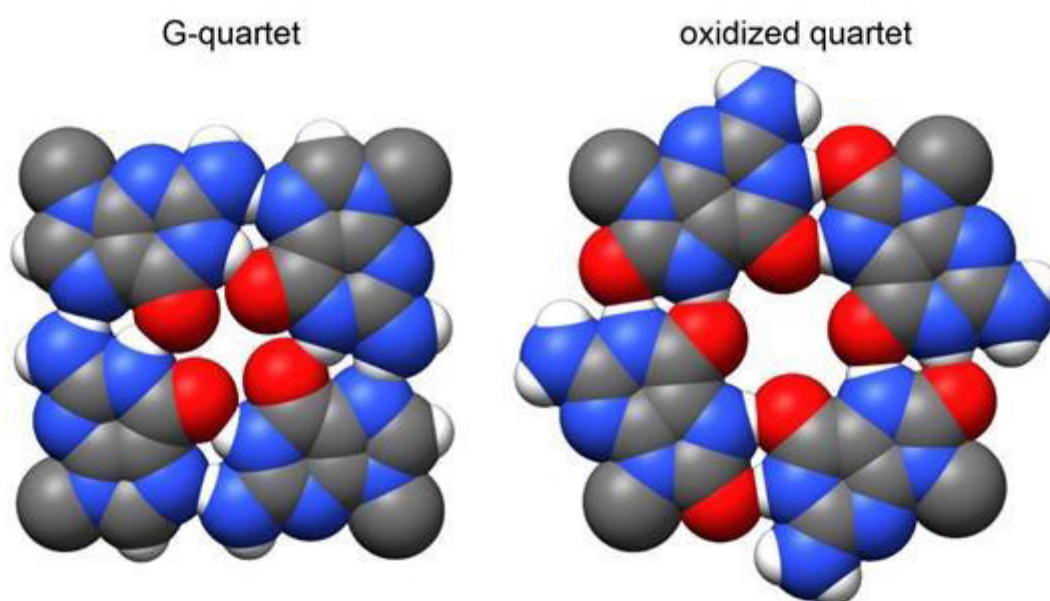
Structural changes caused by an oxidative product of guanine were probed by incorporating 8-oxoguanine into a model oligonucleotide sequence. Using NMR spectroscopy, we determined that oxidized residues do not hinder G-quadruplex formation and that the oxidized residues can form an 8-oxoguanine quartet with a distinct hydrogen-bonding scheme. DFT optimization revealed that the oxidized quartets exhibit a larger central cavity compared to G-quartets, allowing binding of larger cations.[3] In some cases, two species differing only in cation coordination were identified and were found to be in the intermediate exchange regime on the NMR timescale. Our further studies are focused on the effect of guanine oxidation on structural equilibria of dsDNA sequences.

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P-219 - Using ^{17}O solid-state NMR spectroscopy to investigate AlPO-34 and SAPO-34

Miss Emma Borthwick

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Aluminophosphates (AlPOs) are a large class of microporous materials made of alternating AlO_4 and PO_4 tetrahedra. AlPOs, in their undoped condition, have few industrial applications. When other species are doped into the framework, such as silicon resulting in silicoaluminophosphates (SAPOs), this can create active sites, allowing for much wider uses to be found.

AlPO-34 and SAPO-34 possess the chabazite (CHA) framework topology. ^{17}O NMR would be ideal for studying these materials as oxygens link the framework sites, are present in OH groups, and in the water and some of the guest molecules within the pores. Due to its low natural abundance (0.037%), there is a need to enrich materials with ^{17}O to enable spectra to be acquired on a reasonable timescale. Owing to the high cost of isotopically enriched reagents (for example 1 mL of 90% H_2^{17}O (l) costs \sim £2150), there is a need to develop cost-effective and atom-efficient enrichment methods, ideally with lower energy usage. One aim of this work is to use ^{17}O NMR spectroscopy to follow the coking process in industrial catalysts, making the option of post-synthetic enrichment methods desirable.

Here we compare the rate and selectivity of several approaches (including post-synthetic exchange with $^{17}\text{O}_2$ (g), wetness impregnation, slurrying, and steaming) for ^{17}O enrichment of phosphate-based frameworks. The ^{17}O signals in the experimental NMR spectra are assigned using periodic DFT calculations. We show that the substitution of Si into the framework significantly increases the rate of enrichment, but the selectivity of that enrichment varies significantly both with the approach used and the exact conditions employed. Using these approaches we can obtain ^{17}O MQMAS NMR in <24 h using <100 μL of 90 % H_2^{17}O (l). We then apply these approaches to study a series of industrially-relevant SAPO-34 catalysts to understand their catalytic behaviour and coking.

P-017 - Citrullination of arginine-rich regions from RNA-binding proteins regulates their phase separation and nuclear import

Ms Aneta Lenard

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Calcium dependent protein arginine deiminases (PADs) catalyse a conversion of peptidyl-arginine residues to peptidyl-citrulline resulting in a loss of positive charge. The elevated levels of protein citrullination are often seen in patients suffering from multiple sclerosis and several autoimmune, neurodegenerative and cancer diseases. The well-studied PAD4 is involved in chromatin remodelling during inflammation by citrullinating histone proteins, and is the only member of PADs family that contains nuclear localization signal. The recent study reported that multiple RNA-binding proteins (RBPs) contain PAD4-citrullination sites in their disordered arginine-rich regions. Arginine-glycine(-glycine)-rich (RG/RGG) protein regions are highly abundant in RBPs and are involved in a plethora of cellular processes. The misregulation of aberrant liquid-liquid phase separation (LLPS) and membraneless organelles (MLOs) association of RG/RGG regions have been implicated in neurodegenerative diseases. LLPS and MLOs recruitment of these regions is regulated by post-translational modifications, such as arginine methylation and phosphorylation, and by binding to nuclear import receptors, such as transportin-1. Therefore, we hypothesized that citrullination within RG/RGG regions regulates their structural and functional properties. With this project, we aimed at deciphering whether and how PAD4-mediated in vitro citrullination of model RG/RGG regions regulates their phase separation and transportin-1 binding using RG/RGG regions from RBPs FUS, G3BP1, and Nucleoprotein from SARS-Cov2 as paradigms. With the use of solution biomolecular NMR spectroscopy, we demonstrate that arginines in the disordered regions of RBPs are in vitro citrullinated by PAD4. By integrating NMR spectroscopy, DIC microscopy, turbidity and cellular assays, we show that citrullination of RG/RGG regions reduces their RNA-mediated LLPS, RNA-binding, and transportin-1 binding in vitro. Furthermore, we uncovered that methylated arginines are not demethylated or citrullinated by PAD4 in vitro. In conclusion, our findings imply that citrullination of RG/RGG regions regulates their LLPS and transportin-1 mediated chaperoning.

P-249 - Solid-state NMR and Raman study of structural phase transitions in methylhydrazinium-based hybrid perovskite

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Three-dimensional methylhydrazinium ($\text{CH}_3\text{NH}_2\text{NH}_2^+$, MHy) lead halides, related to the famous methylammonium and formamidinium perovskites, are attractive optoelectronic materials crystallizing in polar structures. In this work, ^1H and ^{207}Pb magic-angle spinning (MAS) solid-state NMR and Raman study of the orthorhombic-monoclinic structural phase transition in MHyPbCl_3 is reported.

The temperature-dependent experiments demonstrate that the MHy molecular cations in MHyPbCl_3 are significantly less affected by the temperature-induced structural phase transition compared to the inorganic Pb-Cl framework. This suggests a displacive type of the phase transition dominated by tilting and deformation of the PbCl_6 octahedra. Analysis of the ^{207}Pb MAS NMR spectra reveals the presence of two differently distorted PbCl_6 octahedra and diminishing (increasing) distortion of the less (more) distorted octahedra in the high-temperature orthorhombic phase.

This work was funded by the Research Council of Lithuania (LMTLT) (agreement no. S-MIP-22-73).

P-259 - NMR compatible Bioreactor without Background Signal

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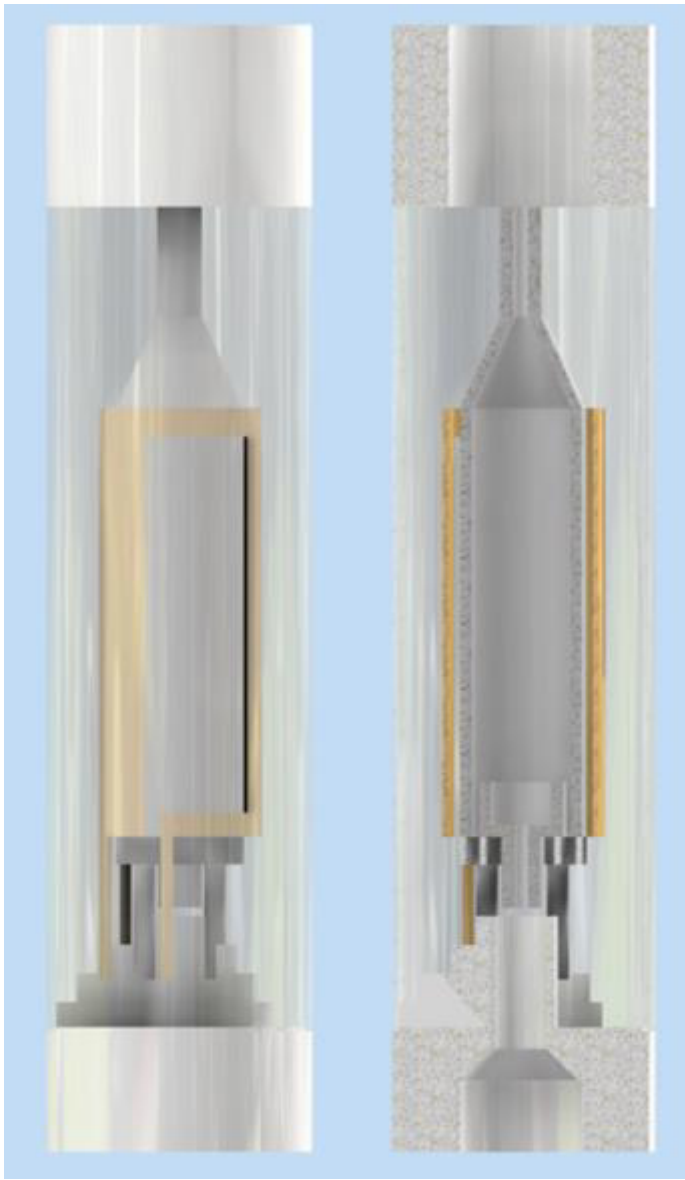
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Studying metabolic pathways or profiles of bacteria through NMR provides exciting opportunities in different fields, including medicine, environmental science, and biology. Since NMR is non-invasive and non-destructive, it allows long term monitoring of chemical processes without having to interrupt and extract samples, or even destroy the samples in the process. Therefore, time-course metabolic measurements of the same sample is possible.

In this work, we present an NMR compatible, rapid manufactured bioreactor for incubation of *Escherichia coli* (Figure 1). It is compatible with a 10 mm coil of a standard high field NMR spectrometer and can be inserted directly into the detection area. This bioreactor ensures the vitality of the organisms and provides a suitable bio- and magnetic field-compatible environment.

The reactor consists of 3D printed ceramic material, and a carbon microfiber matrix for bacterial attachment and growth. Since carbon is a highly biocompatible material, it provides beneficial conditions for the bacteria growth. Furthermore, the material combination enables background signal free hydrogen (¹H) NMR measurements. Since the reactor is 3D printed, it can be individualized to different applications, allowing adjusting the complexity of the systems around it to fit the requirements. Thus, applying a fluidic setup for culture media supply or even other liquids is as possible as simply using it as a sealed bioreactor inside the NMR.

We expect the system to enable the monitoring of bacteria metabolism over longer periods, better correlating to the metabolic rate of *E. coli* cultures. In future designs, medium flow will enable evaluation of the impact of chemicals or substances, like antibiotics, on the metabolic pathway of bacterial cultures.



P-337 - Extracting Chemical Kinetics from a Single Scan NMR Experiment

Miss Annabel Flook

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Solution-phase NMR spectroscopy provides a powerful, yet accessible tool to monitor reaction progress with quantitative concentrations, detailed structural information and suitable sensitivity across several nuclei common in organic reactions. Through mechanistic study, reactions can be evaluated and classified, which can further chemical understanding, support optimisation of known reactions and guide the discovery of new chemical systems. [1]

However, for fast reactions (lifetimes < 10 s), the number of NMR spectra that can be acquired in one reaction becomes a limiting factor. Obtaining meaningful kinetic information for fast reacting systems is more challenging, and requires specialist hardware, such as stopped-flow or rapid-injection coupled with the NMR system. In these cases, experiments must be repeated with increasing pre-scan delay, to obtain suitable data density. This method is both time consuming and susceptible to problems with reproducibility. [1, 2]

In this work, we show that a single NMR experiment holds kinetic information of an irreversible reaction with a lifetime shorter than the acquisition time (5 s). Kinetic data can be extracted by truncation of the start of the FID followed by typical NMR processing (Fourier transform, phasing, baseline correction and integration). This method allows for an increased temporal resolution in the order of 0.1 ms and a window into the reaction kinetics of much faster reactions using a comparable number of experiments and data density to slower reactions.

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Figure: A single FID can be truncated at intervals and upon Fourier transform, spectra are obtained corresponding to changing concentration over time, over milliseconds.

P-265 - Exploring the impact of probiotic supplementation on the faecal metabolome of children with coeliac disease autoimmunity using ¹H NMR spectroscopy

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction. Coeliac disease (CD) is recognized as a lifelong immune-mediated enteropathy manifested as gluten intolerance in individuals carrying specific human leukocyte antigen molecules. In addition to genetics and gluten consumption, other possible factors may trigger the disease. Research suggests that the gut microbiome may be one of them. Probiotics have been suggested as a potential adjunctive therapy.

Aim. Investigate the impact of Lactiplantibacillus plantarum HEAL9 and Lactiseibacillus paracasei 8700:2 supplementation on the faecal metabolome in genetically predisposed children having CD autoimmunity as application of magnetic resonance in clinical studies.

Methods. In the present double-blinded randomized trial, 78 children aged 2–11 years with ongoing CD autoimmunity were recruited by the Celiac disease Prevention with Probiotics (CiPP) study, among which 40 were supplemented with probiotics and 38 with placebo daily for 6 months. Faecal samples were collected every 3 months and analysed using the ¹H NMR for metabolome and 16S rRNA sequencing for bacteriome profiles. The ¹H NMR-based metabolomics approach consisted in spectra acquisition at the proton frequency of 500.18MHz using a 1D NOESY pulse sequence with presaturation (Bruker pulse “noesypr1d”). The spectra were pre-processed with an in-house script consisting of multipoint baseline correction and reduced into predefined bins.

Results. During the 6 months of intervention, the stool concentrations of 4-hydroxyphenylacetate increased in the intervention group as compared to controls, whereas concentrations of threonine, valine, leucine, isoleucine, methionine, phenylalanine, aspartate, and fumarate decreased.

Association between metabolome and microbiome included Pasteurellaceae and Rikenellaceae with glucose and isovalerate.

Conclusions. Probiotic supplementation showed marginal, though significant changes in the levels of amino acids in stool in children genetically predisposed to CD after 6 months of intervention.

Acknowledgements. This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under Grant Agreement No: 874864.

P-001 - Polarization Transfer Methods for Benchtop ^{13}C NMR-Spectroscopy under Continuous Flow

Mr Johnnie Phuong

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Process analytical technologies (PAT) are essential for the effective control and operation of chemical or biological processes. Nuclear magnetic resonance (NMR) spectroscopy is a powerful PAT technique because it is non-invasive and allows fast and calibration-free analysis of complex multi-component mixtures. Only benchtop NMR spectrometers are suitable for process analytics in industry as they are robust and do not require a special infrastructure. However, benchtop NMR instruments have a lower dispersion than high-field instruments. In ^{13}C NMR spectroscopy, this may not be critical, but new problems arise from low signal intensities and premagnetization problems, when flowing samples are analysed. Polarization transfer methods have the potential to solve both problems, because they can enhance the ^{13}C signal and they can improve the premagnetization. However, to the best of our knowledge, polarization transfer methods have never before been applied in benchtop flow ^{13}C NMR spectroscopy. Therefore, in this work, the feasibility of such measurements was shown and their benefits were demonstrated.

Two ternary test mixtures (one with overlapping peaks in the ^1H NMR spectrum and a reference system with well-separated peaks) were studied with a 43 MHz benchtop NMR spectrometer using the polarization transfer sequence PENDANT. The mixtures were quantitatively analysed in stationary and flow experiments. In addition, continuous dilution experiments were performed, mimicking a process monitoring application, and real-time monitoring with ^{13}C NMR spectroscopy with PENDANT in flow experiments was applied. The results were compared with those obtained by standard measurements with ^1H and ^{13}C -spectroscopy and important advantages were found. This demonstrates the high potential of benchtop NMR spectroscopy for applications in process analysis.

P-131 - Deep Neural Network for NMR data analysis

Mr Amir Jahangiri

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

We present a novel approach for processing NMR spectra using a deep neural network based on the WaveNet architecture (WNN). Our method is designed to grasp specific patterns over the entire NMR spectra.

If trained on a fixed non-uniform sampling (NUS) schedule, the WNN benefits from pattern recognition of the corresponding PSF pattern produced by each peak. This results in higher quality of the spectrum and more robust reconstruction relative to results produced by the network trained using random NUS schedules [1].

We demonstrate that the WNN can also successfully perform virtual homo-decoupling in both indirect [1] and direct spectral dimensions. Several other “smart” NMR signal processing will be presented. For example, the WNN can narrow down peaks and improve spectral resolution. Moreover, we demonstrate that our approach often outperforms existing algorithmic methods, and can be used to design new intelligent NMR processing techniques.

Overall, our results highlight the potential of deep learning techniques for improving NMR data analysis.

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P-195 - SABRE Hyperpolarisation of Salpyr Ligands and Their Associated Zn(II) and Cu(II) Complexes

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Signal Amplification By Reversible Exchange (SABRE) is a hyperpolarisation technique that is typically used to polarise small N-heterocyclic molecules. The hyperpolarisation of multidentate ligands and their respective complexes by SABRE has received little attention. Schiff bases, in particular those derived from a salen motif, are therefore of interest. A range of salpyr ligands (Schiff base molecules bearing a pyridyl ring), which have reported biological activity, were synthesised. Ultimately, it was desired to establish how structural complexity affected hyperpolarisation by SABRE. Complexation of the half- and full-salpyr ligands (L1 and L2 respectively) to Cu(II) and Zn(II) further enabled the effect of diamagnetic and paramagnetic metal centres upon polarisation transfer to be measured. These ligands and complexes were analysed by SABRE alongside 3,4-diaminopyridine (DAP) for comparison. The overall enhancements observed for 3,4-DAP and L1 were 484-fold and 133-fold respectively when analysed in d₄-MeOH using a magnetic field of 65 G for polarisation transfer. When d₄-MeOH is exchanged for d₂-DCM, L1 and L2 yielded 26.7- and 13-fold overall enhancements respectively. Comparison of L1 and [ZnL1] was achieved in a 2:1 mixture of d₄-MeOH and d₆-DMSO and yielded enhancements of 15-fold and 15.2-fold respectively. [CuL1] did not show any enhancement; this is presumed to be due to the increased relaxation rate of the hyperpolarised state due to the paramagnetic Cu(II) ion. The structural differences from 3,4-DAP to the full-salpyr L2 yield significant reductions to SABRE activity, however, coordination to Zn(II) did not change the observed enhancements. Half-salpyr L1 can be used in the synthesis of a range of asymmetric salen ligands, and the structure-activity relationship of SABRE may be further investigated.

P-157 - A chip-based C-band ODNP platform

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Overhauser dynamic nuclear polarization (ODNP) is an effective method to boost the intrinsically low sensitivity of chip-based low-field NMR systems. Therefore, in this contribution, we extend one of our most recent NMR systems centered on an NMR-on-a-chip transceiver by ODNP capabilities to improve its achievable sensitivity and limit of detection.

The presented ODNP probe includes three main parts: an Alderman-Grant microwave resonator with an outer diameter of 3 mm, a rotatable guard ring, and a solenoid NMR coil with an outer diameter of 1 mm. The former two are printed on a Kapton polyimide film with a thickness of 25 μm using conductive silver ink and a commercially available PCB printer. The central frequency of the printed MW resonator is 4.58 GHz with a bandwidth of 83 MHz. The rotatable guard ring renders the center frequency tunable with a range of 700 MHz. The used fully-integrated NMR-on-a-chip transceiver features a working frequency between 5 MHz and 770 MHz.

We have performed NMR experiments with and without microwave irradiation to demonstrate the excellent performance of the presented ODNP platform. A maximum enhancement factor of 140 is achieved at a power level of 32.7 dBm delivered to the resonator using a commercial power amplifier. Thanks to the relatively low Q factor of the presented ODNP probe, we were also able to observe the hyperfine splitting and Heisenberg spin exchange on the used TEMPO DNP agent by sweeping the MW frequencies. Finally, we verified experimentally that our system is compatible with a multi-tone MW excitation signal that can provide an additional enhancement factor compared to a standard single-tone excitation for DNP agents displaying a hyperfine splitting. As an example, for a 10 mM TEMPO solution in water, the enhancement factor of a three-tone irradiation compared to a standard single-tone excitation is 2.4.

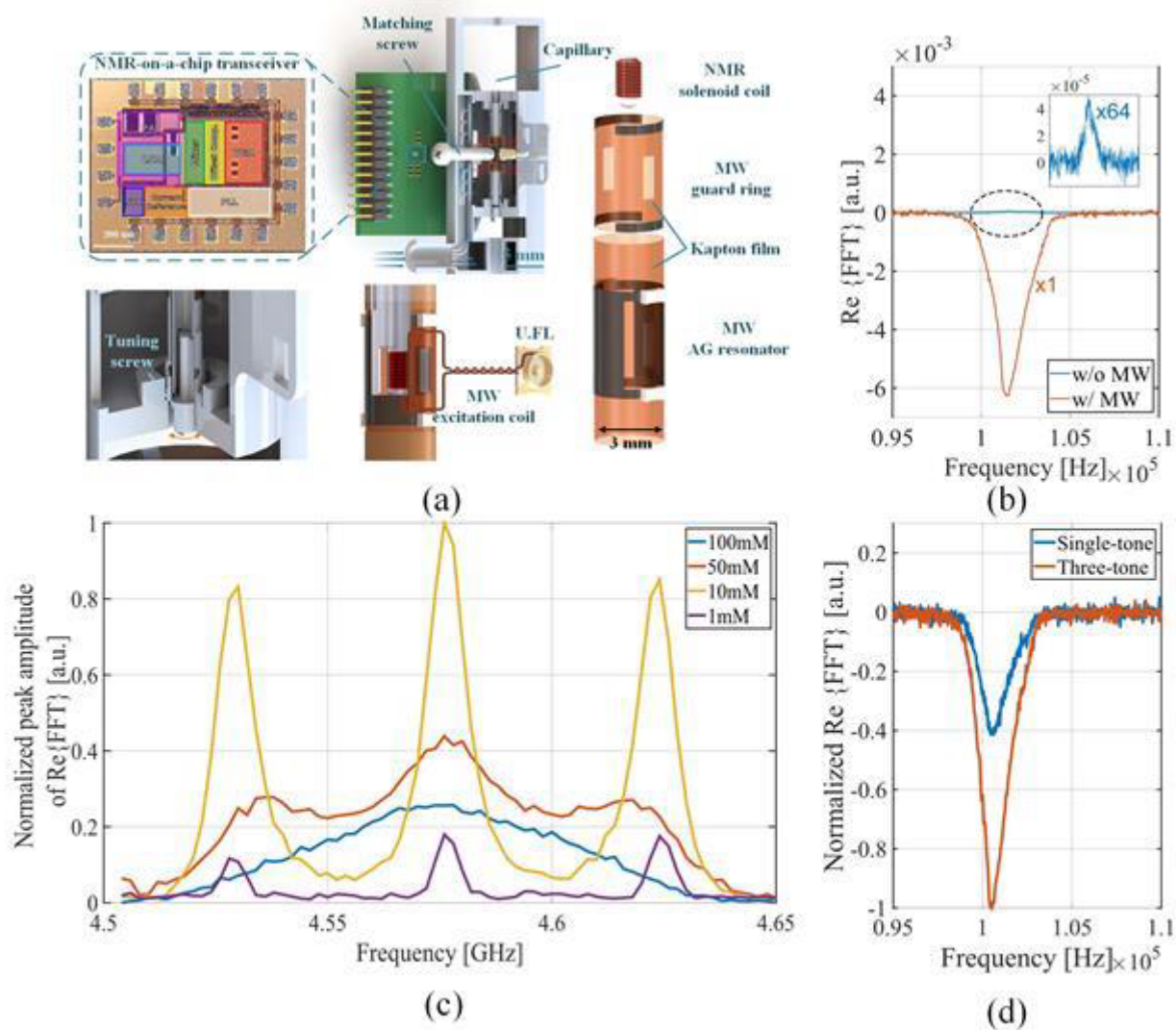


Fig. 1 (a) ODNP probe setup. (b) Real part of the Fourier spectrum of the recorded NMR signal of a sample of TEMPO in water with and without microwave irradiation. (c) Peak amplitude of the recorded NMR signal of a sample of TEMPO in water as a function of MW frequency. (d) Comparison of the NMR spectra obtained using single-tone vs. three-tone MW irradiation.

P-229 - How a tiny switch in silaffine-based peptide linker stereochemistry results in silica rods instead of spheres

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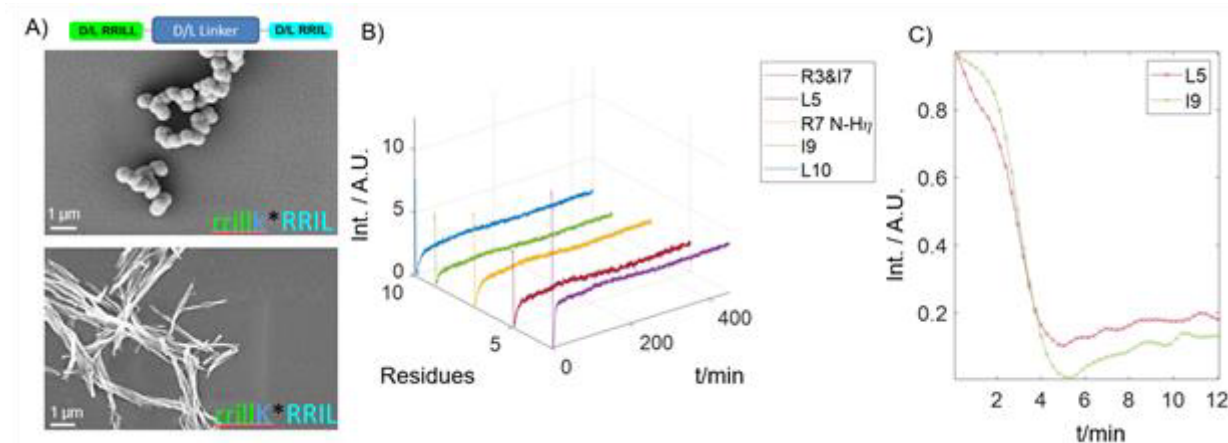
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Nature's ability to shape intricate silica shells under mild conditions serves as an inspiration for rational materials design and as a model system to understand closely related biomineralization processes. Silaffin-based peptide(SBP) are derived from Silaffin 1A₁, a peptide involved in silica shell formation in marine diatoms, by condensing two functional RRIL units by a linker residue. Varying SBP structure and sequence allows for the precipitation of silica tailored to different needs, such as alternative drug administration vehicles.¹ Still, the relation between the chemical structure of SBPs and the morphology of the silica particles they produce is scarcely understood. We present dynamic structural insights into the processes leading to precipitation by real-time NMR spectroscopy. By combining fast-sampling solution-state NMR techniques and MD-simulations, we could elucidate structural changes induced by a switch in linker stereochemistry of the central isopeptide bond in a rrillX*RRIL(X=k,K) peptide, resulting in the precipitation of nanometric needles instead of spheres (Scanning Electron Microscopy(SEM) pictures, Fig.A).

SBPs form supramolecular pre-precipitation assemblies upon exposure to phosphate-containing buffer that facilitate precipitation.² The stereochemistry switch leads to different morphologies in the precipitate - a difference also traceable in the density of the assemblies.¹ Upon exposure of the SBPs assemblies to silicic acid, silica precipitation is initiated. Tracing the changes in intensity in 1D Proton spectroscopy throughout the precipitation event, we observed different kinetics in different residues, showing varying involvement in the process(Fig.B). The initial intensity loss of the central leucine occurs faster than the C-terminal region(Fig.C), a motif conserved from the density in precursors. Merging NMR spectroscopy and MD-simulations enabled precisely defining the relation between structural differences in supramolecular assemblies formed before the precipitation event and tracing their influence as the process unfolds.

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P-019 - Divide-to-conquer a large RNA element: stem-loop element 5 from 5'-UTR of SARS-CoV-2

Ms Klara R. Mertinkus

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

This work shows the potential of solution NMR to enable the characterization of large RNA constructs.

The SARS-CoV-2 virus from the Beta-Coronaviridae family has a large, linear, single-stranded RNA genome. The sequence has regions at its 5' and 3' ends, called untranslated regions (UTR), which are not translated into protein. As these UTRs often form conserved elements, in structure as well as in sequence identity, they are of high interest to characterize. They are potentially involved in RNA-based regulatory functions as well as protein-RNA or RNA-RNA interactions for other cellular or viral purposes.

Stem-loop 5_SL5 (5'-UTR, 5th element) is a ~150-nt large RNA-element. Investigation of this construct by solution NMR proves difficult, due to signal overlap and insufficient resolution. Nevertheless, the chemical shift assignment is possible by using sub-constructs of the big RNA: the divide-to-conquer approach. In this work we show the performed chemical shift assignment of several sub-constructs and the transfer of assignment to the full-length element. Using NMR and SAXS we were able to show the modularity of the system. Aim of the work is an as complete as possible assignment of the large RNA with following structure calculations with the intention of a final 3D model of the RNA.

P-335 - The dipolar field in total correlation spectroscopy

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The dipolar field (DF) – also known as the dipolar or nuclear <i>demagnetizing</i> field – scales with the magnetization and contributes to the magnetic field experienced by the spins, rendering the evolution of the density matrix non-linear. The transverse component of the DF gives rise to a radio-frequency field. While its magnitude is very small under typical liquid state NMR conditions, the DF can induce surprisingly large effects such as multiple spin echos [1] or long-distance inter-molecular cross peaks in 2D spectra [2], in particular after application of pulsed field gradients.

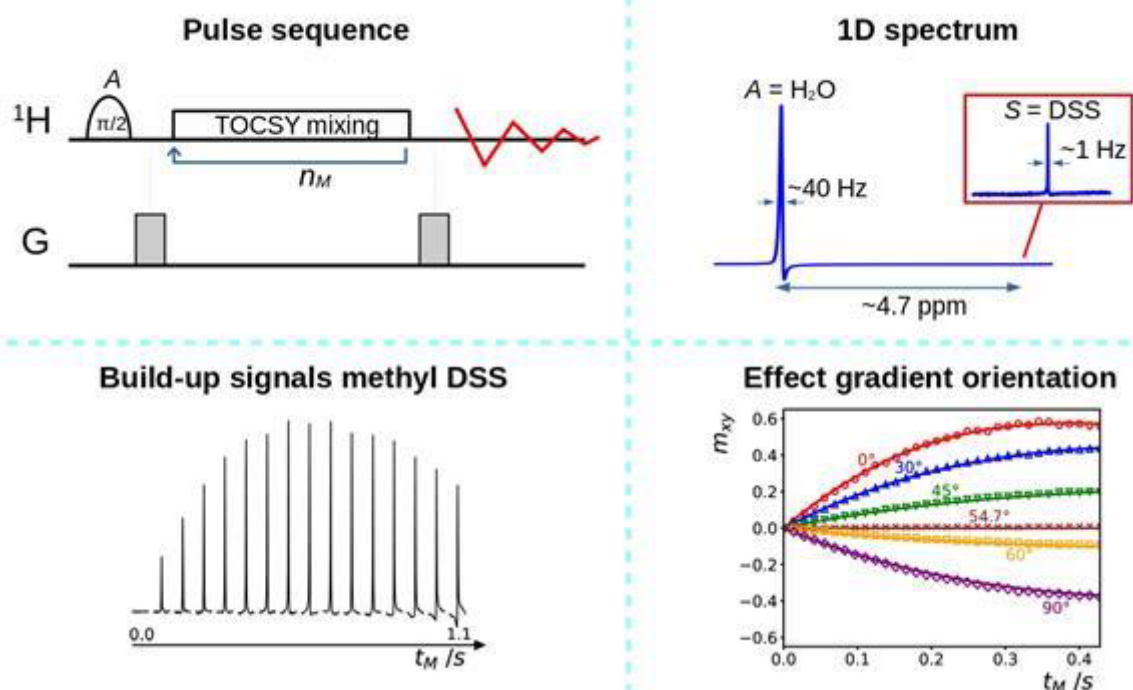
In aforementioned experiments, the DF manifested itself during long free evolution delays, which were interleaved with short hard pulses where it could be neglected. In this work, we investigate – motivated by previous work on radiation damping [3] – the influence of the DF during continuous pulse trains used for homonuclear total correlation spectroscopy. It is shown that, under these circumstances, the DF can mediate inter-molecular transfer of phase coherence from one spin species to another (here, from abundant solvent to dilute solute spins). The efficiency is such that signal intensities close to those of a single pulse experiment can be achieved [4]. A transfer to an opposite coherence order is often privileged. The results are rationalized by numerical simulations with a classical model (using modified Bloch equations) and by a quantum mechanical approach. Several noteworthy features of the coherence transfer will be examined. Finally, the influence of the DF in traditional 2D total correlation spectroscopy will be discussed.

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P-153 - Design and Performance of a Tabletop DNP Polarizer : Towards Recyclable Hyperpolarization

Miss Charlotte Bocquelet

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Hyperpolarization methods provide a way to tackle the inherently low NMR sensitivity and acquire a higher signal intensity in a shorter time. Twenty years ago, dissolution Dynamic Nuclear Polarization (d-DNP) [1] was introduced and is now one of the hyperpolarization methods providing boosts of more than 10'000-fold in sensitivity on a routine basis.

However, this method suffers from two drawbacks narrowing its use. The overall hyperpolarization experiment is i) destructive and ii) single shot. Indeed, the frozen sample, once polarized, is dissolved before being analyzed in liquid state NMR. During this process, it is inevitably diluted, and its signal vanishes within seconds, therefore it cannot be repolarized. On the other hand, the vast majority of NMR experiments rely on coherence selection through phase cycling, and multidimensional analysis, thus requiring numerous consecutive acquisitions, which is impossible today.

We are presently working at turning d-DNP into a new version that will be widely compatible with NMR spectroscopy. It consists in replenishing the DNP hyperpolarization of a sample flowing through a closed loop, without dilution nor contamination using hyperpolarizing silica-based material (HYPSO) as polarizing matrices [2,3] in a compact and helium-free DNP polarizer coupled to a benchtop NMR spectrometer for liquid-state detection.

Here we will present the design of the polarizer, for now equipped with a cryostat for static measurements, a double-tuned $^1\text{H}/^{13}\text{C}$ probe, a Ka-band generator, and a nitrogen auto-refill station. In particular, we show the performances through the DNP enhancement using water-soluble radicals and HYPSO matrices. We will also present our latest progress on implementing fast freeze, melt and flow with the design of a dedicated dual cryostat and heating unit.

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P-077 - Investigation of The Enzymatic Activity of The Peach Allergen Pru p 1

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

The peach allergen Pru p 1, a member of the pathogenesis related protein superfamily PR-10, is involved in plant defense. Pru p 1 is upregulated during pathogen infection and essential for defending the plant against viruses. In addition, PR-10 proteins, which all have common biochemical and structural features, are known to cause allergic reactions to plant food in birch-pollen sensitized individuals. These allergic cross-reactions are caused by structurally similar epitopes in PR-10 proteins, which are recognized by birch-pollen specific IgE antibodies. For Pru p 1, it was found that expression directly correlates with lowered susceptibility for pathogens. Interestingly, this protein has been postulated to possess RNase activity.

Aims and Methods

Knowledge of substrate and low-molecular-weight ligand binding is key for understanding the RNase activity of Pru p 1. In our approach, we used a combination of paramagnetic relaxation measurement and mass spectrometry. A 17-nucleotide RNA molecule was designed that comprises all possible combinations of adjacent nucleotides. Using solid-phase RNA synthesis, paramagnetic tags were introduced on either end of the molecule (3' or 5') to investigate the binding to the allergen. Moreover, mass spectrometry was employed to identify the main cleavage site of this RNA.

Results

The combined NMR data showed that the substrate RNA exhibits weak binding affinity to Pru p 1. Paramagnetic effects and chemical shifts were used to probe the binding site of the RNA and to identify which end is primarily involved in binding. Binding of a proposed low-molecular-weight inhibitor was also probed by paramagnetic relaxation. Mass spectrometry revealed the main cleavage site(s) of the substrate RNA.

Conclusion

Our results confirm that Pru p 1 acts as RNase. The relatively weak affinity for substrate RNA and the observed reaction rate are in accordance with the slow response of peaches to stress.

P-047 - The role of intrinsic disorder in the replication of SARS-CoV-2

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The study of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the outbreak of the COVID-19 pandemic, has become of wide-spread interest. In particular, information about the mechanisms of genome replication is currently lacking. The nucleoprotein (N), a highly dynamic protein formed by three intrinsically disorder regions intercalated by two folded domains, the N- and C- terminal domain (NTD and CTD, respectively)[1], is a fundamental cofactor of the replication machinery. Following replication in double membrane vesicles, the newly synthesized RNA appears to be exported through trans-membrane pores to the cellular lumen prior to be encapsidated by the nucleoprotein. The longest viral protein, nsp3, participates in the formation of these exit pores[2]. Using solution-state NMR combined with SAXS and ITC, we demonstrate that N interacts with the amino-terminal ubiquitin-like domain of nsp3 (Ubl1). We monitor the structure and dynamics of the complex at atomic resolution using PRE and relaxation NMR experiments and show that Ubl1 colocalizes with N, via folding the central disordered domain around Ubl1. We characterize the interaction with RNA and the possible role of Ubl1 in the process of encapsidation. Together with single-point mutations experiments that reflect the adaptive mutations leading to the known more virulent variants, a better understanding of the process of encapsidation could lead to the development of inhibitors of the viral replication machinery.

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P-023 - Molecular mechanism of the selective interaction between a drug-like small molecule and an intrinsically disordered protein by solution NMR

Miss Stase Bielskute

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The intrinsically disordered activation domains of transcription factors play a critical role in regulating gene expression and are therefore promising therapeutic targets. Since intrinsically disordered proteins exist as an ensemble of rapidly interconverting structures identifying small molecules that can target these proteins with selectivity is very challenging. EPI-001, identified by phenotypic screening, was the first small molecule shown to selectively inhibit the androgen receptor (AR) by binding to its intrinsically disordered 57kDa activation domain (AD) [1,2] and an analog of EPI-001 is currently under clinical trials for a late stage of prostate cancer known as castration-resistant prostate cancer (CRPC). The molecular basis of how EPI-001 selectively interacts with its targets remains an open question.

Here we use solution NMR spectroscopy to characterize weak and transient contacts between EPI-001 and the AR AD: we monitored several NMR observables including chemical shifts, paramagnetic relaxation enhancements and NOEs to obtain a very detailed description of how the interaction with EPI-001 reshapes the conformational ensemble of the AR AD, including its multimerization properties, in ways that inhibit its activity as transcription factor. Specifically, we show that EPI-001 interacts selectively with AR, and not with the activation domains of other nuclear hormone receptors, because it has affinity for a sub-domain and identify the specific features of this sub-domain that it targets. Our findings emphasize the potential of integrating different NMR observables measurable by solution-state methods for IDPs with complementary biophysical techniques to address this important challenge for drug discovery [3].

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P-279 - Visualizing Optimally Designed MRI Pulses in KomaMRI

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Magnetic Resonance Imaging (MRI) is an imaging technique commonly used for its ability to image in a non-invasive manner. However, it has its downsides, one of them being it is an expensive tool. Hence, simulating imaging sequences before acquiring the actual image is of the utmost convenience. KomaMRI [1] is a software designed to address this issue. It allows the possibility of simulating the image outcome, which later can be compared to actual experimental data. This work uses Optimal Control Theory (OCT) to design pulses to improve saturation contrast in MRI imaging [2]. The design of these pulses considers typical MRI issues, such as field inhomogeneity. Using KomaMRI as a simulation tool for implementing optimally designed pulses, we can acquire images using the optimally designed pulses and see how they improve contrast and handle said issues. Moreover, KomaMRI allows us to simulate conditions that would be experimentally unfeasible, such as long acquisition types and ideal fields, to test the limits of optimized pulses. This optimization is performed using GRAdient Ascent Pulse Engineering (GRAPE) [3] algorithm, implemented on MATLAB. By defining a cost function that rewards good contrast and robustness against field inhomogeneities, the algorithm can design, step-by-step, the best pulse for each optimization. By Bloch Equation simulation, KomaMRI constructs the simulated images, and it handles the heavy computation by using parallel computing. It also allows the construction of user-defined phantoms, which can be used to simulate samples with different shapes for different probe types, such as a micro-imaging probe. The cost function definition is essential for the process. For different goals, it can be defined and implemented to address issues, for example, signal strength for noisy experiments. Hence, this method can be used for different applications, targeted contrast images, and in the food industry.

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P-241 - Local Structure-Performance Relationships: ^{133}Cs and ^{29}Si Solid State NMR Spectroscopy of an Industrial Catalyst

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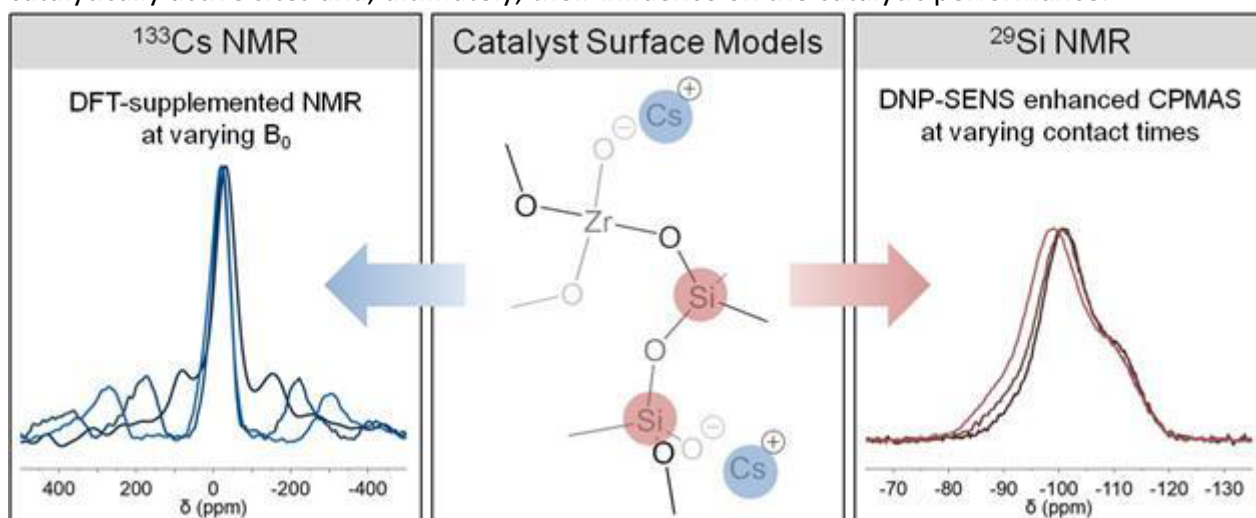
Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Poly methyl methacrylate (pMMA, acrylic) is a lightweight polymer with high impact strength and shatter resistance. Suitable for a range of commercial and scientific applications, it is best known as a substitute material for inorganic glass. Production of the MMA monomer via Mitsubishi Chemical's Alpha Process is a growing technology relying on the use of silica-based heterogeneous catalysts with cesium hydroxide as an active component.

In-depth characterisation of the catalyst surface is required to understand structure-performance relationships. Here, the complex and disordered structure is studied on a local scale with a multinuclear, surface-sensitive solid state NMR spectroscopic approach. Amorphous $\text{Cs}/\text{ZrO}_2/\text{SiO}_2$ samples with varying Cs/Zr contents serve as a systematic model compound library. ^{133}Cs NMR spectroscopy at variable field strengths (7.1 T, 14.2 T, 19.97 T) is employed to characterise the distribution of parameters, with experiments supported by DFT calculations. ^1H - ^{29}Si CPMAS, supplemented by DNP-SENS, reveals surface-sensitive information about the silicon environments.

Asymmetric line shapes seen for ^{133}Cs MAS NMR spectra indicate contributions from more than one site. At varying magnetic field strengths, highly disordered Cs environments are revealed at different levels of resolution. Spectral analysis of dehydrated and hydrated surfaces exposes a dependence of the chemical shift on the presence of water. Based on DFT calculations of crystalline model compounds, the relationships between NMR parameters and the Cs local structure are proposed. Meanwhile, surface-sensitive (DNP-SENS CP MAS) ^{29}Si NMR highlights differences in the local structure around silicon as reflected in chemical shift and linewidth variations that are independent of the CP contact time.

This study offers initial insights into the local structure of Cs and Si in the Alpha Catalyst system. ^{133}Cs and ^{29}Si solid state NMR spectroscopy are promising for an in-depth characterisation of the catalytically active sites and, ultimately, their influence on the catalytic performance.



P-343 - ^{19}F -centered NMR Spectroscopy for the Analysis of Complex Mixtures

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

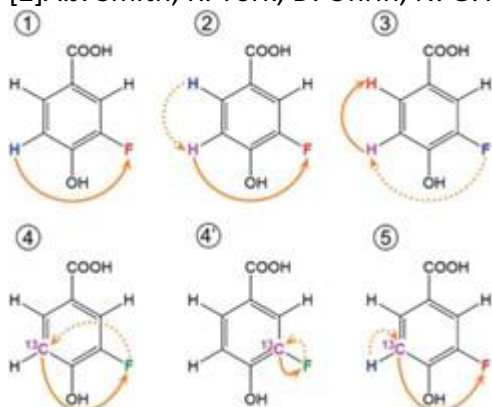
Introduction and aim. Although the number of natural fluorinated compounds is very small, fluorinated pharmaceuticals and agrochemicals are numerous. ^{19}F NMR spectroscopy has a great potential for the structure elucidation of fluorinated organic molecules, starting with their production by chemical or chemoenzymatic reactions, through monitoring their structural integrity, to their biotic and abiotic transformation and ultimate degradation in the environment. Addressing the limitations of existing ^{19}F NMR techniques, we have developed a methodology that uses ^{19}F as a powerful spectroscopic spy to study mixtures of fluorinated molecules.

Methods and results. The proposed ^{19}F -centred NMR analysis [1] utilises the substantial resolution and sensitivity of ^{19}F to obtain a large number of NMR parameters, correlate ^1H , ^{13}C and ^{19}F chemical shifts, values of $J(\text{HF})$, $J(\text{HH})$, and $J(\text{FC})$ coupling constants and sizes of ^{13}C induced ^{19}F isotopic shifts. This was made possible by exploiting the transfer pathways shown in Figure 1. These pathways were used by the following experiments: ① ^{19}F -detected z-filtered ^1H , ^{19}F HETCOR; ② ^1H , ^{19}F TOCSY-HETCOR; ③ ^{19}F , ^1H CP-DIPSI3-DIPSI2; ④ ^{19}F , ^{13}C (^{15}N) HMBC optimised for $n^{\wedge}J(\text{FC})$ ($n^{\wedge}J(\text{FN})$) coupling constants; ④-dash ^{19}F , ^{13}C HMBC optimised for $^1J(\text{FC})$ coupling constants; ⑤ (3,2)D ^1H , ^{13}C , ^{19}F correlation experiment. Dashed and full orange arrows connect the initial and final magnetisation transfer steps, respectively.

We demonstrate the power of the ^{19}F -centred structure determination process by successfully elucidating the structures of chloramination disinfectant by-products of 3-Fluoro-4-hydroxybenzoic acid, which would have been impossible otherwise [2]. This novel NMR approach for the structure elucidation of molecules in complex mixtures represents a major contribution to the analysis of chemical and biological processes involving fluorinated compounds.

[1]A.J. Smith, R. York, D. Uhrin, N. G. A. Bell, RSC Advances 2022, 12(16), 10062-10070 (Hot article).

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P-097 - Insights into RNA G-quadruplex formation in the 5'-UTR of the osteoporosis-related RANKL gene

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Osteoporosis is a bone disease characterized by increased bone resorption, which is a consequence of increased levels of RANKL expression (1). Studies that would reveal molecular mechanisms of regulation of RANKL expression, therefore, offer valuable insights into potential therapeutic strategies. One of the possible ways of gene regulation is by the formation of G-quadruplexes, four-stranded structures known to control gene expression on DNA or RNA levels (2).

We identified a 20-nt long G-rich sequence in the 5'-UTR of the RANKL gene. Our previous studies on DNA constructs based on wild-type sequence (RANwt; 5'-GGGGAGGGAGCGGGAGAGGG) reveal the formation of two topologically distinct G-quadruplexes (3,4). In our recent work, we focused on RNA constructs derived from the rRANwt sequence. rRANwt construct experienced severe signal overlap indicating the formation of diverse conformations. With the use of G-to-U modifications, we were able to isolate four different RNA G-quadruplexes: next to the 'regular' three-layered parallel structure, we identified two different G-quadruplexes with two-nucleotide long bulges and one structure with two concurrently present bulges. In contrast to expectations, all described structures shared similar thermal stabilities and were found in rRANwt in different populations. Additionally, we have demonstrated that the presence of described G-rich sequence influences the expression of the luciferase reporter gene, indicating a possible regulation mechanism of the RANKL gene by the formation of G-quadruplexes in its 5'-UTR.

1. Chin, K.-Y. et al., *J. Clin. Med.*, 11, 6434 (2022).
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3. Lenarčič Živković, M. et al., *Angew. Chem. Int. Ed.*, 57, 15395-15399 (2018).
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This research is funded by Slovenian Research Agency (research core funding No. P1-242). The authors acknowledge the CERIC-ERIC Consortium for access to experimental facilities.

P-365 - Electrolyte design strategies for Li rechargeable battery using PFG and ^{17}O NMR spectroscopic investigation

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Various classes of batteries have been utilized for broad applications in portable electronic devices, electric vehicles and energy storage. However, safety issue in current commercial Li rechargeable batteries is a major obstacle to operate higher capacity battery for electric vehicle applications. Such problems originate from flammable solvent-containing liquid electrolyte. Herein, we evaluate the feasibility of utilizing ionic diffusion properties measured by PFG NMR as a design principle to develop new class of fire-resistant liquid electrolytes. Liquid electrolyte formulation is composed of fire-resistant solvent, secondary solvent, salt and additive. New type of liquid electrolyte molecule with various functional groups and fire-resistance has been synthesized. Carbonate as a second solvent is mixed in to optimize high dielectric permittivity and low viscosity. Correlation between ionic diffusion coefficients, cation transference number and battery performance have been investigated, showing that electrolyte formulation with high Li transference number exhibits high capacity retention. We conducted ^{17}O NMR measurements and simulation to determine Li^+ solvation structure and the interaction between ions and each solvent molecule. Based on these results, we propose how to design molecular structure of electrolyte and electrolyte formulation that allows fast Li ion transport and display good battery performance.

P-359 - Nanobody GPS by PCS: An Efficient NMR Analysis Method for G Protein Coupled Receptors and Other Large Proteins

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While functional motions of biomacromolecules remain often undetected in static crystallographic or cryo-EM structures, NMR chemical shift changes give access to invaluable information about these functional dynamics in solution. Requirement for their interpretation is the assignment of the observed resonances to the corresponding nuclei. Despite assignment strategies based on scalar couplings in multi-dimensional NMR spectroscopy enable complete backbone assignments of up to 80 kDa large proteins, this approach is limited by the fast transverse relaxation rates of large proteins leading to inefficient magnetization transfer, in addition to the impediment of increased resonance overlap. Selective isotope labelling can reduce spectral complexity, but require tedious point mutations to assign the unconnected spins, which may interfere with function.

The presented method here makes use of pseudo contact shifts and their well-defined spatial dependance on the unpaired electron, enabling the assignment of nuclei to the corresponding resonances without measuring their scalar couplings to the rest of the backbone.

The paramagnetic center is introduced by conjugation of the thulium-loaded, rigid DOTA-M7PyridineThiazole variant (Tm-DOTA-M7PT) to single cysteine mutants of nanobodies that specifically bind the β_1 -adrenergic receptor (β_1 AR) in various functional states. The robust antibodies allow spin-labelling at multiple positions enabling triangulation of the receptor atoms and resolving assignment ambiguities, while the high rigidity of the DOTA chelator, together with minimal motion of the tag relative to the protein giving sufficient large PCS to identify nuclei at distances >60 Å. Using this GPS-PCS approach the assignments of valine and tyrosine ^1H - ^{15}N resonances of the β_1 AR in various forms were completed and the detected chemical shift changes revealed so far undetected forces exerted onto the backbone of transmembrane helix 3 (TM3) during activation. The method described is generally applicable to any biomacromolecules in solution for which suitable antibodies exist.¹

[1] J. Am. Chem. Soc. 2022, 144, 47, 21728–21740.

P-143 - Magnetic and relaxation properties of Vanadium(IV) complexes: An integrated ^1H relaxometric, EPR and computational study

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Oxovanadium(IV) complexes ($S = 1/2$, $I = 7/2$) are being actively studied as potential candidates for molecular spin qubits, [1] but have been also proposed as potential MRI contrast agents. [2] In the latter case, the paramagnetic V(IV) complex create a local fluctuating magnetic field that increases the relaxation efficiency of nearby solvent protons. The relaxivity induced by vanadyl(IV) compounds are lower respect low molecular weight gadolinium complexes, but still provide significant relaxation enhancement, particularly at low magnetic field strengths.

We reported an integrated EPR, ^1H relaxometric and computational study that provides information on the magnetic and dynamic properties of vanadyl complexes in solution. [3] The focus is the role of different spatial coordination of the water molecule directly coordinated to the vanadium(IV). So, we selected VO(oxalate)₂, VO(nitrilotriacetate) and VO(acetylacetonato)₂ complexes, while VO(H₂O)₅ and VO(diethylenetriaminepentaacetate) are measured as reference. The complexes were characterized using CW-X-band, pulsed-Q-band EPR spectroscopy and ^1H -NMRD studies in the 0.01 - 120 MHz ^1H Larmor frequency range, in conjunction with DFT calculations. This allowed estimating different parameters that affect the ^1H relaxation times in water solutions, including the distances of closest approach of second-sphere water molecules, the distance between ^1H nuclei of coordinated water molecules and the paramagnetic centre and rotational correlation times. The inner-sphere contribution to ^1H relaxivities was found to be influenced by both scalar and dipolar mechanisms. Water exchange in this series of complexes occurs in the μs timescale, with all complexes showing similar water exchange rates. As an overall, the synergistic combination of experimental and computational studies, represents a significant advance in the characterization of paramagnetic species in solution providing a more complete, accurate and reliable set of structural and dynamic information compared to the traditional approach based on the measurement and analysis of data collected with a single experimental technique.

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- [3] V. Lagostina, F. Carniato, D. Esteban-Gómez, C. Platas-Iglesias, M. Chiesa and M. Botta, *Inorg. Chem. Front.*, 2023

P-123 - Computational chemical shift of xenon in carbon nanotubes

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Carbon nanotubes (CNTs) have gained a lot of interest for their unique electronic and mechanical properties. They have been studied quite extensively both experimentally and computationally using different methods such as nuclear magnetic resonance (NMR) but no computational Xe NMR results for CNTs have been reported so far.

In this study, a computational chemical shift of xenon in proximity of nanotubes with varying diameters is analysed to figure out if there are systematic trends as a function of diameter or chirality of a nanotube.

We use both periodic and non-periodic models of single-wall nanotubes. The structures and the xenon position are optimised. The sites considered are on the axis and inside or outside, close to the surface. One double-wall nanotube is also studied with xenon inside the inner and outside the outer CNT. In addition, a few bundles of two or three nanotubes are studied with xenon on the surface inside or outside one of the CNTs, in the groove between two CNTs or in the interstitial channel in between three nanotubes.

We observe the trends in chemical shift of xenon for nanotubes of different chiralities as a function of diameter of the nanotube. The deviations between different chirality groups are negligible. We consider the variation in chemical shift between different sites of xenon. The differences between chemical shifts in proximity of single nanotubes versus nanotube bundles are discussed.

From these results we can see that Xe NMR is a useful tool for finding the diameter of a CNT for small to mid-sized nanotubes but not for differentiating its chirality. Computational results will help with understanding the experimental results.

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T. Jacklin, R. Khalili, S. Komulainen, J. Vaara, A. M. Kantola, V.-V. Telkki and P. Lantto (2023). Xe-129 NMR chemical shift in carbon nanotubes. Manuscript in preparation.

P-243 - Resorcinarene cages: a new class of potential ^{129}Xe biosensors with unique chemical shift and exchange dynamics

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Introduction

Xenon biosensors have gained a lot of attention in recent years.[1] These biosensors include a cage encapsulating xenon atom functionalized to bind a specific target. This enables sensitive, background-free molecular imaging. There are, however, several problems with the current cage compounds: toxicity, difficult synthetization, low yield etc. Therefore, there is a demand for new types of cage structures which would be affordable, easily synthesizable and efficient.

Aims

To introduce a class of potential biosensor cage structures based on two easily synthesizable resorcinarene macrocycles bridged either by aliphatic carbon chains or piperazines.[2]

Methods

We investigate their potential as biosensors by combining DFT calculations together with ^{129}Xe chemical exchange saturation transfer (CEST) and T_2 relaxation experiments.

Results

Thermally averaged first principles DFT calculations predicted an extraordinarily high chemical shift (345 ppm) of ^{129}Xe in the aliphatically bridged resorcinarene cage ABR-6. The shift is outside the typical range of ^{129}Xe resonances, thus providing unambiguous contrast. DFT also predicted two ^{129}Xe resonances for the piperazine-bridged PBR-3 cages corresponding to single and double loading of xenon. The computations were confirmed by NMR experiments in methanol. The cages show fast Xe exchange rates (12,000–49,000 s⁻¹), resulting in a high CEST response regardless of the low binding constant (0.09–3 M⁻¹).

Conclusions

Combining state-of-the-art dynamical modeling of ^{129}Xe NMR chemical shift with modern experimental methods provides valuable information on Xe encapsulation in bridged resorcinarene cages. The sensitivity of the biosensor is strongly affected by the properties of the cages and both cages presented here exhibit promising features for biosensor applications.

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P-201 - Cross-Polarization for Bullet-Dynamic Nuclear Polarization

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Bullet DNP [1,2] uses pressurised helium gas to rapidly transfer the frozen hyperpolarized sample from the polarizer magnet to a secondary magnet. The hyperpolarized material is only dissolved upon arrival. The dissolution happens in ambient temperature environment which allows for comparatively low dilution and limits the associated signal loss.

DNP buildup times for low gamma nuclei can be substantially longer than for ¹H. Cross-polarization can be employed in the polarizer to shorten buildup times for low gamma nuclei: ¹H polarization is built up rapidly and repeatedly transferred to the target isotope. This approach has been successfully used with Dissolution-DNP [3] and its popularity is increasing.

We report our progress on cross-polarisation in the context of bullet DNP using a cross-coil DNP probe design in which two separate orthogonal coils are used to create two independent radio-frequency fields. We discuss the trade-offs between different RF circuit designs and arching in the helium environment.

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P-397 - Triple resonance HFC experiments

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Introduction

Conventionally, triple-resonance experiments require not only a special NMR probe that can be simultaneously tuned to all three frequencies of interest, but also three dedicated RF channels in the spectrometer console. However, recent advances in spectrometer architecture have enabled the HFC configuration using only two channels, albeit with some limitations which will be discussed here.

Aims

We demonstrate examples of the unique HFC capabilities of modern two-channel JEOL NMR spectrometers.

Methods

NMR experiments were carried out using simplest two-channel configuration of the recently introduced ECZL spectrometer series equipped with a triple-resonance ROYAL-HFX probe. NMR spectra were processed and analysed using JASON. A time-sharing method was utilized for the high frequency nuclei, ¹H and ¹⁹F, while ¹³C radiofrequency pulses were applied on the other dedicated channel.

Results

Experiments were evaluated by observing either proton, fluorine or carbon while double decoupling the other two nuclides. Selective decoupling techniques were also explored to demonstrate rapid collection of structural information. The limitations imposed by the utilization of time-sharing on the high frequency will be discussed.

Conclusions

Triple-resonance experiments such as HFC can be carried out using the simplest two-channel configuration of JEOL ECZL spectrometers. The only additional hardware requirement compared to the most basic configuration is the NMR probe.

P-323 - Ultra-Fast and Time-Resolved Laplace NMR methods in chemistry, material and food sciences

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Introduction

The Laplace NMR can be significantly accelerated by using two techniques:

- Ultra-Fast (UF) Laplace NMR
- Time-resolved (TR) Laplace NMR

Both methods are based on different approaches and represent somehow orthogonal properties, making it crucial to carefully select proper method for system

Aims

In this work we present the variety of application of UF and TR Laplace methods, including:

1. In-situ photo-polymerization monitoring.
2. Hyperpolarized molecules exchange between immiscible solvents.
3. Butter manufacturing.
4. Internal structure studies of gelatin methacrylates (GelMA).

Methods

Ad.1. We have studied the photopolymerization of bis-anthracene derivatives in situ by combining the TR Diffusion NMR and TR non-uniformly-sampled HSQC. The combination of both methods allowed to follow the concentration changes of each mer during the process [1].

Ad.2. By Utilizing the UF Diffusion Exchange Spectroscopy (DEXSY) we were able to monitor the exchange between system of two immiscible solvents. Additionally to overcome the sensitivity issues we utilized the parahydrogen based hyperpolarization.

Ad.3. The properties of butter depend on the ration between the crystallized and non-crystallized fat in the cream before churning. The ratio is controlled by the specific temperature changes of the cream. We utilize UF-DT2 methods to follow the changes in the fat fraction during this process.

Ad.4. GelMa is used in novel approaches for 3d printing of bionic organs. We utilize TR-restricted diffusion [2] methods for fast analysis of the internal pore structure of this material.

Results and conclusions

We demonstrated the applicability of UF and TR Laplace NMR ranging from chemistry to food processing. We have shown that with proper approach the multidimensional Laplace NMR can be a powerful tool to study dynamic processes.

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P-233 - Gas diffusion and relaxation within ordered Al₂O₃ aerogels

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Introduction: Gas diffusion in nanoporous materials correlates with key properties for applications. NMR studies of diffusion and magnetic relaxation in ordered Al₂O₃ aerogels are jointly made [1] at low temperature in Kazan and room temperature in Paris.

Aim: Strongly reduced ³He gas diffusion and diffusion anisotropy (Fig. a) were found in ordered aerogels at 4.2 K [1]. Data deviate from expectations within Knudsen models. This behaviour was attributed to the action of the aerogel wall attractive potential on gas atom dynamics. The hereby reported room temperature studies may confirm this scenario.

Methods: ³He gas is laser-polarised (at ≈ 1 mbar) for high sensitivity, then compressed into a low- or medium-density [2] aerogel sample (Fig. b). Each measurement uses a few mbar of ³He mixed with 0-1 bar of buffer gas. NMR is performed at 1.3 mT, using pulsed gradients and CPMG sequences or small flip angles for T₂ or T₁ measurements, respectively. Experimental details for low temperature studies are given in [2].

Results: Polarisation circa 50% typically yield SNRs of order 1000. Very long relaxation times (minutes) are achieved with no applied gradients. Changes in T₂ with gradients and timings are investigated and effective diffusion coefficients Deff are inferred (Fig. c). Results on the pressure and orientation dependence of Deff will be presented and discussed, as well as data for gradient-induced T₁s.

Discussion: Hyperpolarisation provides excellent sensitivity for ³He NMR, even at low gas pressure and weak magnetic field. Polarisation is well preserved in Al₂O₃ aerogel samples thanks to slow wall relaxation on this material. Final conclusions on actual processes restricting gas diffusion are expected to be derived from data obtained with aerogels of different densities and comparison with low-temperature results and with suitable numerical models.

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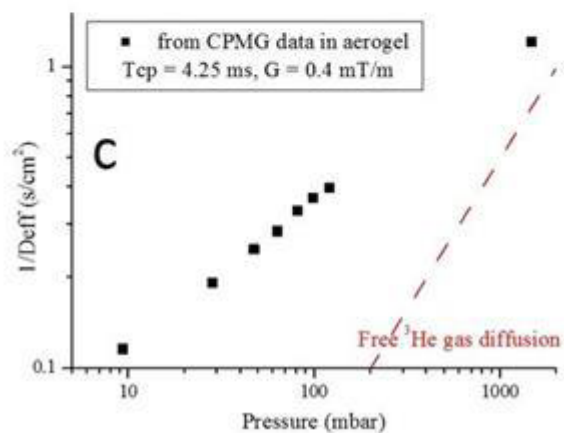
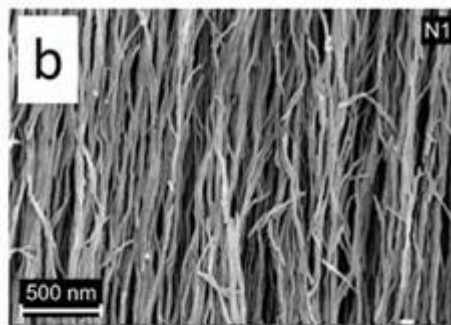
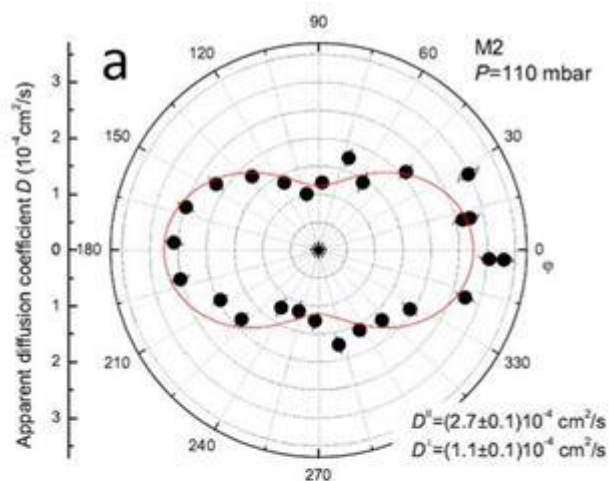


Fig. 1 a: Diffusion anisotropy of ^3He gas in compressed Al_2O_3 aerogel at 4.2 K [1].

b: SEM images of uncompressed aerogel [1].

c: Example of pressure dependence of diffusion coefficients in aerogel at room temperature.

P-189 - Progress in polarization of ^3He atoms in a magnetized plasma (PAMP)

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Introduction

Hyperpolarization techniques allow a strong increase in NMR sensitivity, most often by many orders of magnitude. The recently discovered PAMP technique yields significant ^3He polarization at high magnetic field strength, simply obtained by firing a strong rf discharge in the gas¹. It is a promising alternative to laser-based hyperpolarization in pure helium gas by metastability exchange optical pumping². The physical mechanism of PAMP remains to be firmly established.

Aim

The reported new investigations aim at further exploration of PAMP dynamics and conditions for improved efficiency, as well as at a deeper insight on the polarization build-up process.

Methods

^3He gas contained in sealed glass cells³ of different shapes was polarized in a magnetized plasma from room to 200°C temperature at 3.66 T.⁴ Filling pressures lied in the few millibars range and rf powers of up to 60 W were applied to a wired coil to sustain the discharge. Nuclear polarization was monitored by NMR. Simultaneously, the optical fluorescence spectrum of the plasma and the cell temperature were recorded.

Results:

A set of ^3He polarization build-up curves were obtained for a wide range of applied rf power. Correlations between the ^3He steady-state polarization values and the atomic oxygen impurity number density and cell temperature measurements were observed. A ^3He polarization as high as 8% was reached.

Discussion

The collected data indicate that higher polarization values should be achievable with higher rf discharge power and gas temperature. Further investigations of PAMP and in-depth studies with complementary diagnostic tools are planned⁵. They will assess the potential of this technique for a variety of NMR-based applications. They will also help for the development of suitable models of PAMP.

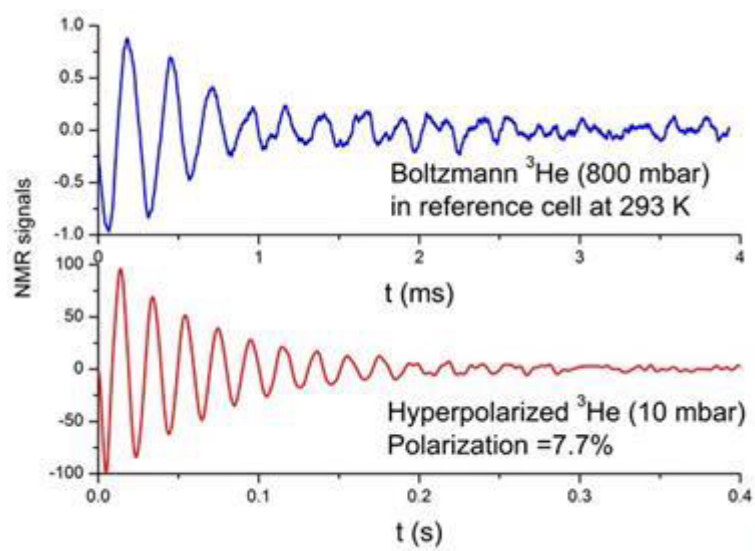
¹A. Maul et al., doi.org/10.1103/PhysRevA.98.063405

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³A. Makarchenko et al., doi.org/10.1134/S0020441221050213

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⁵HELPING project, anr.fr/Project-ANR-20-CE30-0021



P-339 - Ultrafast COSY for structural analysis of small molecules

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Structural analysis by NMR spectroscopy relies on a wide range of 2D experiments. In their conventional form, 2D NMR experiments are time-consuming as they require the acquisition of a large number of increments in order to sample the indirect dimension with sufficient resolution. This can lead to a significant load of the spectrometers. Many methods have been developed by the NMR community to accelerate 2D data acquisition, such as non-uniform sampling (NUS), Hadamard encoding, fast-repetition methods, or aliasing. Among these methods, ultrafast 2D NMR (UF) allows the acquisition of the entire indirect dimension in a single scan, by combining spatial encoding of the indirect dimension with spectroscopic imaging (EPSI).

The COSY method is one of the most widely used 2D experiments for the structural elucidation of small molecules. In this preliminary study, we explored the potential of the UF COSY experiment for structural analysis and we optimised the parameters of the pulse sequence to obtain a complete 2D spectrum in less than 30 seconds.

We worked with cyclosporine A as a standard yet complex test sample. Several aspects were addressed: optimisation of the parameters to obtain a complete map, sensitivity, phase cycling, impact of J-modulation.

It was thus possible to record a UF COSY spectrum covering the entire spectral range of cyclosporine A (9 ppm) in 25 seconds. However, some of the correlation spots could not be obtained at this stage. Several avenues are being explored to try to remedy this, including the use of alternative COSY-based UF pulse sequences, to better understand the effects that modulate signal intensities in UF 2D NMR. These initial tests will be extended to other homonuclear techniques (TOCSY, diffusion, etc.) and applied to structural analysis, as well as to the monitoring of non-equilibrium mixtures.

P-351- Revisiting dynamics in DNA by high-resolution relaxometry and molecular dynamics simulations.

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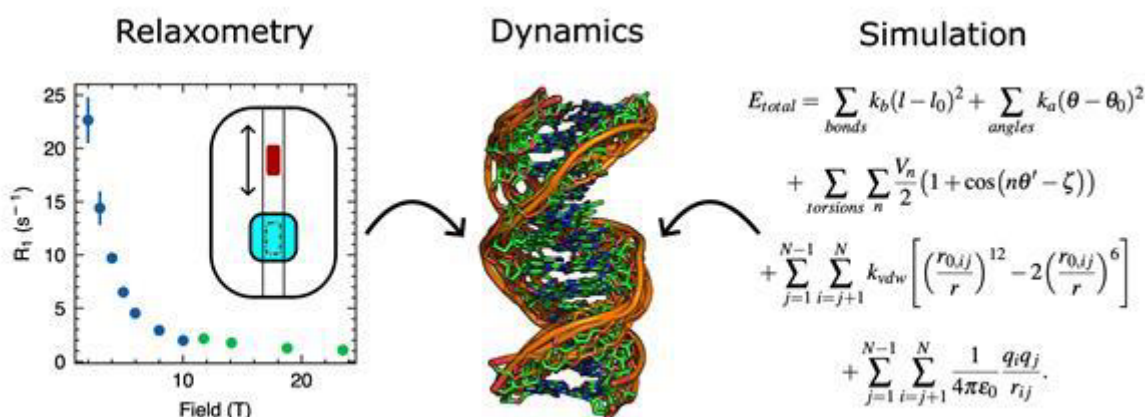
Introduction: Molecular motions in nucleic acids are vital for their biological functions and determine how and when they interact with their surroundings. While CEST, R1ρ and CPMG experiments are exploited to study μs-ms motions, few experiments target motions occurring on the nanosecond time scale. High Resolution Relaxometry (HRR) has emerged as a unique technique to probe motions occurring on this timescale by recording multiple low field longitudinal relaxation rates.

Aims: This study aims to develop methods to use HRR and high-field spin relaxation experiments to probe the local motions in nucleic acids and interpret them with molecular dynamics trajectories. We will identify the motions occurring on the ns time scale and provide a detailed picture of these motions in the sugar backbone and the nucleobases.

Methods: We have measured carbon-13 relaxation in a 12 base pair DNA helix at natural abundance at sites distributed across the sugar backbone and the nucleobases: low-field longitudinal relaxation rates from 2 T to 10 T with a new prototype sample shuttle, and high-field rates (R1, R2, 13C-{1H} NOE) at fields from 11 T to 23 T (500 Mhz-1GHz proton frequency). These relaxation rates are interpreted with ~70 μs molecular dynamics simulations.

Results: We have used the experimental rates and MD simulations to build models that describe the modes of motion and the interactions leading to relaxation. With these models, we can identify the forcefields and water models that most accurately describe the motions occurring within our DNA helix, and in turn describe the motions themselves.

Conclusions: We have recorded the most extensive set of relaxation rates to date on a DNA duplex and demonstrated that the low- and high-field relaxation rates can be combined with MD simulations to characterize internal motion in nucleic acids.



P-375 - The SARS-CoV-2 ORF7b protein interacts with human cellular proteins displaying transmembrane leucine zippers

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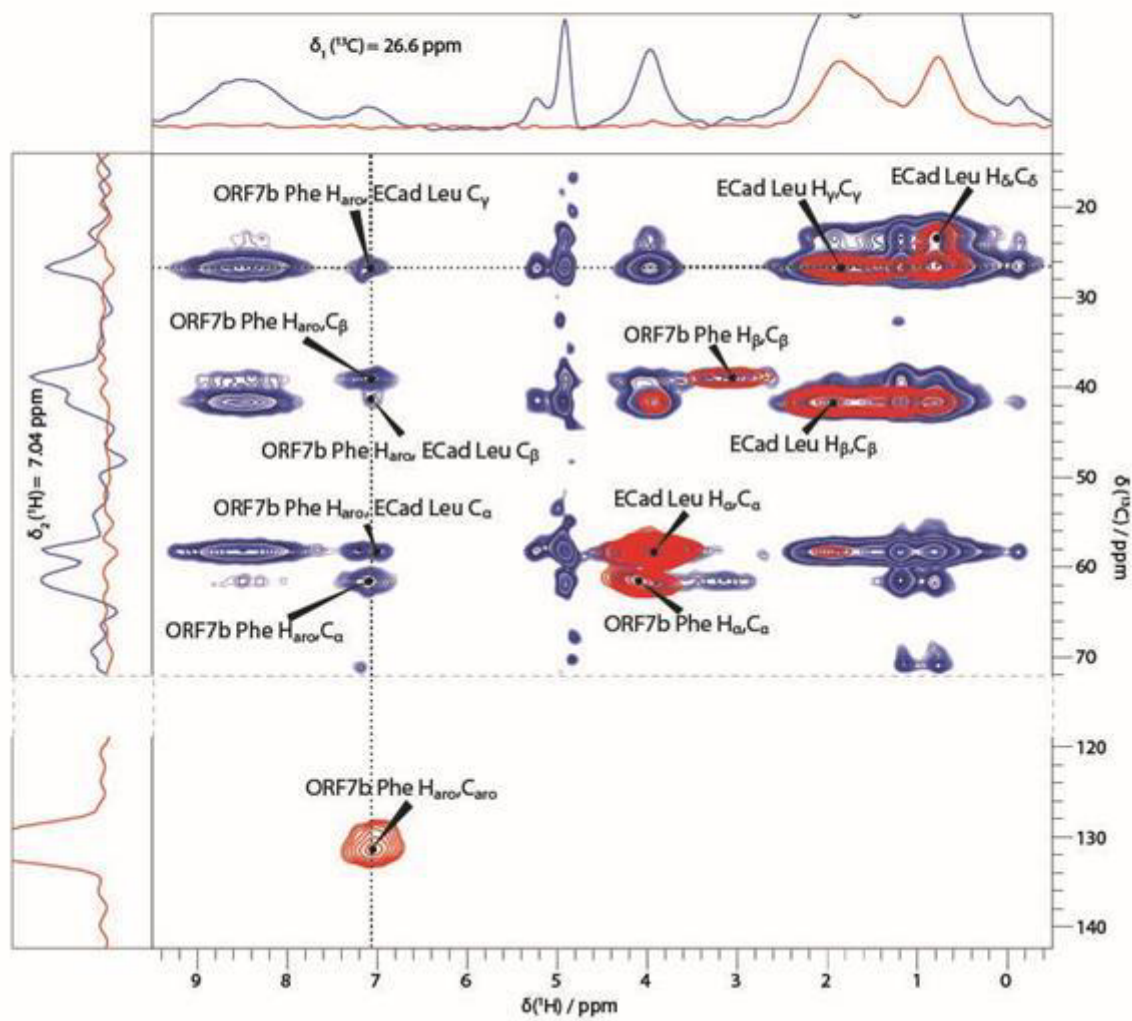
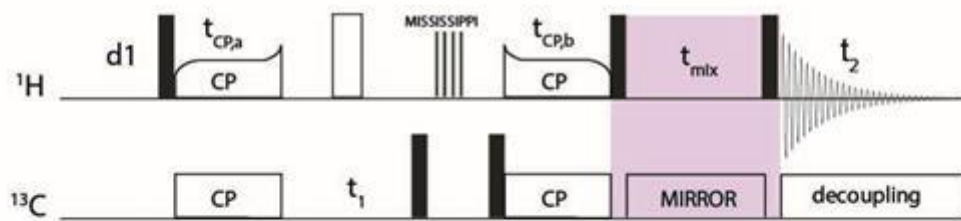
The genome of SARS-CoV-2 coronavirus encodes a number of non-structural accessory proteins whose function during the infection is not entirely understood. One of those, ORF7b, a small-size transmembrane protein, is proposed to interact with human transmembrane proteins like phospholamban (a key regulator of cardiac contractility) and E-cadherin (involved in epithelial cell-cell adhesion) based on its amino acid sequence similarity.

Here we study ORF7b by solid-state NMR and pull-down experiments. We start from an AlphaFold model and collect experimental data from solid-state NMR spectroscopy. The multimerization and the formation of heteromultimers together with human E-cadherin and phospholamban are investigated by NMR and biochemical methods.

The samples are produced using wheat germ cell-free protein synthesis followed by affinity purification and reconstitution into ERGIC membrane mimetics, allowing us to utilise selective isotopic labelling schemes needed due to the broadened and poorly dispersed ¹H resonance lines. Various ¹H detected fast MAS (100 kHz) solid-state NMR techniques were used including ¹H-¹H transfer by the reverse MIRROR scheme.

The structure prediction of ORF7b by AlphaFold being entirely helical with a flexible N- and C-terminal region was experimentally evidenced by secondary chemical shifts. Residues are partially detected in CP-based spectra suggesting that it has flexible tail regions out of the membrane. The multimerization of ORF7b was confirmed by the presence of ¹H-¹H transfer in a mixture of Leu-labelled and Phe-labelled samples in a 2D hChH-MIRROR spectrum. The same approach demonstrated the interaction of Leu in E-cadherin and Phe in ORF7b. Furthermore, multimers were shown by crosslink experiments, and interaction with both E-cadherin and phospholamban was evidenced by a pull-down assay upon co-expression of the interacting proteins in the cell-free system.

The possible interaction of ORF7b helical multimers with phospholamban or E-cadherin could play an important role in several heart and olfactory system related symptoms of COVID-19.



P-367 - Effects of molecular motion on recoupling under MAS in solid state NMR

Ms Kathrin Aebischer

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Solid state NMR spectroscopy is an important technique to obtain information about molecular motion with atomic resolution which is essential for the understanding of biological systems. One commonly used approach to study dynamic processes is the measurement of order parameters from incompletely averaged anisotropic interactions such as dipolar couplings, CSA tensors or quadrupolar couplings. The molecular motion will introduce stochastic fluctuations and the observation of the averaged interaction gives insight into the amplitude of the underlying dynamic process. Under MAS, this usually requires the use of a recoupling sequence that reintroduces for example the dipolar coupling.

We investigated the effects of dynamics on the apparent recoupling behavior for a variety of recoupling sequences including CP, wPARS, and REDOR by performing numerical simulations. Using different kinetic jump models to mimic molecular motion allows us to characterize the effects of parameters such as the MAS frequency, the geometry of the spin system and the recoupling sequence used. This approach enables us to study both the recoupling efficiency of the pulse sequence in question as well as the time scales of the dynamic processes that lead to a scaling of the interaction.

We observe that the range of motional time scales that result in an averaged dipolar coupling depends on the coupling strength and the recoupling sequence used, while the MAS frequency or rf-field amplitudes mostly affect the intermediate exchange regime. Characterizing the molecular motion in this intermediate regime is, however, challenging and depends strongly on the length of the observation window. These observations from simulations show the complexity of the interference between the different time-dependent processes that are present when considering heteronuclear recoupling under MAS in dynamic systems.

P-003 - Construction of a low-field benchtop MRI system for general applications

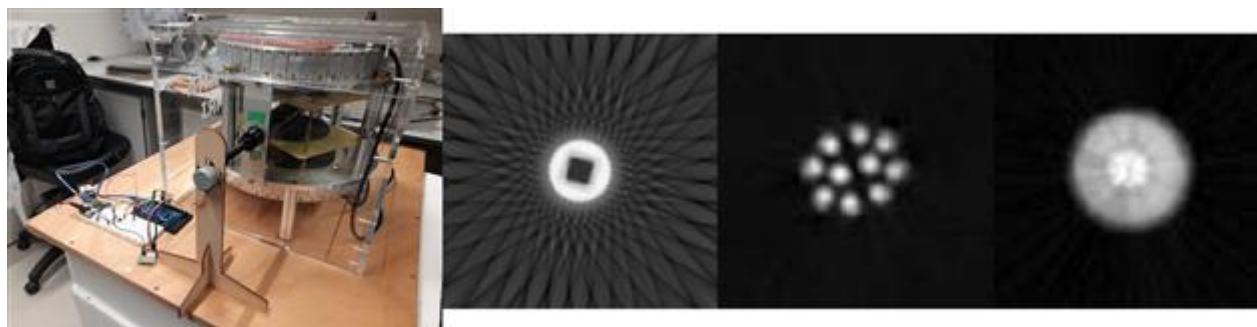
Dr Rodrigo De Oliveira Silva¹, Mr T. Rodriguez², Dr R. Jolivet³, Dr Luis Mario Fraile⁴, Dr M. Kowalska⁵, Dr J-N. Hyacinthe⁶, Dr J. Rivoire⁷, Dr S. Pallada⁶, Prof. Dimitrios Sakellariou¹

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Low-field NMR instruments are made of an assembly of magnetic pieces that are placed together to generate the desired magnetic field in a specific region known as the “sweet spot”. The design of these instruments can be object-oriented, thus avoiding the concept of one instrument that fits all. In this project, an H-shape 2 MHz magnet containing a 3 cm diameter bore at the centre of the pole pieces was designed to be applied in hyphenated MRI experiments with other techniques (e.g. CT, PET, etc.). Furthermore, these construction constraints imposed further design constraints for the gradients and shimming coils designs which are not found in commercially available instrumental solutions.

Here, it is presented the resulting MRI system composed of the magnet, probe, and gradients. The sweet spot is intended to perform images inside a sphere of 2 cm diameter, having less than 200 ppm homogeneity (without the shimming coils). Moreover, back-projection reconstruction images were recorded with this system, showing its capabilities to perform MRI experiments. Preliminary results can be observed in the figures below, from the left showing the magnet system, the image of a rectangular phantom with a square 1 cm cross-section surrounded by water, the image of an ensemble of 5 mm NMR tubes, filled with liquid and 2 cm in length, and a 5 mm carrot slice.



P-227 - Upcycling of textile waste into high added value products

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Global textile fiber production is predicted to reach 149 million tons by 2030, with waste textile expected to exceed 90 million tons annually [1,2]. Sadly, only 20% is recycled and rest is either burned or sent to landfills causing emissions. This alone is huge burden for environment, without accounting burden from growing cotton. Furthermore, the options for upcycling cellulose-based textile waste are limited [3].

Aerogels are considered as lightest solid materials, which have complex porous structure (over 90% porosity) and variety of exceptional features including thermal and mechanical properties. Therefore, aerogels are considered as ideal materials for several application e.g. adsorbents and delivery matrices [3]. However, their complex structure makes characterization challenging, and many available methods are unsuitable due to their limitations, such as requiring high compressive forces that can alter or destroy the structure [4].

NMR is one of the most powerful tools in chemical sciences. NMR provides versatile dynamic, structural and chemical information. NMR cryoporometry can be used to study porous structures in nanometer scale as melting point of a liquid in small pore is lower than bulk liquid, and melting point depression is inversely proportional to pore size. Walls of the porous material restrict the diffusion of liquid inside the material. Therefore, the diffusion information can be used to study micrometer-scale pores, as well as the pore connectivity [4].

In this presentation we exploit NMR cryoporometry and diffusion NMR to characterize various aerogels prepared from regenerated cellulose dissolved in ionic liquid or NaOH solutions and then dried after preparation step. Other optical methods were used as a reference.

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- [2] H. Dahlbo, et al. *Sustain.Prod.Consum.* 9, 44-57, (2017).
- [3] M. Négrier, et al. *RSC Sustain.*, (2023).
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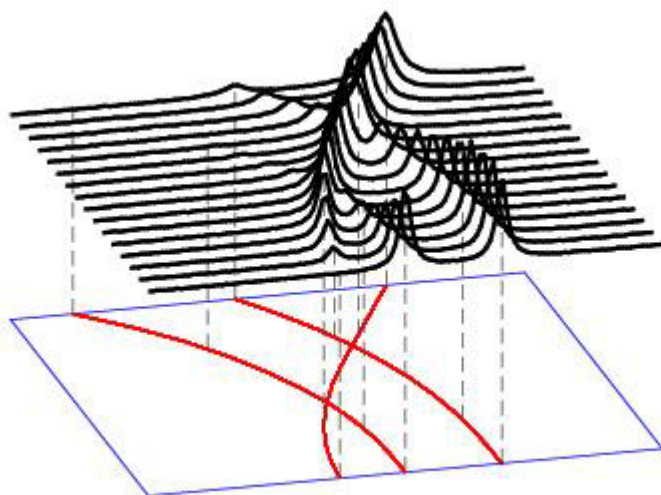
P-137 - AI-based signal tracking of NMR time series

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Hard modeling of NMR spectra using a Gauss-Lorentz peak model is an effective way to reduce dimensionality. In this manner high-dimensional measured data are reduced to low-dimensional information such as peak centers, amplitudes or peak widths. Since these parameters can typically be assumed to be smooth functions in time, cubic splines can be used to interpolate these parameter functions, ensuring correct behavior in situations where peaks may cross, see J. Magn. Reson., 339 (2022) 107212. However, since the underlying nonlinear optimization is computationally expensive, a machine learning approach is used for much faster parameter prediction. Neural networks are trained on randomly generated data sets. Since these neural networks are very specialized (e.g. predicting parameters of exactly 3 peaks in a given spectral window), it is suitable to combine both methods. Results are presented on model and experimental data sets.



P-327 - Towards integrated control of a flow reactor and a high-field NMR spectrometer

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In flow chemistry, reactants are pumped in continuous streams, where the reaction occurs in a temperature-controlled segment, called reactor. Automated reactors are nowadays used to assist synthetic chemists in their daily assignments; from self-optimizing reactors to whole autonomous flow optimization systems. Different in-line analysis methods have been used, such as IR spectroscopy, HPLC or benchtop NMR, to track reaction yields or conversion and seek optimal experimental conditions, while monitoring the chemical reactions on the fly. High-field NMR is a powerful tool for real-time monitoring, and its higher resolution and sensitivity is needed for reactions that cannot be addressed with benchtop NMR. The expanding suite of flow NMR methods creates an opportunity to use high-field NMR as a detector for automated flow chemistry.

In this work, we describe the integrated control of a flow chemical reactor and a high-field NMR spectrometer, as a way to obtain detailed and real-time information on the outcome of flow reactions. Several challenges have to be tackled. First, the mixture under study has to be delivered from the reactor to the NMR spectrometer. Then, the experiments have to be created, launched, monitored and their acquired spectra investigated in an automated and integrated approach.

We connected a custom flow reactor to a commercial flow tube (Bruker, InsightMR) inserted into a 500 MHz spectrometer (Bruker, AvanceIII). To perform experiments autonomously, we developed a MATLAB-based graphical user interface (GUI) to remotely control the high-field spectrometer. The GUI can be used to program experiments, and collect and analyse spectra. It is also able to track and detect deliveries of the mixture that is subsequently studied. This interface is designed to be integrated in a software for reaction control and optimization.

We will describe the design of the program, the experimental challenges and how to overcome them and examples of applications.

P-173 - The interplay between SABRE mechanism and Partially Negative Line of ortho-hydrogen

Mr Marek Czarnota

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Hyperpolarization methods provide a significant enhancement of the sensitivity of the NMR spectroscopy. One of the most promising hyperpolarization methods is Signal Amplification By Reversible Exchange (SABRE) which exploits the properties of hydrogen, and we will focus on this method.

Aims

We want to understand the interplay between the SABRE mechanism and the Partially Negative Line (PNL) of a hydrogen molecule produced from a parahydrogen molecule in SABRE.

Methods

We prepared a sample containing pyridine and iridium catalyst(Ir(COD)(IMes)Cl) dissolved in benzene. We added parahydrogen(a mixture enriched in parahydrogen spin isomer) to the sample. Then, we shook the tube to mix its contents. Immediately after, we put the sample in the NMR spectrometer to measure the ^1H NMR spectrum. After hyperpolarization relaxation, we took the sample out of the spectrometer and repeated this procedure a few times. We used benzene as a solvent instead of more common ones to slow down the processes occurring during the activation of the catalyst so we could observe them.

Results

In the first spectrum, we have already observed the PNL signal with a small negative part. In consecutive measurements, the intensity of the PNL signal grew and reached a maximum around the second and third spectrum. Then, in the next few spectra, the PNL effect was decreasing. Eventually, only "normal" Lorentzian hyperpolarized hydrogen signal was visible after several measurements. Additionally, we analyzed the hydride region. Several hydride signals were present there. By looking at changes in their intensity, we saw the correlation of two of them with the PNL effect intensity.

Conclusions

We have demonstrated that the PNL of hydrogen can be generated by applying the SABRE method. The PNL can be observed during the activation of the catalyst so that it can be associated with forming a specific transition structure of the catalyst.

P-361 - Ultra-selective 1D clean in-phase correlation spectroscopy

Mr Daniel Taylor

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

¹H NMR spectra contain a wealth of information about molecular structure and conformation, but extracting this information can be challenging where there is signal overlap caused by poor chemical shift dispersion and signal multiplicity. In such cases, 2D homonuclear correlation NMR methods increase spectral resolution and add connectivity information, but these experiments are time-consuming and often fail to resolve severe signal overlap. Targeted information can be obtained much more quickly using 1D selective NMR experiments, but these require the target signal to be well resolved. Where signals are significantly overlapped, the pseudo-2D CSSF (chemical shift selective filter)¹ experiment provides better signal discrimination, but it requires extended experiment time and non-trivial experiment setup.

The GEMSTONE (gradient-enhanced multiplet-selective targeted-observation NMR experiment)² pulse sequence element provides CSSF selectivity in a 1D experiment. We have previously used this technique in combination with 1D NOESY² and 1D TOCSY³ to identify through-space and through-bond connectivities to nuclei whose NMR signals overlap. TOCSY methods disperse magnetisation throughout an entire spin system, which is useful for identifying complete spin systems within a molecule but is ambiguous in the assignment of individual NMR resonances. Here, we extend our previous work by combining GEMSTONE and 1D CLIP-COSY (clean in-phase COSY)⁴ to obtain only correlations to spins which are directly coupled to that excited. The new method is complementary to GEMSTONE 1D TOCSY, where both direct and relayed correlations are typically observed. We demonstrate the utility of this new experiment with lasalocid (an antibacterial feed additive) and cyclosporin (an immunosuppressant), the ¹H NMR spectra of which contain regions of severe signal overlap such that conventional selective excitation is not possible.

¹ J. Magn. Reson., 1988, 78, 582–587.

² Angew. Chem., Int. Ed., 2021, 60, 666–669.

³ Chem. Commun., 2021, 57, 2368–2371.

⁴ Angew. Chem., Int. Ed., 2016, 55, 7655–7659.

P-163 - Remote NMR (R-NMR): Moving NMR infrastructures to remote access capabilities

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Within the EU project Remote-NMR (R-NMR), we wish to set the common ground needed for allowing all users to perform NMR measurements remotely with an experience comparable to on-site access. While routine NMR applications are established in every university and in industry, more specialized applications are often performed in research infrastructures offering access and support to local and external users. During the pandemic access to these infrastructures, in particular by external users, has dropped significantly highlighting the need to establish standardized procedures for remote spectrometer access. R-NMR is an inclusive network of European NMR-infrastructure with the aim of establishing protocols for remote NMR-usage, including dissemination of research protocols, sample shipment and CO₂-monitoring.

We present the results of two surveys within the user community and the community of NMR facility managers focused on if and how remote NMR is possible, including sample shipment procedures. Our progress towards establishing standardized methodologies will be also shown.

REMOTE NMR (R-NMR)



MOVING NMR INFRASTRUCTURES TO REMOTE ACCESS CAPABILITIES



Review: Remote NMR Landscape


↓

Define: Procedures

↓

Implement: Standardized Remote NMR Procedures

www.r-nmr.eu
[@RemoteNMR_eu](https://twitter.com/RemoteNMR_eu)



This project has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement N. 101058595

P-247 - In situ observation of the bulk erosion of polylactide using MRI

Mr Jacob Golding

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Introduction: Biopolymers, such as polylactide (PLA), are used extensively as medical implants, because they are biocompatible. Knowledge of the degradation mechanism of these plastics is essential for implant design and placement. It is known that PLA can undergo either surface or bulk erosion.^{1,2} The ratio between surface and bulk erosion is believed to be controlled by the rate of hydrolysis and solvent diffusion.³

Aim: 1H MRI provides an opportunity to visualise the degradation of PLA and distinguish between surface and bulk erosion for the first time. Solvent diffusion and hydrolysis processes in PLA have been investigated to establish better understanding of the degradation mechanism.

Methods: 3 mm diameter rods of PLA were degraded in a range of solvents and 1H spin-echo MR images were acquired, in situ, at intervals of 8.5 minutes over a 5-day period.

Results: 1H MR images show initial solvent ingress into the rod (Fig. 1a), followed by saturation after 18 hours (Fig. 1b). Cracks in the PLA structure were observed after 84 hours (Fig. 1c).

Conclusions: Degradation of PLA has been visualised over time. This is the first-time that bulk erosion of a solid polymer has been observed, in situ, followed by cracking of the polymer structure. MRI has been shown to be able to distinguish between the different degradation mechanisms. Future research will investigate how changing the conditions of degradation will impact the degradation mechanism.

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2. Jong et al., *Polymer*, 2001, 42, 2795.

3. Nostrum et al., *Polymer*, 2004, 45, 6779.

P-187 - Removing NMR barriers in oligopeptide detection with parahydrogen hyperpolarization

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Introduction

Oligopeptides are small biopolymers with a wide array of biological properties. They are of interest as biomarkers or mediators of disease and as drug candidates or drug transporters. However, the complex nature of biological matrices and the low abundance of oligopeptides makes the analysis in their working environment challenging. Herein we show how parahydrogen hyperpolarization application scope can be extended to sensitivity enhanced NMR chemosensing of unmodified oligopeptides in solution.

Aims

This work aims to detect oligopeptides with high-field non-hydrogenative PHIP (HF-nhPHIP)¹ and gives insight into how the organometallic parahydrogen hyperpolarization catalyst interacts with biopolymers. The involved catalyst-analyte complexes are elucidated by NMR and DFT calculations.

Methods

We use HF-nhPHIP for chemoselective oligopeptide detection. HF-nhPHIP does not require sample manipulation like labelling or sidearm addition. It detects parahydrogen derived hydrides of short-lived oligopeptide-iridium complexes and works quantitatively orders of magnitude below the usual NMR limit of detection.

Results

We show how unmodified oligopeptides ligate to iridium catalyst and form peptide-iridium complexes detectable by HF-nhPHIP chemosensor. We present the binding configurations of alanine oligopeptides supported by a regular NMR and DFT study. We demonstrate that oligopeptides thermodynamically prefer bidentate binding and form complexes, which resonate in a unique spectral region free from background signals.

Conclusions

The present work gives insight into how the versatile Ir-IMes chemosensor system interacts with complex biological molecules with multiple ligation sites. The distinguishable chemical shifts can aid fast decisions in complex mixture analysis, including determining the composition of the sample, following biological processes, and quantifying analytes. Further development of the approach could lead to practically applicable non-residue specific oligopeptides detection method.

¹Fraser, R., et.al., Acc.Chem.Res. (2022)

P-299 - Ab initio measurement of pH and pKa in aqueous-organic solvent mixtures by ^1H NMR without external calibration

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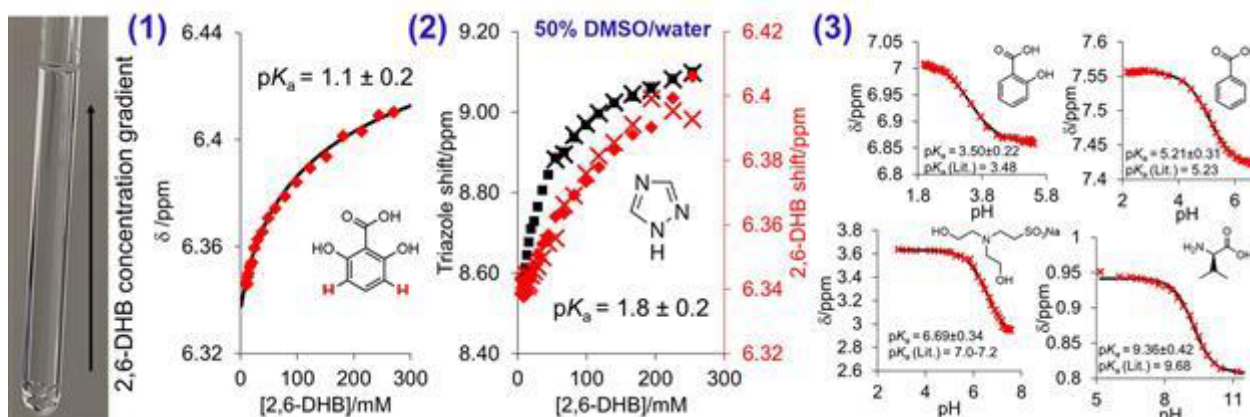
Introduction: Aqueous-organic solvent mixtures are commonly used for reactions or analyses where the components of a system are insoluble in pure water, for example to determine the pKa of small molecule drugs. However, at present the accurate measurement of pH and pKa in aqueous-organic solvents is challenging and fraught with experimental pitfalls. pH electrodes suffer from unknown errors and even damage when placed in these solvents due to incompatibility with the electrode filling solution.(1) Reliable literature pKa data on which a pH scale can be based is also very sparse. Here, we demonstrate how the pKa of organic molecules can be measured in aqueous-organic solvents directly and conveniently by ^1H NMR in any solvent composition, temperature or deuteration level without external calibration.

Methods: We first create a concentration gradient of 2,6-dihydroxybenzoic acid (2,6-DHB) in an NMR tube. By monitoring the ^1H chemical shift and concentration of 2,6-DHB by ^1H chemical shift imaging, we obtain the pKa of 2,6-DHB in the solvent mixture of interest in a single sample via fundamental equations of dissociation. We have pioneered the use of similar approaches to determine $\text{Ca}^{2+}/\text{Mg}^{2+}$ association in aqueous systems.(2) The pKa of 2,6-DHB is used to determine the pKa of 1,2,4-triazole in a self-consistent fashion, and then molecules with higher pKa until we can measure the pH between 1 and 11 via their ^1H NMR shifts and thus determine the pKa of unknown analyte molecules.

Results: Using 50% (v/v) DMSO/water, 1-propanol/water and 30% acetonitrile/water as exemplar mixtures, we obtain pKa values within 0.4 units of literature values. Our highly efficient, single-sample approach offers experimenters unprecedented access to pKa and pH in aqueous-organic solvents, be they supramolecular chemists, drug-discovery scientists or chemical biologists.

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P-181 - Parahydrogen-Induced Polarization for Organ-on-a-Chip Research

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Organ-on-a-chip (OOC) platforms are microfluidic devices in which cells can be cultured in a 3D environment to replicate the structure and function of human organs.[1] These microfluidic devices allow experiments to be carried out with a high degree of repeatability and experimental control, and provide a route to personalized medicine and possibly even a means to replace animal testing. Many diseases affect cellular metabolism (e.g., cancers), and the changes in metabolic flux provide a marker to track the disease progression and response to treatment. In this work we aim to develop an OOC platform to study the metabolic activity of human organoid samples. Magnetic resonance imaging (MRI) is an ideal spectroscopic method for this, as a noninvasive method that provides a unique signature for individual metabolites, but the sensitivity must be improved.

Parahydrogen-induced polarization (PHIP) is a technique that can be used to hyperpolarize molecules in solution, to enhance their MRI signals by a factor of around 100,000. In our lab we are using PHIP to hyperpolarize the metabolites fumarate and pyruvate; two molecules that are used for in vivo imaging.[2] This involves chemically-reacting a precursor molecule with parahydrogen gas to yield a hyperpolarized product, and then purifying it from the reaction solution, ready for perfusion into organoids.

PHIP carries significant advantages for coupling with OOC microfluidics: both methods are famously low-cost and yield a high experiment turnover rate. Using PHIP, it is possible to produce hyperpolarized solutions every few minutes, offering the possibility for pseudo-continuous metabolic monitoring. We are developing this platform to study disease progression and treatment response in DM1 muscular dystrophy organoids.

1. C. M. Leung, P. de Haan, K. Ronaldson-Bouchard et al., Nat. Rev. Methods Primers 2022, 2, 33
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P-349 - NMR Study on Alkali-metal Cations Mediated NanoParticles Assembly

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Inorganic nanoparticles (NPs) attract great research interest due to their unique properties, which derive from a combination of their intrinsic characteristics such as size, shape, chemical composition, and ability to engineer physical properties by a variety of surface ligands.

Surface ligands may control size and shape of NPs during the synthesis as well as acting as a template for NPs assembly. For instance, specific interaction of surface negatively charged ligands with alkali metal cations affects formation of NPs assembly.

Since the most of alkali metals have high abundance of non-zero nuclear spin isotopes, NMR spectroscopy and relaxometry are powerful tools for tracking chemical environment of alkali-metals cations, their mobility, and interactions with neighboring entities.

We report on ⁷Li and ¹³³Cs NMR study of alkali-metal cations mediated nanoparticles assembly in simple aqueous media. The experimental data provide unambiguous evidences of the formation of NPs assemblies.

The ¹³³Cs NMR spectra revealed peak broadening, strong chemical shift, and moderation of exchange between bound and free Cs⁺ ions. All these effects were interpreted in terms of NPs assembly formation on the addition of Cs⁺ ions into NPs aqueous solution. On the other hand, the ⁷Li NMR spectra do not reveal any spectral changes on addition of Li⁺ cation into the NPs solution, which indicates no NPs assembling.

Measurements of ⁷Li and ¹³³Cs nuclear spin-lattice relaxation times (T₁) in various aqueous solution revealed significant acceleration of the relaxation rates on the complexation of alkali metals by ligands in comparison with those in the chloride solution of those cations. This occurs due to prevailing contribution of nuclear quadrupole interaction appeared on complexation. At the same time NPs assembling practically does not affect nuclear relaxation pointing out no (or insignificant) changes in cations' local environment on assembling.

P-135 - Molecular Dynamics with Orientational Constraints -to investigate the conformational landscapes of biomolecules

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

In both bio(macro)molecules and small molecules, structure and dynamics conspire in creation of molecular conformational landscapes. Mapping the landscape is important to uncover function of (bio)molecular machineries implicated in mechanisms of diseases (1). Anisotropic NMR data, such as residual dipolar couplings (RDCs), is sensitive to dynamics occurring on ps-ms timescales. In the presence of flexibility, however, the translation of RDCs into conformational information has traditionally not been straightforward. Recently, Molecular Dynamics simulations with Orientational Constraints (MDOC) have been proposed (2, 3). The use of RDCs as orientational constraints accelerates MD simulations allowing to capture dynamics of up to ms timescale motions, effectively enabling quantification and visualization of complex long timescale dynamics (2, 3).

Here, we explore the use of MDOC simulations with RDCs as experimental restraints to describe the conformational landscape of biomolecules. To test the potential of the method, we first investigated isopinocampheol and a trisaccharide raffinose, two small molecules with different degrees of flexibility. In each case, we aimed to measure a rich dataset of RDCs, including long-range ¹H-¹H and ¹³C-¹H RDCs (4). Next, we applied the methodology to a disordered peptide sequence derived from non-structural protein 5A of Hepatitis C Virus, whose conformational landscape is relevant for understanding its viral replication machinery (5). The RDC-restrained MDOC simulations provide insights on the accessible states by the (macro)molecules, allowing for thorough verification of their conformational landscapes with underlying fluctuations occurring on ps to ms timescales.

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P-345 - Quantitative PSYCHE NMR vs qNMR – The possibility for the direct quantitation of components in mixtures

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Quantitative (qNMR) has proven a useful analysis tool for many years but is of limited use in the case of mixtures with complex multiplets or overlapping signals. We decided to envisage the use of a Pure Shift technique (PSYCHE NMR) as a way to provide decoupled peaks that were suitable to quantify components in biologically relevant mixtures.

Aims

To quantify a series of commonly occurring, biologically relevant compounds and metabolites using internally referenced quantitative PSYCHE NMR and compare the precision and accuracy with that achieved using conventional qNMR. Also, to investigate the effect of various parameters and evaluate their impact on spectroscopic signal overlap in mixtures in addition to statistical variation.

Method

Samples of the three analytes were prepared in triplicate with three iterations of each NMR experiment conducted for each sample. Approximately 5mg of each analyte and internal reference standard were accurately weighed in aluminium crucibles. The weight was determined three times and the average value calculated. Crucibles were added to a clear glass vial and 750µL of DMSO-d₆ was added. The solution was then vortexed for 30s and 600µL of the solution mixture was transferred to a 5mm NMR tube for analysis.

Results

The parameters, which generated spectral data with accurate purity and clean spectra involved excitation sculpting, optimisation of swept pulse flip angle and receiver gain. According to two-tailed F tests, there was no significant difference between both methods. The obtained data indicates that our PSYCHE method fulfils the criteria of a quantitative analytical technique.

Conclusions

This study indicates that PSYCHE can be used quantitatively over the whole spectral width. This optimised method offers a reliable and unique approach to allow acquisition of considerably cleaner high resolution PSYCHE NMR spectra, where artefacts are reduced while simultaneously producing precise quantitative results.

P-169 - Simplifying NMR spectra by triple resonance experiments with Multi Frequency Drive System

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

【Introduction】

Organic compounds containing nuclei such as phosphorus and boron often exhibit spectral complexity and reduced sensitivity due to J couplings between hydrogen and carbon with these nuclei in NMR analysis. To address this issue, triple resonance experiments are commonly used. However, the configuration of triple resonance spectrometers can be an obstacle to instrument installation due to its complication.

【Aims】

To overcome this, we developed a triple resonance system called Multi Frequency Drive System (MFDS).

MFDS assigns different nuclear frequencies up to four frequency sources in a single channel. For example, when using newly developed triple resonance probe, ROYALPROBE™ P+, the frequencies of ³¹P and X are assigned to a single channel (LF channel). The mixed radio frequency pulse output is divided by a diplexer, which enables input to each port of the probe. As a result, we achieved to triple resonance measurements even with a 2-channel spectrometer.

【Methods】

To demonstrate the effectiveness of MFDS, we prepared borane complexes as catalysts for asymmetric synthesis and phosphonium salts as phase transfer catalysts, and conducted various solution NMR measurements using a standard 2-channel JEOL JNM-ECZL600G spectrometer equipped with the ROYALPROBE™ P+.

【Results】

³¹P and ¹H spectra are affected by coupling with the spin-3/2 boron (¹¹B) nucleus can complicate the interpretation of interactions and further reduce the sensitivity of signals. These complicated NMR spectra are simplified and signal intensities are enhanced by MFDS driven triple resonance measurements of ¹H, ³¹P, and ¹¹B.

【Conclusions】

By using MFDS, various NMR spectra can be simplified and sensitivity can be enhanced, similar to conventional triple resonance experiments.

P-341 - Towards geometric double-quantum excitation in oxygen-18 enriched oxalate and squarate

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

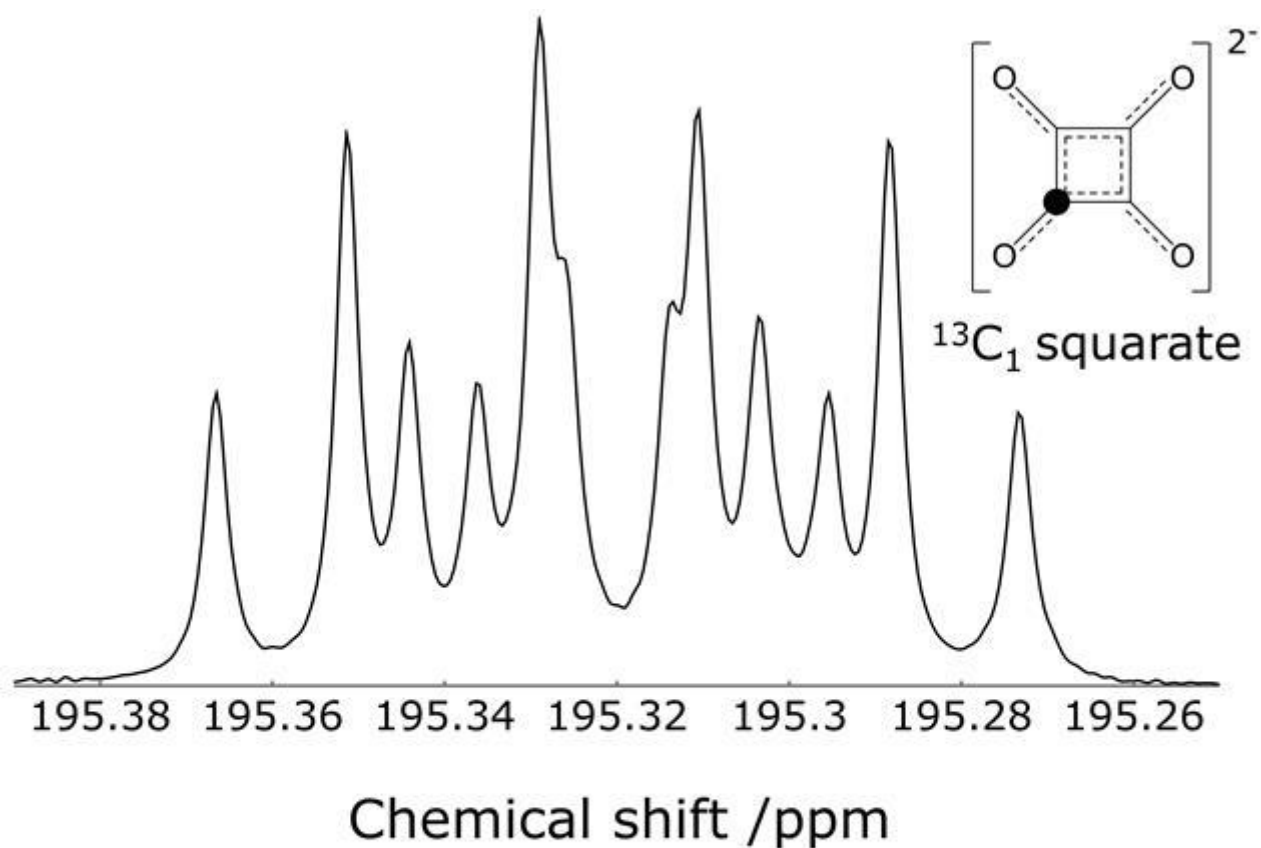
Nuclear singlet order is protected against common relaxation mechanisms, and decays slower than longitudinal order. Under favourable conditions, a ¹³C₂-singlet lifetime of more than one hour has been measured in a room-temperature solution¹. Extended singlet order decay times are anticipated for coupled ¹³C-pairs in small and highly symmetrical molecules, two promising candidates being oxalate and squarate.

To allow access to singlet order, the magnetic equivalence of the system is broken by ¹⁸O-enrichment². We observe isotope shifts and coupling patterns due to ¹⁸O-substitution, including strong “dynamic” isotope shift effects³, caused by the perturbation of the acid-base equilibrium from ¹⁸O-substitution. Proton exchange at the oxygen atom is responsible for these unusual effects, and known to induce premature singlet relaxation in ¹⁸O-enriched ¹³C₂-oxalate, and ¹⁸O-enriched 1,2-¹³C₂-squarate⁴. There is however a more symmetrical ¹³C₂-squarate configuration — 1,3-¹³C₂-squarate — which has not been examined by singlet NMR yet, due to difficulties in synthesis.

To study this highly symmetrical molecule, we plan to implement a recently devised double-quantum excitation method⁵, that exploits the geometric Aharonov-Anandan phase. It demonstrated higher efficiency in exciting double-quantum coherences in systems at near-magnetic equivalence, compared to standard techniques. Our aim is to apply the geometric double-quantum excitation procedure to devise a filter, isolating signals arising exclusively from ¹³C₂-isotopologues present within an ¹⁸O-enriched ¹³C₁-squarate sample.

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Room temperature ^{13}C spectrum of ^{18}O -enriched $^{13}\text{C}_1$ squarate in D_2O at 9.4 T. There are twelve inequivalent ^{18}O -isotopologues, each giving rise to a different chemical shift.

P-381 - Cross-polarisation in Rotating Quadrupolar Solids using Phase-modulated Pulses

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

Cross-polarization (CP) phenomenon has become an integral part of solid-state nuclear magnetic resonance for the study of nuclei with low gyromagnetic ratios and natural abundance. Continuous wave CP and its variants have become regular techniques to transfer polarization of highly sensitive and abundant nuclei (^1H , ^{19}F) to low sensitive spin-1/2 nuclei (such as ^{13}C , ^{15}N) under static and MAS conditions. However, enhancement of quadrupolar spins under MAS conditions is still desired [1]. The quadrupolar interaction under sample rotation becomes time-dependent and, therefore, renders the spin-locking of quadrupolar magnetization inefficient.

Aim:

To develop a new efficient and robust pulse sequence for cross-polarization from ^1H to half-integer quadrupolar nuclei under MAS conditions.

Method:

A proposed sequence for CPMAS in quadrupolar spin systems is shown in the figure. The pulse sequence instead of using a continuous spin-lock on the quadrupolar channel employs a phase-modulated locking while a simple continuous lock is active on ^1H channel. This sequence gives a transverse-to-longitudinal type of transfer, hence a 90° pulse is used after locking on the quadrupolar channel.

Results:

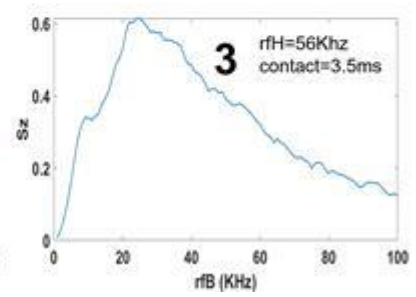
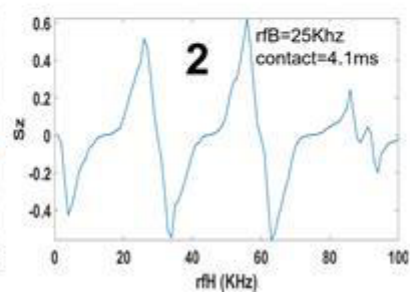
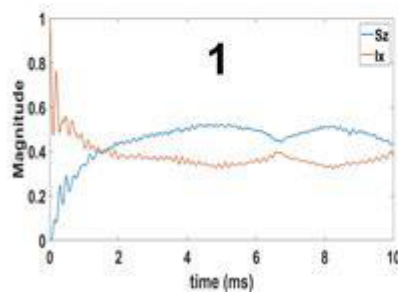
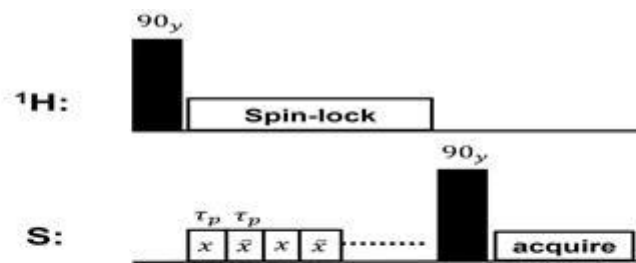
Simulations [2] of spin-locking are shown below the sequence for an isolated pair of ^1H and ^{11}B with 5 kHz dipolar coupling, 2.57 MHz quadrupolar coupling (upto second order), and 0.1 value of asymmetry parameter. The initial density operator in each simulation contains only proton transverse magnetization (I_x) with zero contribution from ^{11}B . Plot 1: trajectories during the locking period. Plot 2 and 3: the Hartmann-Hahn matching curves.

Conclusion:

Theoretical analysis and simulations suggest that the proposed sequence may effect a transverse-to-longitudinal type of cross-polarization in rotating quadrupolar solids. A detailed study will be presented at the conference.

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P-269 - Generic authenticity screening of whisky by NMR spectroscopy.

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

For many high-value products, it can occur that counterfeiters try to sell inferior goods under a false name. By means of NMR spectroscopy, it is possible to analyze the origin and quality of a large number of foodstuffs on the basis of their metabolite composition. One advantage is that NMR spectra of such complex mixtures of metabolites represent a multiparametric system that can contain a variety of information. In combination with chemometric methods, it is possible to visualize this information and highlight relevant features. The signal intensities of single features directly provide information about the concentration of individual metabolites. This combination of information allows a comprehensive analysis of products even with highly complex histories and enables multiple interpretations based on a single measurement. The higher the number of different parameters the larger the set of samples that is required. In the case of whisky distilled from grain of different origins, distilled in different stills, stored in a wide variety of casks, in different locations for different periods of time, there are many parameters that contribute to quality.

In this work, ¹H NMR spectroscopy with water and ethanol suppression of whisky was used to compare a few of these different parameters and identify patterns, for example to make statements about the origin of different whiskies, within Scotland but also from other countries. Further aspects are, for example, the storage period or the type or types of barrels used for storage. Therefore, the study presented here could provide a simple and fast reliable tool to detect counterfeits and mislabeling more easily, even in the case of products with such complex background.

P-257 - NMR proteo-metabolomics: A rising technology for the quantification of acute-phase inflammation proteins from human serum/plasma

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

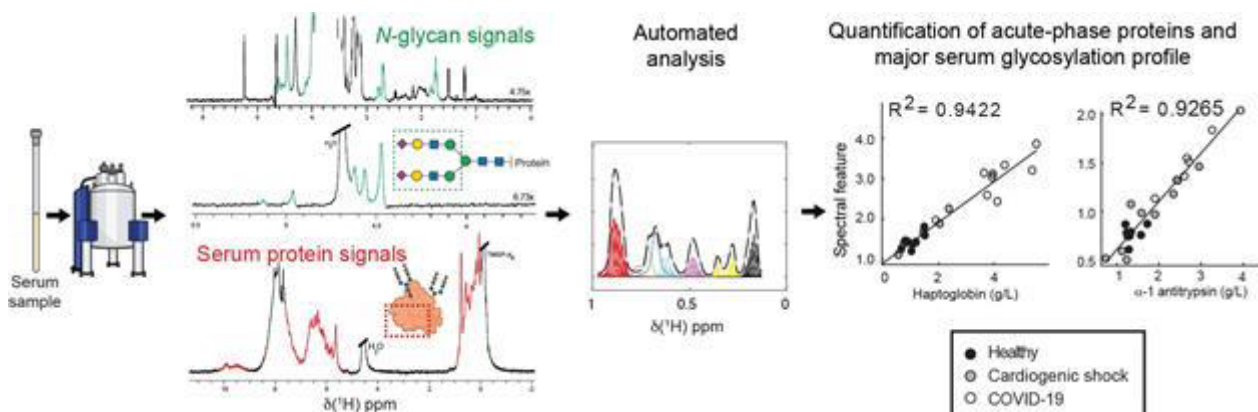
Introduction: Nuclear Magnetic Resonance (NMR) spectra of human serum and plasma show, besides metabolites and lipoproteins, two characteristic signals termed GlycA and B arising from the acetyl groups of glycoprotein glycans. These resonances are associated with acute phase proteins and constitute good markers for inflammatory processes. Recently, we have reported a comprehensive assignment of glycoprotein glycan NMR signals observed in human serum/plasma, showing that GlycA and GlycB signals originate from Neu5Ac and GlcNAc moieties from N-glycans, respectively. Here, we present a novel NMR proteo-metabolomics platform for the quantification of serum/plasma acute-phase inflammation proteins and their major glycosylation profiles.

Aims: Development of NMR methods that allow a further characterization of several acute phase inflammation proteins along with their glycosylation profile. Evaluation of the diagnostic potential of glycoprotein markers obtained from 10-20 min long NMR measurements in different disease settings.

Methods: Preparation of serum/plasma samples follows Bruker IVDr standards, allowing straightforward clinical routine measurements. The proteo-metabolomics platform offers a toolbox of diffusion edited, J-edited, T₂- and scalar coupling-filtered NMR experiments able to isolate structural-reporter N-glycan signals from glycoproteins directly from serum samples. N-glycan profiles are combined with specific protein signals to allow quantification of acute-phase inflammation glycoproteins. Spectral acquisition, FID processing and data analysis are fully automated.

Results: As a test system we have analyzed COVID-19 and cardio-shock patients against healthy controls. COVID-19 were clearly separated from those of healthy controls solely based on their serum glycoprotein concentrations. In fact, conventionally determined concentrations of acute phase glycoproteins such as α -1-antitrypsin, α -1-acid glycoprotein, ceruloplasmin, complement factors C3, C4 and H, haptoglobin, hemopexin and transferrin correlate well with distinct features in NMR spectra (R^2 up to 0.9422, p-value <0.001), allowing their simultaneous quantification.

Conclusions: This work demonstrates the overall potential of NMR proteo-metabolomics as a rising technology for complex diagnostic blood analysis.



P-149 - Investigating the structure of a fluoride sensing riboswitch by PELDOR and ^{19}F ENDOR measurements

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The fluoride sensing riboswitch from *Thermotoga petrophila* binds fluoride ions in a 1:1 ratio. To counteract the electrostatic repulsion between the negatively charged fluoride ion and the negatively charged phosphate backbone the fluoride ion is coordinated by three magnesium ions.[1]

To date, only the crystal structure of the fluoride riboswitch is known. Using a combined approach of site directed spin labelling with nitroxide spin labels, PELDOR measurements, and ^{19}F ENDOR measurements at 34 and 94 GHz we could gather insight into the structural changes of the overall fold of the fluoride riboswitch upon fluoride binding and into the solution structure of this riboswitch in particular with respect to the fluoride binding pocket.

Investigating the riboswitch structure in the fluoride-free, a magnesium-bound, and the fluoride-bound state using PELDOR measurements we found that the riboswitch forms a preorganised structure even before encapsulation of the fluoride ion.

We further investigated the position of the fluoride ion in the solution structure by ^{19}F ENDOR measurements.[2] Due to the use of nitroxide spin labels orientation selection had to be taken into account. We show the use of rotamer clouds derived from *in silico* spin labelling as basis for simulating the ^{19}F ENDOR spectra.

These investigations demonstrate that distance determinations are feasible in such a system opening the possibility to trilaterate the fluoride position in the solution structure of the fluoride riboswitch. Those results also show that the fluoride ion is tightly coordinated to a specific position in the solution structure.

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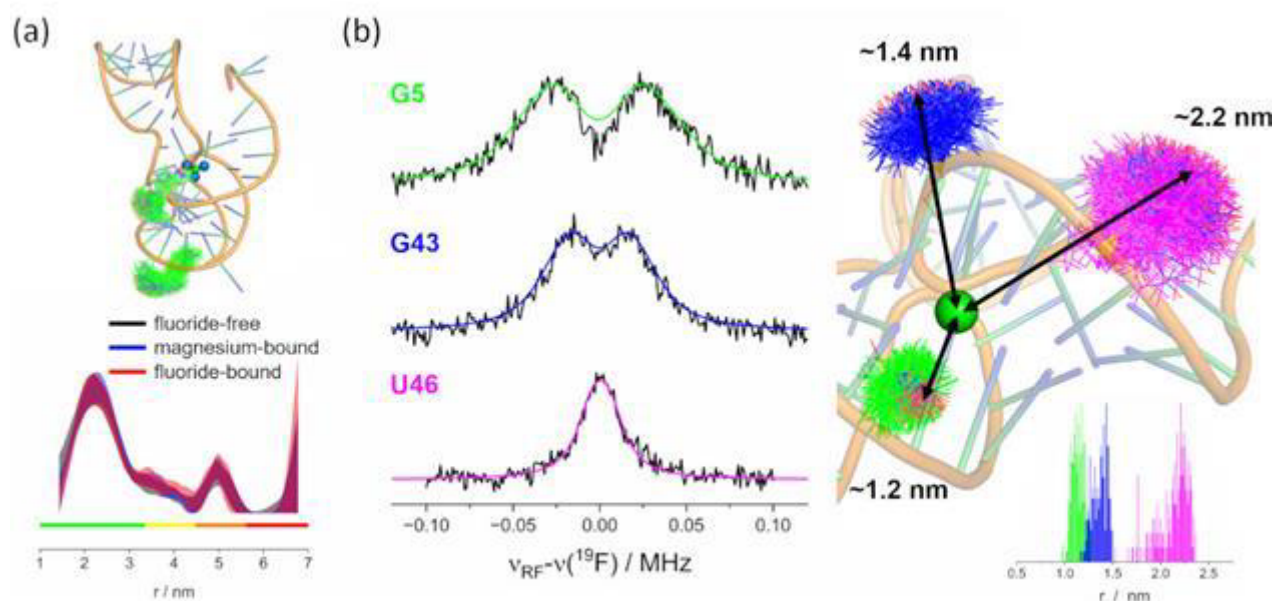


Figure 1: PELDOR distance distributions (a) and ^{19}F ENDOR measurements (b) of nitroxide labelled fluoride riboswitch constructs. Coloured lines are simulations based on *in silico* spin labelling.

P-217 - Large ^{31}P -NMR enhancements in liquid state dynamic nuclear polarization

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Dynamic nuclear polarization (DNP) can increase the low sensitivity of nuclear magnetic resonance (NMR). Electron spin polarization is transferred from an organic radical to coupled target nuclei under microwave irradiation. In the liquid state, nuclear polarization builds up through a cross-relaxation process called Overhauser effect, which is driven by time modulation of the electron-nuclear hyperfine coupling.

Besides ^1H and ^{13}C , ^{31}P is also an attractive nucleus in NMR. The molecule triphenylphosphine (PPh_3) shows large ^{31}P -NMR signal enhancements ($\epsilon > 150$) for magnetic fields ranging from 0.3 T to 14 T when doped with BDPA as polarizing agent (PA). To shed light into this unusual field dependence of the enhancement, we compared the ^{31}P -DNP performance of two PAs, BDPA and nitroxide radical TEMPONE (TN), on two target molecules PPh_3 and triphenylphosphine oxide (TPPO) in different solvents at 1.2 T. The results show a large ^{31}P -NMR signal enhancement for BDPA/ PPh_3 in benzene ($\epsilon = 360 \pm 36$), which decreases upon exchange of the solvent or the PA ($\epsilon = 21 \pm 3$ for TN). No enhancement is observed for TPPO as the target molecule.

With the help of DFT calculations, the experimental observations were rationalized as follows: 1) PPh_3 tends to form a weak complex through non-covalent interaction, which leads to short distances between ^{31}P and the allyl group of BDPA and therefore a large hyperfine coupling ($\Delta A_{\text{iso}} > 13$ MHz). 2) The efficiency of TN as PA is hampered by the larger distance to ^{31}P and a reduced hyperfine coupling. 3) In the case of TPPO, the oxygen prevents a close approach of PA and ^{31}P leading to negligible hyperfine couplings.

Our study in ^{31}P -DNP showed that a radical and target molecule non-covalent interaction can be effective for large NMR signal enhancements independent on the magnetic field.

M. Reinhard et al., PCCP, 2023, 25, 822-828.

P-387 - Firsts insights into Na insertion and SEI formation in organic carboxylate anodes using solid-state NMR spectroscopy and DFT calculations

Mr Heitor Seleghini

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The growing demand and cost increase of Lithium-ion batteries (LIBs) has prompted intense research on the development of similar technologies based on more abundant and cheaper elements, with one of the alternatives being the use of Na as a replacement for Li. Many of the materials used in LIBs cannot be used in Sodium-ion batteries (NIBs), posing a significant problem for anode materials, as Na atom intercalation is often unfavourable and materials can suffer from significant volume changes and mechanical stress as a result of the intercalation.

One possible class of materials that can be used as anodes in NIBs are conjugated sodium carboxylates, which do not exhibit large volume changes or significant modifications to the long-range structure with Na intercalation, but still require improvement with regard to their conductivity, cycling stability and energy density. Such materials are usually synthesised in a single step from the reaction of an organic acid with sodium hydroxide, and the electrochemical insertion of Na occurs together with the reduction of the organic backbone.

Aiming to understand the process of electrochemical sodiation and desodiation in sodium benzenedicarboxylate (Na_2BDC), which can be used as the anode in a NIB, together with the side reactions that lead to the formation of the solid electrolyte interphase (SEI), an ex-situ study of the charging and discharging process was performed using multinuclear and multidimensional solid-state NMR spectroscopy, alongside ab initio random structure searching (AIRSS) and DFT calculations to help understand the structural changes taking place. From such techniques, it was possible to determine the species that form the SEI, together with the structural changes caused by the insertion and removal of sodium on the Na_2BDC anode.

P-275 - Magic Angle Spinning Imaging: Deciphering the Structure of Pathological Calcifications

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction. Pathological calcifications (kidney stones, KS, based mainly on hydrated calcium oxalates and calcium phosphates) are among the most difficult natural materials to characterize at the atomic level due to their inherent chemical and structural complexity (corresponding to organic-inorganic hybrids). Due to huge related societal health costs (more than 800 million euros in France), physicians and nephrologists are most interested in new diagnosis tools based on advanced spectroscopies.

Aims and methods. Currently, the use of standard MRI is strictly limited in hospitals. Quoting Brisbane et al. [1]: "... Using standard MRI sequences, kidney stones appear as a non-specific void". For the first time in the nephrologist's community, we have implemented rotating pulsed field gradients imaging techniques (Magic Angle Spinning MRI in the solid state) for the detailed study of KS including ¹H, ³¹P 3D and 2D slice-selected images.

Results. In a first step, 3D phantoms including solid apatite, brushite and struvite (calcium phosphates) were successfully imaged under MAS conditions. In a second step, Chemical Shift Imaging (CSI) on human KS allowed to resolve spatially the presence of calcium phosphates (apatite and brushite) and organic species such as proteins and fatty acids in selected solid samples. The major advantage of solid state MAS imaging is that several filters such as T2*, T1ρ(¹H) in cross polarization experiments (CP), CP contact time, can be used as many contrast parameters to obtain MAS MR images of KS ... clearly not looking at "non-specific voids"!...

Conclusions. A new imaging tool of investigation has been developed for the specific studies of pathological calcifications. The next challenge is to implement natural abundance ¹³C MAS MRI to spatially resolve hydrated calcium oxalates (the main inorganic components of KS).

[1] W. Brisbane et al., Nat. Rev. Urol., 2016, 13, 654.7, 7:8224.

P-119 - Optimal NMR investigation of Intrinsically Disordered Proteins at 1.2 GHz through ^{13}C -detected experiments

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Nuclear magnetic resonance (NMR) spectroscopy in solution has a wide range of applications, owing to its capacity to investigate molecules and biomolecules at the atomic level. Instruments with a ^1H Larmor frequency of up to 1.2 GHz are now available in a few facilities, allowing an increase in resolution and sensitivity[1]. The increased magnetic field, together with ^{13}C -detection, offers opportunities to expand the complexity of Intrinsically Disordered Proteins or Regions (IDPs/IDRs) that can be investigated[2]. The advantages, however, are accompanied by challenges that need to be overcome when conducting experiments at such a high field.

Aim

We delineate an optimal procedure for the acquisition of ^{13}C -detected experiments at 1.2 GHz on IDPs/IDRs.

Methods

All the NMR experiments were acquired on a Bruker spectrometer operating at 28.2 T equipped with a cryogenically cooled probe head optimized for ^{13}C -direct detection (TXO). The optimized experiments included the H α -CACO experiment, various variants exploiting the ^{13}C '- ^{15}N correlation (CON, H α flip-CON[3], HNBEST-CON[4]), and two different multiple receiver experiments (CON//HN, CON//H α -CAN)[5].

Results and conclusion

The procedure was developed by exploiting a well-known system, α -synuclein. It consists of several steps, beginning with the measurement of relaxation properties of the starting and detected nuclei. The next step was to choose pulses that irradiated the full spectral window without putting too much energy into the probe. An additional consideration concerns selection of the type of NMR tube, which is crucial for controlling sample's ionic strength. We further applied the procedure to investigate two challenging IDRs: CBP-ID4 (207 residues) and a portion of BRCA1 (681 residues). In both cases, the experiments provided informative results, demonstrating the suitability of the protocol for investigating these systems at very high magnetic field.

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P-039 - NMR reveals morphology-dependent interactions between α -synuclein monomers and fibrils.

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Amyloid fibrils may adopt different morphologies depending on the solution conditions and the protein sequence. Here, we show that two chemically identical but morphologically distinct α -synuclein fibrils, A and B, can form under identical conditions. NMR measurements show that the monomers interact differently with the different fibril surfaces. Only a small part of the N-terminus of the monomer interacts with the fibril surface of morphology A, compared to a larger part of the monomer for morphology B. Differences in ThT binding and mesoscopic structures support the presence of two morphologies with different surface properties. The NMR measurements also show that monomers have a lower solubility in the presence of fibrils of morphology B than in the presence of A, indicating that fibrils of morphology B are thermodynamically more stable. Consequently, at prolonged incubation time, fibrils of morphology B remained B, while initially monomorphic sample of morphology A gradually transformed to B.

P-363 - Method for determining pKa of NMR invisible small molecules using spatially-resolved ^1H NMR through pH gradient and proton absorption parameters

Mr Haider Hussain

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction: The acid dissociation constant of a molecule has a significant impact on its chemical and biological activity.[1] NMR spectroscopy is a powerful tool for determining the pKa of molecules using chemical shift information, given that the average chemical shifts of all the measurable NMR-active nuclei are expected to reflect the fractional protonation of each ionisable group of a molecule.[2] Typically, NMR pKa measurements are done using multiple 1D NMR experiments in which the chemical shift of the analyte is measured as a function of pH. This approach can be time consuming and laborious.

Methods: We previously obtained pKa values using pH gradated NMR samples and ^1H chemical shift imaging (CSI) to obtain titration curves in “single-shot” NMR experiments.[3] Here, we present an extension of this method that allows analysis of NMR-invisible molecules using CSI 2D ^1H NMR via the quantity of protons absorbed by a basic indicator. We implement a new fitting function for the NMR data that allows determination of the pKa and concentration of an analyte from the slope and y-intercept of a linear plot. We investigated a collection of small molecules, both visible and invisible, of a wide range of pKa values using a variety of base indicators and obtained pKa values within 0.4 units of literature value.

Conclusion: Our technique has potential for obtaining pKa of inorganic ions, polymeric and colloidal systems despite their poor NMR visibility.

References

- [1] Sandra et al. TrAC., 2007, 96, 1043-1061.
- [2] Gift et al. J. Chem. Educ., 2012, 89, 1458-1460
- [3] Wallace et al., Anal. Chem., 2018, 90, 4160-4166.

P-095 - Insights into functional interactions of a GPCR, enabled by methyl labelling in mammalian cells.

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

G-protein-coupled receptors (GPCRs) are major drug targets of roughly half of all prescriptions and have enormous therapeutic potential. NMR is ideally suited to study ligand and effector binding as well as the dynamic conformational equilibria related to GPCR function.

However, to obtain correctly folded GPCRs for NMR studies, expression in mammalian cell culture is often required, where labelling schemes are not as established as in lower expression systems.

Methods and Results

We therefore aimed at establishing isotope labelling protocols for mammalian cells, and successfully achieved several labeling patterns including ILV-methyl-¹³C labeling. For the latter, extracts from *E. coli* bacteria fed with labelled precursors were added to human cell culture.

With these protocols, we were able to produce human β_1 -Adrenergic Receptor (β_1 AR), a pharmaceutically highly relevant GPCR, and demonstrated functional binding to G proteins, using ILV-methyl-¹³C labelled mini-G protein surrogates. In these experiments we observed significantly different binding affinities for mini-Gs and mini-Go, confirming mini-Gs as the principal signaling partner for β_1 AR.

To study the effect of β -blockers, a novel assay was established. The potency of different antagonists was examined based on their ability to dissociate the β_1 AR-mini-Gs activation complex.

Conclusions

Several labelling schemes for proteins expressed in mammalian cells were introduced, such as ILV-methyl-¹³C labeling. This provides insight into binding of a receptor to small molecules and protein interactors. In addition, a new functional assay for G-protein displacement was introduced, which bridges the gap between measurements of ligand affinity and cellular function readout.

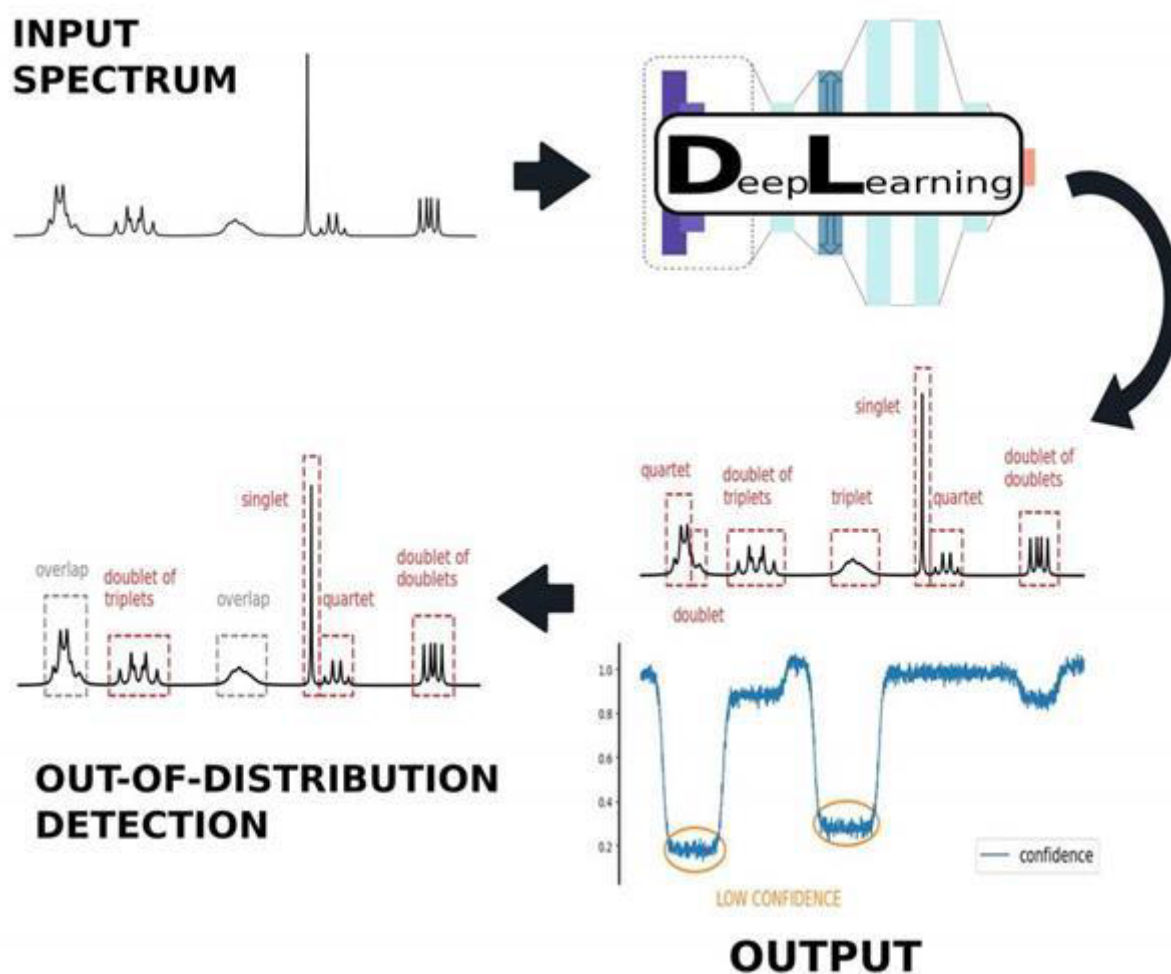
P-129 - Automatic Classification of Signal Regions in 1D NMR Spectra with Deep Learning

Ms Giulia Fischetti¹, Mr Nicolas Schmid^{2,3}, Dr. Simon Bruderer⁴, Dr. Andreas Henrici², Dr Bjoern Heitmann⁴, Prof Guido Caldarelli¹, Prof Alessandro Scarso¹, Professor Dirk Wilhelm²

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

From the NMR discovery, efforts were devoted to introducing an automatic procedure that can ease the chemical compounds characterization while ensuring consistency of the results across the scientific community. This is still an open problem that received renewed attention after the advent of deep learning. Here, we present a novel supervised deep learning framework that can mimic the work of an expert spectroscopist who annotates one-dimensional NMR spectra produced by small molecules to retrieve information on their structure. Considering only the spectrum, our model detects and classifies multiplets with up to four coupling constants for their splitting phenotype. For training, we generate a dedicated set of a hundred thousand spectra with a regularization procedure that ensures consistency among the multiplet phenotypes represented. Our network produces a point-by-point class prediction supported by a confidence score. The prediction uncertainty allows the detection of overlapping and composite signals effectively. The network's performance was evaluated against an experimental testing set composed of forty-eight proton NMR spectra of small molecules annotated by experts. The results suggest that our framework can deal with anomalies and unclear signals while correctly classifying multiplets and detecting overlapping peaks.



P-065 - How RNA methylations change the folding landscape

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

How RNA methylations change the folding landscape

Stefan Hilber, Christoph Kreutz*

Introduction and Aims

The ribosome is a macromolecular complex that plays a crucial role in the biological protein synthesis. It is one of the most conserved and highly developed molecular machines of the cell, which is composed of two subunits (50S and 30S subunit). Even if the respective ribosomal subunits consist of several huge ribosomal proteins, the rRNA is the key to translation. Methylation of nucleotides in such ribosomal RNAs is an omnipresent phenomenon that takes place in all living organisms and can affect the properties of RNA secondary structures [1].

To better understand the influence of methylated nucleotides on the structural and dynamic features of rRNA, several constructs mimicking the 16S ribosomal A site RNA of *E. coli*, which serves as an interaction site for the mini-helix formed by the mRNA codon and the tRNA anticodon, were investigated. Moreover, this system acts as mediator of the translational process and is known as a target for aminoglycoside antibiotics.

Methods and Results

To this end, solution-state nuclear magnetic resonance spectroscopy serves as a powerful tool to give unique insights on the structural and dynamical effects of methylations on the folding landscape of 16S A site rRNA on an atomic level under physiological conditions. The modifications and stable isotope (SI) labels required for the NMR experiments were incorporated by solid phase synthesis. We were able to assign resonances and elucidate the excited state of a 27 nt long 16S A-site RNA with and without methylations.

Conclusion and Outlook

We successfully incorporated modifications and SI labels at specific positions in larger sequences to better mimic the 16s rRNA and investigate structural features and dynamics.

[1] Chugunova, A. et al. *Nat Chem Biol* 14, 226–235 (2018).

P-287 - Stabilization of H-Bonded Base Pairs as a Function of Hydrogen-Bonding Alternation

Miss Zuzana Osifová

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Canonical nucleobases form base-pairs in two main geometries – Watson-Crick pairing dominates in static functions of nucleic acids and Hoogsteen pairing contributes to their dynamic functions. In cell, the adoption of a bonding geometry depends on many factors, e.g. stacking of the nucleobases or backbone conformation. In contrary, the preferred geometry of intermolecular complexes of nucleobases in solution is mainly driven by hydrogen-bonding interactions. Previously, we found that methylated adenines offering two hydrogen bonds to the thymine counterpart, prefer to form Hoogsteen-like complexes in methanol. Now, we extend the hydrogen-bonding pattern of the purine moiety by substitution in positions 2 or 8 to discuss the bonding preferences of three-hydrogen-bonded complexes in both geometries.

Aims

The aim of this project is to determine whether adoption of Watson-Crick or Hoogsteen bonding geometry substantially influences stabilization of triply-bonded base pairs in solution.

Methods

The presented experimental findings are based on changes in ^1H NMR spectra induced by the addition of suitable H-bonding partners. DFT-derived computations produced free energies of complexation, geometries and chemical shielding.

Results

Triply H-bonded complexes formed two distinguishable groups based on differences in the alternation of H-bond donors (D) and acceptors (A). ADA-Type complexes were stable enough to generate a separate set of NMR signals at 180 K. Complexation/decomplexation processes at higher temperatures resulted in averaged signals. Complexes with three H-bonds of the DDA-type generated well-distinguished separate NMR signals across a broad temperature range. Our observation of separate signals of these complexes confirmed their greater stability.

Conclusions

We found that the key aspects of base-pair stabilization in solution are the count of H-bonds and the alternation of H-bonding donors and acceptors. There is no additional benefit in adopting particular geometry.

Acknowledgements: This work was supported by Charles University Grant Agency (grant no. 302922) and GACR (grant no. 22-15374S).

P-013 - EV-olution of Diagnostics: Exploring the Diagnostic Potential of Extracellular Vesicles with Benchtop NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Reported as the most frequently diagnosed cancer in males across 112 countries, prostate cancer accounts for 3.8% of all cancer deaths globally. Early detection and treatment can significantly improve patient outcomes, with a 100% 5-year survival rate when diagnosed at stage 1, compared to 49% at stage 4. Prostate specific antigen (PSA) tests are routinely used for screening; however, due to their limited accuracy, PSA tests often result in false-positive diagnoses leading to overtreatment and unnecessary biopsies.

As a proposed alternative, metabolomic techniques have the potential to analyse liquid biopsies for the presence of tumour derivatives in biological fluids including metabolites and extracellular vesicles (EVs). Found in all biofluids, the term 'extracellular vesicle' refers to any non-replicating nanoparticle delimited by a lipid bilayer that is synthesised and released by a cell. Known to play a role in cancer development and metastasis, EVs also have the potential to provide valuable information for the diagnosis of prostate cancer when coupled with metabolomic analysis.

Current metabolomic techniques utilise high-field NMR (HF-NMR) facilities for the analysis of biofluid metabolites and their relative concentrations. However, widespread use of HF-NMR is restricted by the large size and cost of the spectrometer. Consequently, smaller spectrometers, such as benchtop nuclear magnetic resonance (bNMR) spectrometers, have been introduced to overcome these limitations and facilitate the incorporation of metabolomics into diagnostic pathways without the need for invasive diagnostic procedures such as tumour biopsies. As such, the aim of this project is to investigate the feasibility of using bNMR as a metabolomic tool for the analysis of isolated prostate cancer EVs as a biomarker for cancer diagnostics.

EVs were isolated from conditioned cell media of prostate cancer cell lines (DU145) using size exclusion chromatography. NMR spectra of the EVs were acquired using an Oxford Instruments 60Mhz X-Pulse benchtop NMR spectrometer.

P-357 - Field-dependent relaxation in ^{13}C -enriched pyruvates.

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

A state that is well protected from some common relaxation mechanisms and has a lifetime, much longer than the usual relaxation time constant T_1 , is called a long-lived state. The use of long-lived states in NMR spectroscopy and imaging presents many opportunities, such as hyperpolarised molecular imaging. Among the substrates that can be used as a probe for hyperpolarized NMR and MRI, pyruvate gained much attention. Passing the ^{13}C magnetization through singlet order in [1,2- $^{13}\text{C}_2$]-pyruvate was done a long time ago [1,2], but only in a single magnetic field. The main goal of this work was to determine the relevant time constants of [1,2 - $^{13}\text{C}_2$]-pyruvate as a function of magnetic field, using a precise sample shuttle.

An 80mM aqueous [1,2- $^{13}\text{C}_2$]-pyruvate sample was subjected to a pulse sequence for making "singlet precursor order" and a field-cycling procedure, using a shuttling system, to make a singlet order. The [1,2- $^{13}\text{C}_2$]-pyruvate was prepared in a state of singlet order in low magnetic field and then transferred adiabatically to high field for observation (Fig.1). The field dependence of LLS time constant was measured. The influence of the presence of paramagnetic oxygen in non-degassed sample to a ^{13}C singlet lifetime of [1,2- $^{13}\text{C}_2$]-pyruvate was investigated as well.

Pyruvate, as a key metabolic intermediate, is widely used probe for MRI. The most known and useful for metabolism studies is 1- ^{13}C -pyruvate, but there are a few studies where 2- ^{13}C -pyruvate and 3- ^{13}C -pyruvate were used for other in-vivo MRI applications. In this work is also presented a comparison of T_1 for [1,2- $^{13}\text{C}_2$]-pyruvate, [1- ^{13}C]-pyruvate, [2- ^{13}C]-pyruvate and [3- ^{13}C]-pyruvate.

[1]Tayler MCD, et al. "Direct enhancement of nuclear singlet order by dynamicnuclear polarization." J. Am. Chem. Soc. 2012; 134: 7668–7671.

[2]Irene Marco-Rius et al. "Hyperpolarized singlet lifetimes of pyruvate in human blood and in the mouse". In: NMR in Biomedicine 26.12 (2013), pp. 1696–1704.

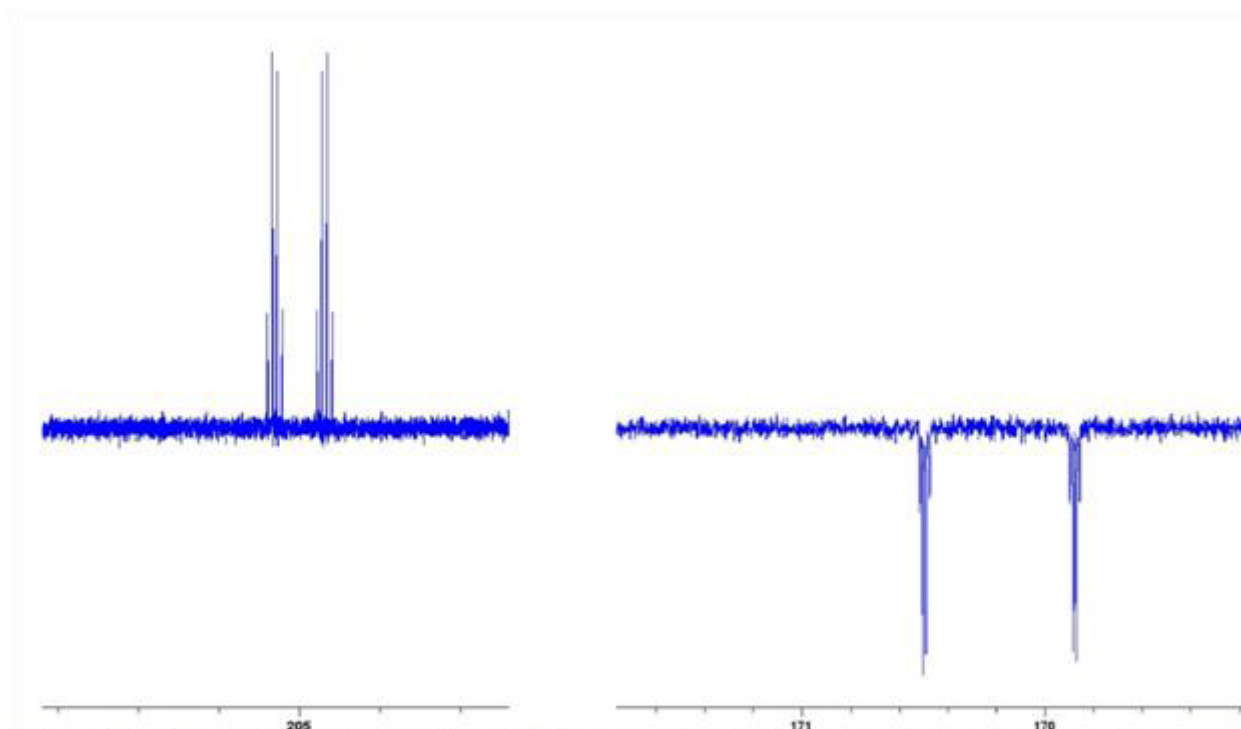


Figure 1. The ^{13}C spectrum of a sample of [1,2- $^{13}\text{C}_2$]-pyruvate which was prepared in a state of singlet order in low field and transferred adiabatically to high field for observation.

P-253 - Comparative Study of Commercial Silicon-based Anode Active Materials for Li Rechargeable Battery

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

With increasing demands of longer use of electric vehicles and energy storage systems, the use of high capacity active materials and their higher loading are required to increase the energy density of lithium-ion batteries (LIBs). Si anode active material has been receiving tremendous attention due to its ultra-high theoretical specific capacity. However, up to now, just a few percentage of Si active materials have been employed as a Si-graphite composite for industrial anodes. Since various types of Si active materials are available, there is a strong need for systematic and comparative studies on the compositional and interfacial structures and battery performance of different Si. Herein, we investigate the correlation between material's structural properties, SEI formation behavior of different industrial Si anode active materials and half-cell performance by solid state NMR, X-ray photoelectron spectroscopy (XPS), impedance measurements and electrochemistry. The materials compositions are characterized as SiO_x ($x \approx 1.2$), carbon-coated Si, and bare Si (with different particle sizes). Surface analysis results reveal that LiF-dominant SEI layer provides a good passivation of anode surface and improvement in the capacity retention. Based on electrochemical and spectroscopic characterization results, silicon oxide is relatively beneficial beyond Si materials in the aspects of high structural stability and cycling stability, meeting better the needs of practical applications. This is mainly due to more effective accommodation of a large volume change by the formation of active Li-Si alloy cores embedded in an inactive matrix consisting of Li-silicates and lithium oxide than carbon matrix of Si-C composite or bare nano-to-micro Si. In addition, formation of SEI layer with good passivation property and low impedance is mandatory. This work gives insight into the rational design of well-working Si-graphite anode at a high Si content for high energy density LIBs.

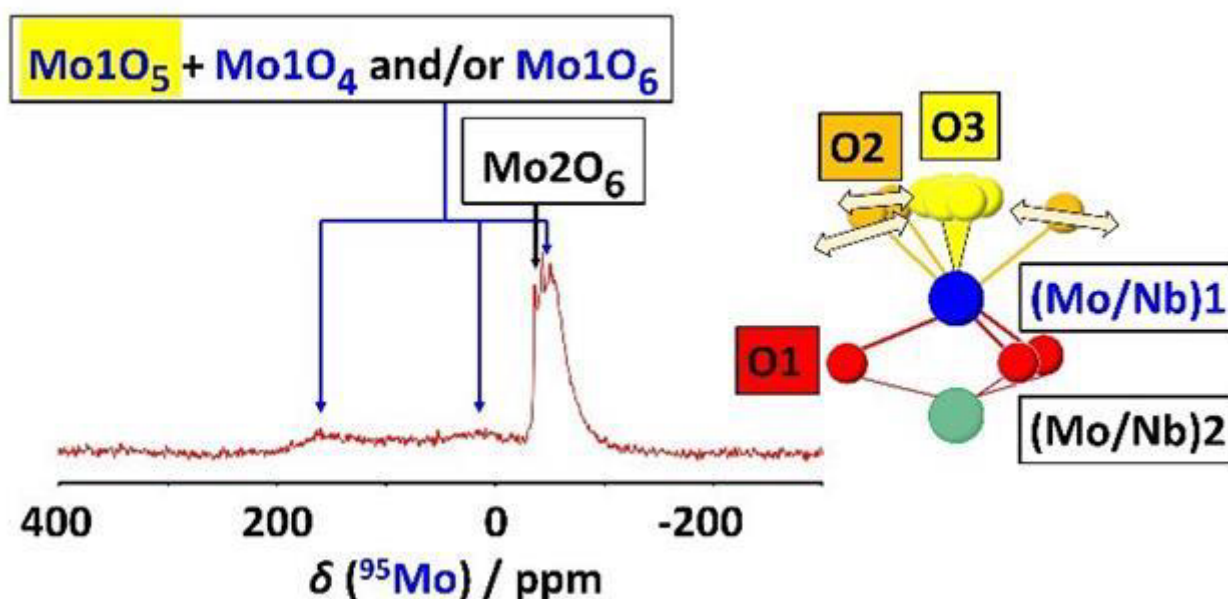
P-401 - Different Local Structures of Mo and Nb Polyhedra in the Oxide-Ion-Conducting Oxide Ba₃MoNbO_{8.5} Revealed by Solid-State NMR Measurements

Dr MASATAKA TANSO¹, Dr Atsushi Goto¹, Mr Shinobu Ohki¹, Dr Yuuki Mogami¹, Mr Yuichi Sakuda², Mr Yuta Yasui², Dr Taito Murakami², Dr Kotaro Fujii², Dr Takahiro Iijima³, Professor Masatomo Yashima²

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The oxide-ion conductor Ba₃MoNbO_{8.5} and their related oxides are important groups of materials because of their high ionic conductivity. The structure of the ion-conducting layer of these materials has not been clarified because of their complex structure and the difficulty in distinguishing between Mo and Nb. In this study, we separately detected ⁹⁵Mo and ⁹³Nb by solid-state nuclear magnetic resonance (NMR) measurements to directly observe the Mo and Nb coordination in the high-oxide-ion conductor Ba₃MoNbO_{8.5}. The results showed that the number of revealed peaks was different for ⁹³Nb and ⁹⁵Mo. For the two chemical shifts from ⁹³Nb NMR, the more intense peak was attributed to a NbO₆ octahedron in the conducting layer, while the less intense peak was ascribed to a NbO₄ tetrahedron in the conducting layer or a NbO₆ octahedron in the nonconducting layer. Four peaks were observed in the ⁹⁵Mo NMR of the ⁹⁵Mo-enriched sample. One peak was attributed to the MoO₆ octahedron in the nonconducting layer. The other three peaks attributed to the conducting layer were only interpreted by assigning either one or two of them to the MoO₅ polyhedra, which are speculated to play an important role in ionic conduction. The present work has demonstrated that the analysis of the local structure of Mo–O and Nb–O polyhedra by NMR is an important tool for understanding the nature of ionic conduction because it provides element-independent information. It is therefore expected to contribute to the further development of oxide-ion conductors.



P-215 - Towards Recyclable DNP Hyperpolarization via Radical Immobilization in Polarizing Solids

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

The use of hyperpolarizing solids in dissolution dynamic nuclear polarization (dDNP) offers an elegant way to generate radical- and glassing-agent-free hyperpolarized analytes to boost NMR sensitivity by many orders of magnitude.[1] Immobilization of paramagnetic radicals ensures efficient hyperpolarization transfer to nearby nuclear spins confined in a porous solid matrix, while minimizing paramagnetic relaxation after dilution and final dissolution to the liquid state.

However, the proposed benefits of hyperpolarizing solids are currently still being reviewed in context of a traditional dDNP perspective. Hyperpolarization proceeds at 1-4 K at 5-7 T and dissolution is performed by introducing hot pressured deuterated water.[2] The overall hyperpolarization experiment therefore again remains i) destructive and ii) of single-shot character narrowing its use and application potential.

We are currently working on a recyclable DNP alternative without dilution nor radical contamination based on a heterogenous stationary phase of silica-based polarizing matrices (HYPSO) acting as a packed-bed hyperpolarization reactor positioned in a compact (1T) and helium-free DNP polarizer. The hyperpolarization reactor is responsible for freezing, hyperpolarizing, and melting the analyte, which afterwards is pumped through a closed loop coupled to a benchtop NMR spectrometer for liquid-state detection.

Here, we report and analyze the performance of HYPSO matrices to enhance sensitivity in ¹H and ¹³C (CP) of impregnated analytes at 1T and 77K, the conditions which will be present in the final design of the recyclable DNP setup. An effort was made to rationalize the active DNP mechanism prominent in these solid conditions by based on recording DNP spectra at different frequency modulation amplitudes. Optimal pore design and radical concentration for the best DNP performance was investigated by chemically tailoring the porous matrix to our needs.

[1] Cavailles, M. et al. *Angewandte Chemie*. 130, 7575-7579 (2018).

[2] Ardenkjaer-Larsen, J. H. et al. *Proc. Natl. Acad. Sci.* 100, 10158–10163 (2003).

P-117 - Developing a mechanism for the biomineralization of magnetite nanoparticles by Magnetosome Membrane Specific protein F (MmsF) via NMR

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

Magnetite nanoparticles (MNPs) are a widely adopted technology, limited by current synthesis methods. . Magnetotactic bacteria, such as *M. Magneticum*, can biomineralize MNPs precisely through membrane bound organelles termed magnetosomes. The proteins employed by the magnetosome, such as MmsF, have been shown as promising additives for improving chemical synthesis of MNPs.

Aims

In this project, I aim to use an artificial protein construct named MmsFcc to study the functional loop sequence (DRDDEFVYFHAKQ) of Magnetosome membrane specific F (MmsF). I aim to probe the metal binding capacity of MmsF, and solve its structure via NMR. Furthermore, I plan to utilise non-functional and rescue point mutants of MmsFcc to provide residue specific functional information.

Methods

MmsFcc and its mutants is purified via the N-terminal histag. A HSQC assignment of MmsFcc, and its mutants (D1S, F9Q, F6Y/F9Q) will be completed. Metal titrations will be performed to provide residue specific binding data. This data will be combined with solved structures to create a model of MmsF mediated biomineralisation.

Results

I have completed an assignment for D1S. Structural calculations are currently being performed for D1S. Metal titrations have been completed for WT and D1S. An assignment of WT is being performed. I plan to repeat this for F6Y and F6Y/F9Q.

Conclusion

N/A

P-309 - Hyperpolarization of long-lived states and coherences in chains of methylene protons

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

P-297 - Drug discovery of small molecules that target RNA

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Fragment-based screening (FBS) by NMR has become a central tool in the pharmaceutical industry to identify new ligands that bind to selected biological targets. NMR is particularly suited to this task since the screening process takes place on a sample in solution under conditions where both the target and the ligands are very close to their physiological state. Typically, both ¹H and ¹⁹F nuclei are used for FBS by NMR. Nowadays, most pharmaceutical groups have incorporated NMR screening strategies into their drug discovery and drug design programs.

While the usual targets of these programs are classically enzymes or protein receptors, noncoding RNAs, which have proven roles in disease and in the regulation of biochemical pathways, are increasingly becoming a therapeutic target of choice.

We combine FBS by NMR with machine learning to accelerate drug discovery of molecules that interact with RNA targets. To this end, we developed new methods for screening (orthogonal and paramagnetic libraries), fast and unambiguous resonance assignment using isotopically labeled RNA, as well as characterisation of hydrogen bonds. Several examples of our portfolio are shown.

P-083 - Unraveling the hidden moves of p38g with High-Resolution Relaxometry

Miss Ana Paula Aguilar Alva

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction:

The p38 mitogen activated protein kinases (MAPK) participate in the adaptation of cells to stress and are involved in the regulation of a myriad of cellular processes, accommodating a broad range of substrates [1]. As such, p38 MAPK play roles in many diseases, including cancer and diabetes, and have become targets for drug discovery [2]. The regulation of these proteins by phosphorylation has been described from a biological perspective, yet the molecular mechanism is not a simple structural switch. p38 γ is the only p38 MAPK with a PDZ binding motif which gives p38 γ a unique importance in protein-protein interactions [1].

Aims:

The objective of this study is to quantify the conformational ensemble and nanosecond dynamics of p38 γ at atomic resolution.

Methods:

Traditional relaxation experiments performed at high fields (>10 T) are not sufficient to fully characterize nanosecond motions [3]. We complement high-field relaxation measurements on ¹³C methyl bearing side chains with High-Resolution Relaxometry (HRR) experiments at low fields in order to get a more complete coverage of the spectral density function. We use a new shuttle system that achieves fast transfer of the sample down to 36 mT in as little as 60 ms.

Results:

Nuclear Magnetic Relaxation Dispersion (NMRD) profiles are obtained from the relaxometry experiments and contribute to further understanding of the conformational landscape and dynamics of p38 γ .

Conclusions:

We obtained a first map of the dynamics of p38 γ at nanosecond timescales. Further analysis will require the use of molecular dynamics simulations to better understand the conformational dynamics of p38 γ .

[1] P. Maisonneuve, et. al. The Journal of biological chemistry, vol. 291, no. 32, p. 16699–16708, 2016.

[2] A. Escós, et. al. Front. Cell Dev. Biol., vol. 4, no. 31, 2016.

[3] A. A. Smith, et. al. Journal of Biomolecular NMR. vol. 75, p. 119-131. 2021

P-005 - Challenges and solutions for accurate magnetization reversal and tipping outside the RWA

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Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Introduction

In low static field, with a linear rather than circular polarized rf field, a breakdown of the rotating-wave approximation (RWA) may occur and lead to significant deviations from expected trajectories on the Bloch sphere. The effects depend strongly on several factors including detuning (via the static field or rf frequency) and pulse characteristics (phase, amplitude, shape, or transients).

Aims

Using simulations and experiments, we seek to explore the many combined effects that one must consider when using pulsed linear rf fields outside the RWA. We aim to develop practical strategies that can be employed in this regime to generate accurate tip angles.

Methods

Relaxation and diffusion were ignored in simulations. The Bloch equation was solved via numerical integration to compute the magnetization vector in the laboratory frame. Either nominal or recorded coil currents were used as inputs in the simulations. Experiments were performed in a home-made 3-mT MRI system¹ with wired rf coils², using laser-polarized ³He gas or thermally-polarized water samples.

Results

The desired terminus can be achieved through the application of a suitable phase-dependent Bloch-Siegert shift, notably different from that of cw NMR, and appropriate consideration of pulse timings². The figure displays selected simulation results obtained for rectangular Π pulses: rf-driven North-South trajectories, terminus points, and Bloch-Siegert shifts needed to reach the South pole. Experimental data are in good agreement with expectations. They are very sensitive to fine details in coil currents and rf fields.

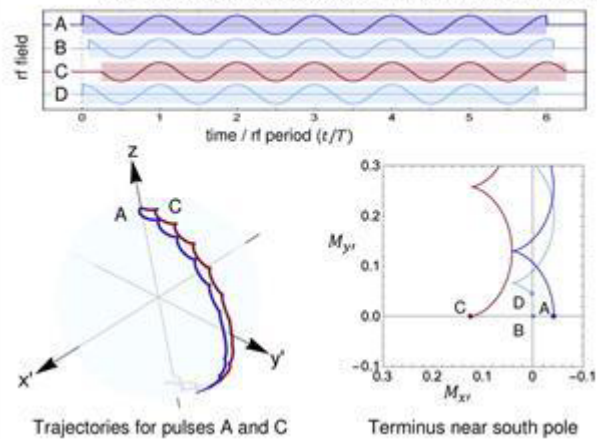
Conclusions

For any pulse duration and start phase, a shift from resonance and a small change in rf amplitude can be used to compensate for inaccuracies induced by operation outside the RWA. Practical considerations arising from this work and prospects for further investigations will be described.

¹Nacher et.al., J. Magn. Reson 310 (2020) 106638

²Bidinosti et.al., J. Magn. Reson 345 (2022) 107306

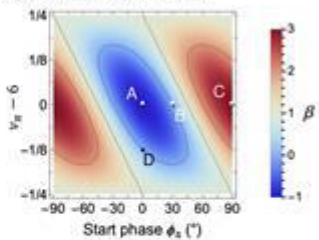
Outside the RWA: Rectangular π -pulses of equal areas ($B_1 t_\pi$) but different start phases (ϕ_s) and durations ($t_\pi = \nu_\pi T$) lead to different trajectories on the Bloch sphere and do not all produce accurate magnetization reversal.



Bloch-Siegert shift
for rf pulses:

$$\delta B_p = \beta \left(\frac{B_\omega}{16\nu_\pi^2} \right)$$

where $|\gamma|B_\omega = 2\pi/T$.



	ϕ_s	ν_π	β	rf amplitude
A	0°	6	-1	nominal B_1
B	30°	6	0	nominal B_1
C	90°	6	3	nominal B_1
D	0°	5 + 7/8	0	increase B_1 by 1.5%

Conditions required to achieve terminus at the south pole

P-391 - Integrated solid- and solution-state NMR approach to investigate binding mode of toxin to potassium channel

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¹Fmp Berlin, Berlin, Germany, ²Humboldt Universität Berlin, Berlin, Germany

Poster Session 1, Hall 1 & 2, SEC, July 10, 2023, 13:45 - 15:45

Peptide toxins can be found in many different organisms, and are known for their distinct biological functions. Toxins derived from scorpions, including for example charybdotoxin (CTX), noxiustoxin and kaliotoxin, are prominent inhibitors acting on potassium channels [1]. With their help, a multitude of structural information on these channels were obtained in the past [2,3], and especially in electrophysiological measurements, they are widely used for defining the orientation of the respective channel in the membrane, as they interact with only one side of the channel blocking the ion flux through the lipid bilayer [4].

In our study, we focus on the binding of CTX, a 37 amino acid protein from *Leiurus quinquestriatus*, to MthK. MthK is a Ca²⁺ activated potassium channel from archaeon *Methanobacterium thermoautotrophicum*. Showing a high degree of homology to eukaryotic BK channels [5], MthK serves as a model for those in several studies. Gaining insights will therefore help to understand inhibition and binding in eukaryotic K⁺ channels. We use solid-state magic-angle-spinning (MAS) NMR to characterize the channel-toxin complex, working in proteoliposomes to investigate the system in a near-native environment. With fast-spinning proton detected experiments, we were able to obtain highly resolved spectra, not just from the MthK with and without bound CTX, but also from the toxin itself in its bound state. To detect structural differences, the chemical shifts were compared to solution NMR spectra of the free CTX in solution. With this integrated approach, we were able to resolve several structural details of the complex, leading to deep insight into the system, especially the binding of the toxin. Using our results, following experiments for example on the behavior of K⁺ and other ions such as ammonium [6] in presence of the blocked channel are possible.

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P-400 - Zwitterionic or Not? Fast and Reliable Structure Determination by Combining Crystal Structure Prediction and Solid-State NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

When it comes to crystal structure determination, computational approaches such as Crystal Structure Prediction (CSP) have gained more and more attention. It is well known that the coupling of CSP with solid-state NMR (SSNMR) greatly enhances the performance and the accuracy of the predictive method, leading to the so-called CSP-NMR crystallography (CSP-NMRX). Here, we present the successful application of CSP-NMRX to determine the crystal structure of three structural isomers of pyridine dicarboxylic acid, namely quinolinic, dipicolinic and dinicotinic acids, which can be in a zwitterionic form, or not, in the solid state. Mono- and bi-dimensional SSNMR spectra were used to determine the correct molecular structure (i.e., zwitterionic or not) and the local molecular arrangement; at the end, the RMSEs between experimental and computed ¹H and ¹³C chemical shifts allowed the selection of the correct predicted structure for each system. Interestingly, while quinolinic and dipicolinic acids are zwitterionic and non-zwitterionic, respectively, in the solid state, dinicotinic acid exhibits in its crystal structure a “zwitterionic-non-zwitterionic continuum state” in which the proton is shared between the carboxylic moiety and the pyridinic nitrogen. Fancier SSNMR experiments were carried out, i.e., ¹⁴N-¹H Phase-Modulated (PM) pulse and Rotational-Echo Saturation-Pulse Double-Resonance (RESPDOR), to provide an accurate N–H distance value confirming the hybrid nature of the molecule. The CSP-NMRX method showed a remarkable match between the selected structures and the experimental ones. The correct molecular input provided by SSNMR reduced the number of CSP calculations to be performed, leading to different predicted structures, while RMSEs provided an independent parameter with respect to the computed energy for the selection of the best candidate.

P-206 - Hyperpolarized solution-state NMR spectroscopy via intermolecular NOE with parahydrogen-polarized source molecules

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Nuclear spin hyperpolarization provides a promising route to overcome the challenges imposed by the limited sensitivity of NMR spectroscopy. Recently, we have demonstrated that solutions of highly polarized source molecules can be created by dissolution of optically polarized pentacene-doped naphthalene crystals. This in turn enabled a transfer of magnetization to target molecules via intermolecular cross-relaxation at room temperature, moderate magnetic fields (60 MHz) and opened an alternative mechanism for hyperpolarization of external nuclei of the target molecules [1]. We called the method Hyperpolarized NOE System (HYPNOESYS) which so far have led to enhancements up to 2600 over 60 MHz, 50 over 400 MHz, and 40 over 600 MHz.

In order to make this technique more accessible, we extended our work by using Para-Hydrogen Induced Polarization (PHIP) as a tool to produce highly magnetized source molecules. The magnetization is attained by converting parahydrogen-derived singlet order using low-field polarization transfer protocols. We apply this technique on (1-¹³C,₆D)-dimethyl maleate which is produced by reacting (1-¹³C,₆D)-dimethyl acetylenedicarboxylate with parahydrogen in acetone-d₆.

The polarized solution is mixed with a solution of target molecules, where polarization is transferred in the same way via intermolecular NOE. Current work is focused on maximizing the achievable magnetization on (1-¹³C,₆D)-dimethyl maleate. We observe that under normal experimental conditions the achievable magnetization is limited and we hypothesize this is linked to the dipolar field effect. We will discuss such obstacles and recent advancements in using both hyperpolarized ¹H on naphthalene and on (1-¹³C,₆D)-dimethyl maleate as sources for hyperpolarizing target molecules in solution state. This will include transferring polarization to other nuclei, suppressing the source background, and strategies to improve enhancements with PHIP-based HYPNOESYS.

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P-152 - Minimizing the perturbation of the applied magnetic field by optimizing solid-state NMR probe structures

Mrs Jasmin Schönzart

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The NMR probe is likely the most specialized part of the NMR spectrometer with respect to the sample and is often the only component that needs to be exchanged when switching between different NMR samples (e.g. liquid, solids, gels) and experiments (e.g. static, spinning). It is essential that the probe locates the sample in the most homogeneous part of the magnetic field within the magnet, to allow for the best recorded spectra possible. In an ideal case, the probe itself would not perturb the applied magnetic field, yet effects of perturbation can be observed, which is why shimming of the probe is essential before every NMR experiment. In this work we want to present ways to minimize the perturbation of the applied magnetic field by optimizing solid-state NMR probe structures. This is achieved by a combination of simulations and experiments, which take susceptibilities of all parts in the NMR probe into account to then study the net effect upon the sample. Based on those results, modifications to NMR probes can be made, to minimize unwanted contributions. The smaller the perturbation of the applied magnetic field, the narrower the observed possible linewidth and the higher the resolution of the experiment.

P-362 - Light-coupled NMR spectroscopy without optical fibres

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Light is critical for sustaining life on earth, and is an important source of energy: a lot of chemical processes rely on light. Solution NMR spectroscopy is an extremely versatile analytical technique allowing to monitor chemical reactions in situ and in operando, however technical difficulties existed until recently in delivering strong, uniform and multi-coloured light to the sample inside the magnet. Recently we suggested a new way to illuminate NMR samples in situ, using an approach that we named NMRtorch, where LEDs are positioned right at the top of special NMR tubes, avoiding the need in using optical fibres and lasers, and making the measurement process more convenient and easy [1]. The resulting illumination is uniform and powerful. The universality and the power of this approach for some photoresponsive systems have been demonstrated and presented before [1]. We expand on that work and demonstrate that the approach works well in other areas, such as the fast and controlled release of photocaged molecules using in situ illumination with UV-A light generated by an LED [2], photostability studies of proteins, observing live the change in oligomerization state of photoreceptor proteins such as CarH upon illumination, or differential response to illumination in a family of azobenzene variants. We will discuss the implementations and the most up-to-date developments in NMRtorch approach for the high-field and benchtop spectrometers.

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P-198 - Off-the-Shelf Gd(III) Compounds as Efficient High-Spin Metal Ion Polarising Agents for Magic Angle Spinning Dynamic Nuclear Polarisation

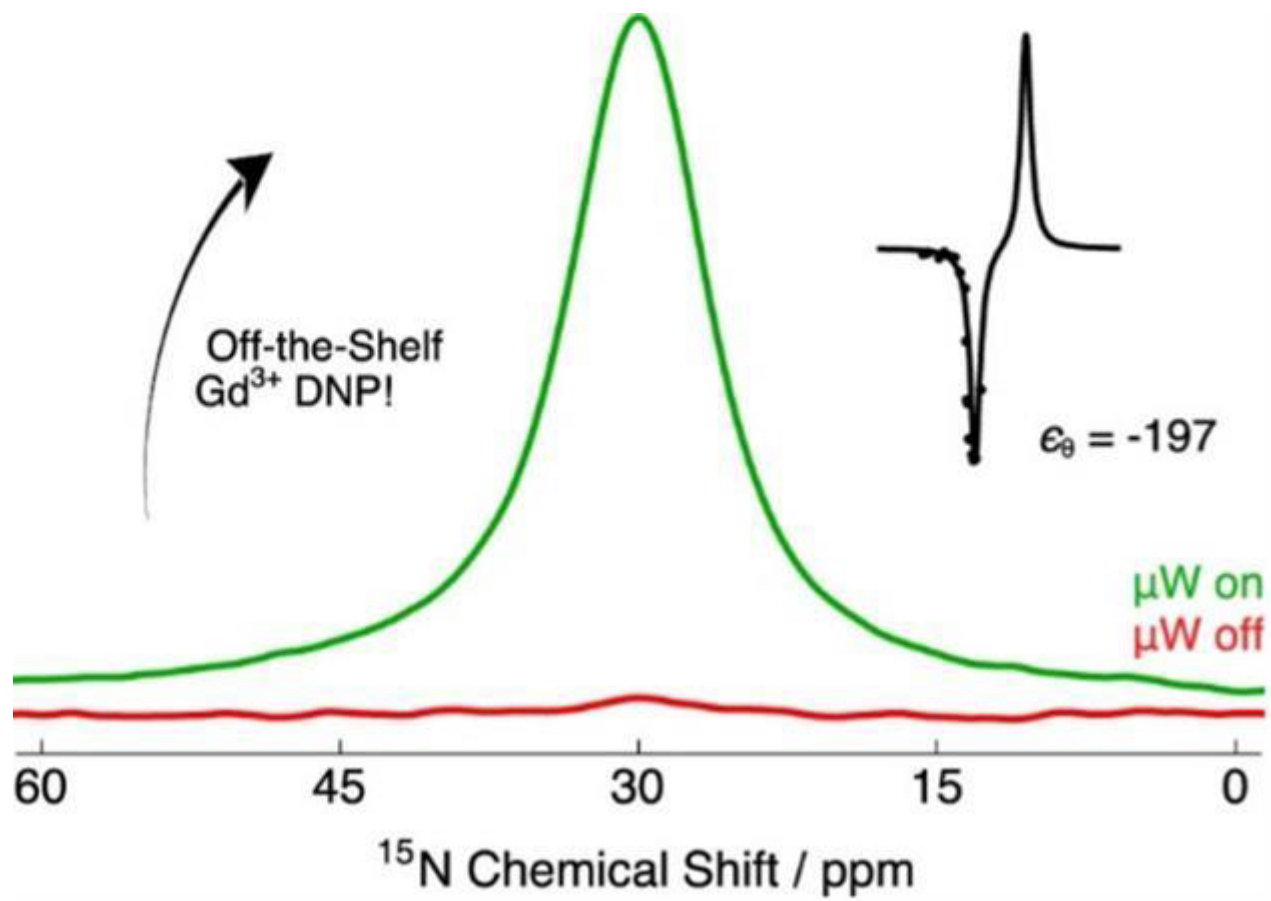
Dr. Daniel J. Cheney¹, Dr Stuart Elliott², Mr Benjamin Duff¹, Dr. Ashlea R. Taylor-Hughes¹, Mr. John P. Corley¹, Dr. Subhradip Paul³, Dr. Paolo Cerreia Vioglio³, Dr. Adam Brookfield⁴, Dr. Shane Pawsey⁵, Dr. David Gajan⁶, Professor Helen C. Aspinall¹, Dr. Anne LESAGE⁶, Professor Frédéric Blanc¹

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Significant solid-state NMR sensitivity enhancements may be achieved using DNP,¹ with much of its progress being driven by the rational design of efficient biradical-based polarising agents.² However, these are typically not commercially available, and often require lengthy, expensive, and low-yielding synthesis. Based on previous work using paramagnetic metal ion complexes (such as [Gd(dota)(H₂O)]⁻ and [Gd(tpatcn)]^{3,4}), we introduced the use of Gd(NO₃)₃ as an easily accessible and inexpensive “off-the-shelf” polarising agent without the need for chemical synthesis.⁵ We have demonstrated that appreciable sensitivity enhancements for ¹³C and ¹⁵N in [2-¹³C,¹⁵N]-glycine may be achieved at both 9.4 T (–35 for ¹³C and –197 for ¹⁵N) and 14.1 T (–20 and –68). Analysis of the DNP enhancement profiles and electron paramagnetic resonance spectra revealed that the solid effect is the dominant polarisation transfer mechanism. Current work is looking at investigating the impact of the Gd(III) concentration and source to optimise these promising enhancements further, and render the DNP methodology more accessible for the wider NMR community.

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P-092 - Cytosine methylation in the bcl-2 promoter region: a study on G-quadruplex formation and thermodynamic stability

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Cytosine methylation by the enzyme DNA methyltransferase (DNMT) leads to the formation of 5-methylcytidine (mC)¹. This epigenetic modification is generally associated with transcriptional silencing and is the most-studied epigenetic DNA modification in higher eukaryotes. Importantly, the introduction of an epigenetic modification into the gene promoter region could affect the DNA local structure and its stability².

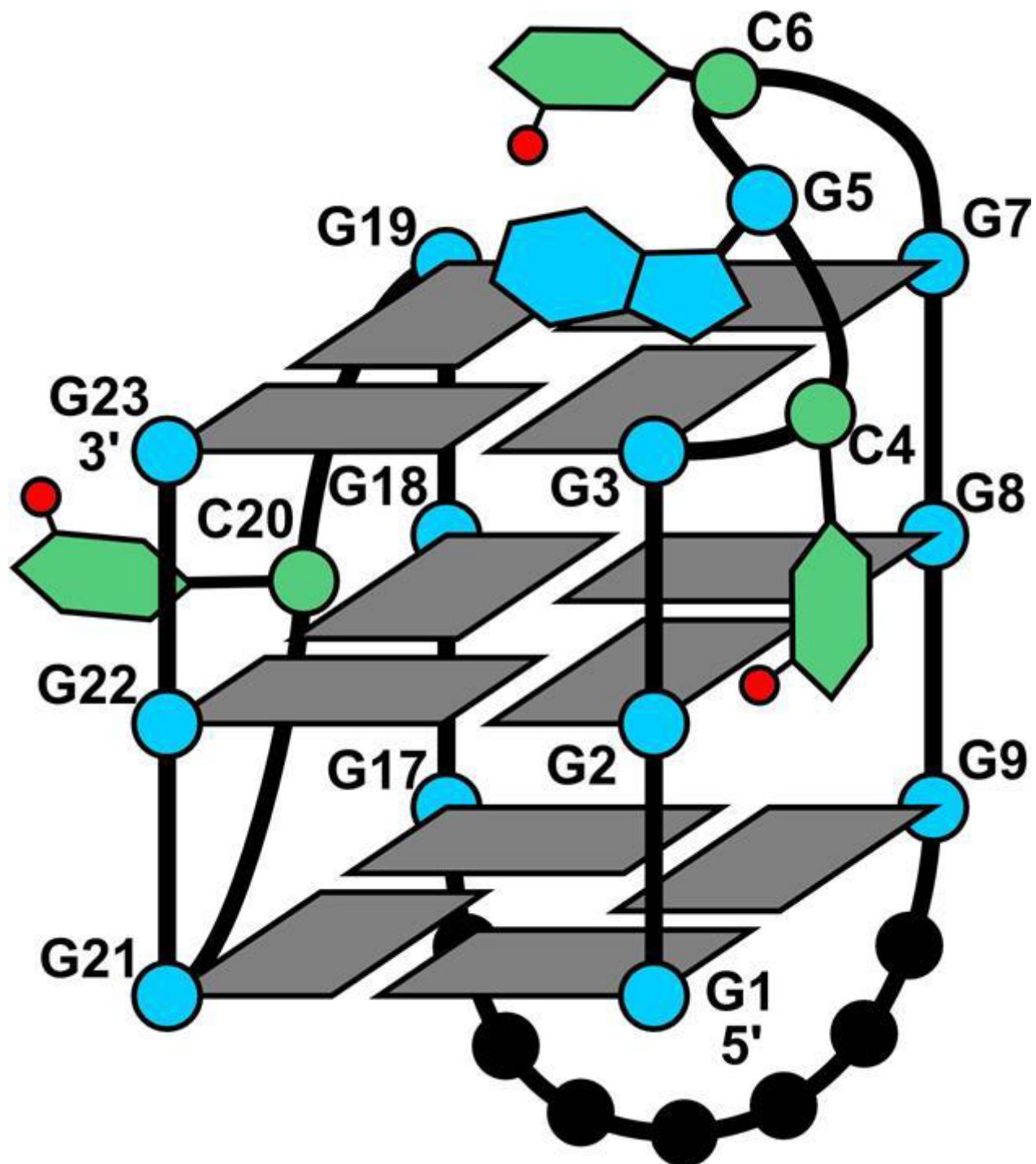
Bcl-2 (B-cell lymphoma 2), an anti-apoptotic protein, controls carcinoma growth in many tumors. Our intention was to acquire a deeper understanding of how epigenetic modification affects the structure and stability of the bcl2mid G-quadruplex³. Importantly, the effects of epigenetic modifications on noncanonical structures, are poorly studied. We commenced a study of the impact of introduction of mC on the folding of G-rich region of bcl-2 promoter. Special focus was given to substitution of the cytosines with mC at positions C4 and C6 in the lateral loop and C20 in the propeller loop.

Our results based mainly on 1D ¹H, ³¹P and 2D ¹H-¹H NOESY complemented with CD spectroscopy and differential dynamic calorimetry (DSC) data show that cytosine methylation does not impair folding of oligonucleotides into G-quadruplex structures. Moreover, single substitutions of cytosines with mC are well tolerated, and the original topology of the G-quadruplex is retained. However, we observed some local structural rearrangements in cytosine methylation at positions C4 and C6, which are also reflected in the changed thermodynamic stability. This is consistent with the fact that the first loop region (C4-G5-C6) defines and stabilizes the structure of bcl2Mid through its specific interaction with the core G-quartets.

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P-096 - Unraveling the dynamics of the G-quadruplex/RG-rich peptide complexes by NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Interactions between proteins and nucleic acids are crucial for the regulation of many cellular pathways. However, exact mechanisms at the atomic level are often still poorly understood due to difficulties in vitro mimicking of intracellular conditions that are needed for breakthrough structural studies.

One example of such important biological interactions are the ones between non-canonical nucleic acid secondary structures called G-quadruplexes and the arginine/glycine-rich (RGG/RG) domains of DNA/RNA binding proteins.¹ G-quadruplexes are structurally diverse and capable of performing a broad range of cellular functions, most notably regulation of gene expression, which may be facilitated by the binding of various DNA or RNA processing proteins. Nucleolin, a multifunctional nucleolar protein, contains an intrinsically disordered C-terminal RG/RGG-rich domain. It plays a role in various cellular functions and is also capable of G-quadruplex binding.²

We investigated the interaction between the nucleolin-derived RG/RGG-rich peptides and the parallel DNA G-quadruplex adopted by the oligonucleotide with four d(G₄C₂) hexanucleotide repeats, that are characteristic for the gene C9orf72 and the onset of ALS neurodegenerative disease.³ We showed that the investigated interaction is weak and the binding is influenced even by the smallest differences in the amino acid sequence of RG/RGG-peptides, while a specific amino acid sequence may be responsible for the major contribution towards the binding affinity. Folding of the oligonucleotide into the G-quadruplex during temperature annealing is also potentially affected by the presence of the peptides, resulting in altered G-quadruplex topology. Our results may become of greater interest considering the importance of the investigated interaction for the development of ALS and FTD diseases.

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P-028 - Insights into Horizontal Gene Transfer from NMR Spectroscopy – Elucidating a Novel Molecular Regulation Mechanism of Natural Bacterial Transformation

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Natural transformation is closely related to the rise of antibiotic resistances in bacteria.[1] Recently, the discovery of the protein RocC in *Legionella pneumophila* and its interaction partner, the highly conserved 66 nucleotide long sRNA RocR, shed first light on a completely novel regulation mechanism of the translation of the relevant proteins. The formed RocR-RocC complex binds the 5' UTR of mRNAs coding the uptake system, inhibiting their translation, thus acting as a negative regulator.[2] Despite recent efforts, the structural and dynamic properties of the functional 66 nt RocR in complex with the protein remain elusive.[3]

Results

In this work we present our latest insights into RocR structure and dynamics obtained from NMR studies of site specifically stable isotope-labelled RocR, RocC and RocC-RocR complex samples. The pseudoknot motif identified previously is involved in a slow exchange dynamic on the chemical shift timescale which we studied extensively by NMR and presume is crucial for the molecular mechanism of gene regulation.[4] Furthermore, we used sophisticated site-specific stable isotope labelling patterns in combination with the previously reported resonance assignment and PRE NMR to study the annealing of mRNA to the regulating complex in-vitro for the first time.

Conclusion and Outlook

To the authors' knowledge such challenging systems have, if ever, only been studied scarcely by solution NMR spectroscopy, due to the multifaceted challenges posed by them. Our results on the formation of this >50 kDa RNA-RNA-protein complex highlight the potential of site-specific stable isotope labelling strategies in this regard.

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P-062 - Structure analysis of the cocaine DNA aptamer upon binding quinine by site specific labeling and solution NMR-spectroscopy

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Aims

The binding mechanism of the cocaine-binding aptamer has been thoroughly studied and served as a model system for different biosensor applications. Aptamers are very selective for binding their specific substrate. The cocaine-binding aptamer, however, has been found not only to bind other substrates but these even with a higher affinity than cocaine itself. While it binds cocaine only in the low micromolar range quinine is bound with a dissociation constant in the nanomolar range. According to competitive-binding studies both quinine and cocaine bind at the same site of the aptamer. In order to analyze the exact binding mechanism of the cocaine-binding aptamer it is of high interest to also study this binding of quinine. [1]

Methods and Results

In order to elucidate the solution structure of the aptamer in complex with quinine we used site specific ²H, ¹³C, ¹⁵N stable isotope labeling of the DNA aptamer for assignment purposes. By titrating quinine to the aptamer and consecutive NMR measurements the structural changes of the aptamer could be observed. Additionally, we could assign all imino-signals via the ¹H-¹H NOESY experiments and ¹⁵N labeling in the apo and bound state.

Outlook

We are currently working on the elucidation of the key interactions between the aptamer and the ligand in the bound, which will result in the solution structure determination of the binary complex. On top of that we want to analyze the dynamics of the cocaine DNA aptamer in the apo state.

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P-038 - Efficient methods of amino-acid type recognition for resonance assignment of intrinsically disordered proteins

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

NMR spectroscopy is the most powerful technique for atomic-scale investigation of intrinsically disordered proteins (IDPs). It offers details on the object's transient structure, dynamics, and interactions. The first step of any NMR-based study is resonance assignment. This task might be challenging due to IDPs' very dynamic nature, which leads to the extremely narrow chemical shift range. As a result, finding the sequential connectivities is very complicated and the majority of spin system chains that are produced are short. In this case, accurate amino-acid type recognition is essential for mapping the chains on the protein sequence.

TSAR [1] is a program for automatic resonance assignment of IDPs using data from high-dimensional (4D, 5D) experiments processed with Sparse Multidimensional Fourier Transform [2]. In its original version TSAR utilized BMRB [3] and IDPs-derived [4] statistical data.

Aims

The aim was to improve the methods of amino-acid type recognition in order to facilitate mapping of short spin-system chains on the protein sequence.

Methods

The TSAR program has been developed to employ Linear Discriminant Analysis [5] as well as chemical shifts predicted for a given IDP by the POTENCI [6] tool.

Results & Conclusion

Employment of these sophisticated methods allowed more accurate resonance assignment (increase of correct and decrease of incorrect assignments), opening up an avenue to interesting information on IDPs.

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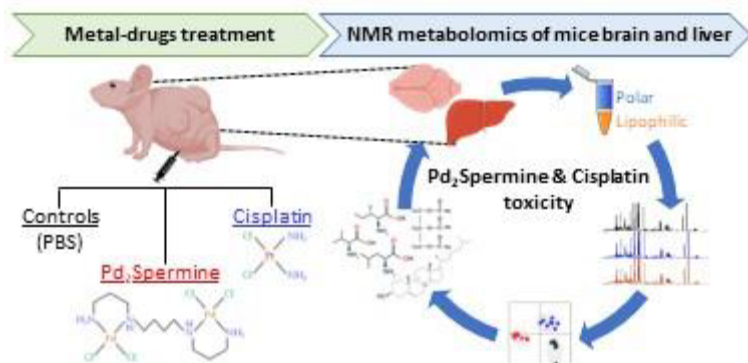
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The clinical use of cisplatin (cDDP) in chemotherapy regimens is often limited by severe deleterious effects, such as nephrotoxicity, hepatotoxicity and neurotoxicity. Therefore, alternative transition-metal drugs have been studied and, interestingly, the polynuclear palladium(II) chelate with spermine (Pd2Spm) has displayed promising in vitro and in vivo antitumor properties as well as favorable in vivo pharmacokinetics and biodistribution. Further knowledge on the metabolic response of tumors and organs, including the brain, is required to adequately assess drug efficacy and toxicity.

Main funding: CICECO-Aveiro Institute of Materials project (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020); Portuguese National NMR Network (PTNMR) project no. 022161; T.J.C. PhD grant SFRH/BD/145920/2019 (FCT).



P-098 - Effect of crowding agents on the nanosecond dynamics in a disordered protein.

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Intrinsically disordered proteins (IDP) and protein regions (IDR) are very abundant in the proteome of eukaryotes. Often with low sequence complexity, these proteins and domains display nonetheless important biological functions such as chaperone activity, signal transduction and site-specific binding. One key factor for the function of IDPs and IDRs is their dynamics.

Picosecond-nanosecond motions of several IDPs have been determined by relaxation measurements mostly in water. In the presence of crowding agents that mimic the cellular environment, nanosecond motions were shown to become slower in the model IDPs SeV-NTAIL and MKK4 1-86 . In other IDRs, molecular crowding stabilizes well-defined local conformations . Here, we question the effect of crowding agents on nanosecond dynamics of the C-terminal IDR from the protein XRCC4 (X4-CTR). XRCC4 is a homodimeric scaffolding protein of the human non-homologous end joining (NHEJ) DNA double-strand break (DSB) repair pathway, recruiting DNA-Ligase IV to the DSB site.

Aims:

To get further insight on the effect of crowding on dynamics in X4-CTR, we use high-resolution relaxometry, as low-field measurements are expected to be exquisitely sensitive to nanosecond motions .

Results:

We use a new prototype of fast sample shuttle, with enhanced sensitivity, to measure residue-specific ¹⁵N longitudinal relaxation rates in X4-CTR over 2 orders of magnitude of magnetic field (from 40 mT to 14.1 T), in presence and absence of the crowding agent.

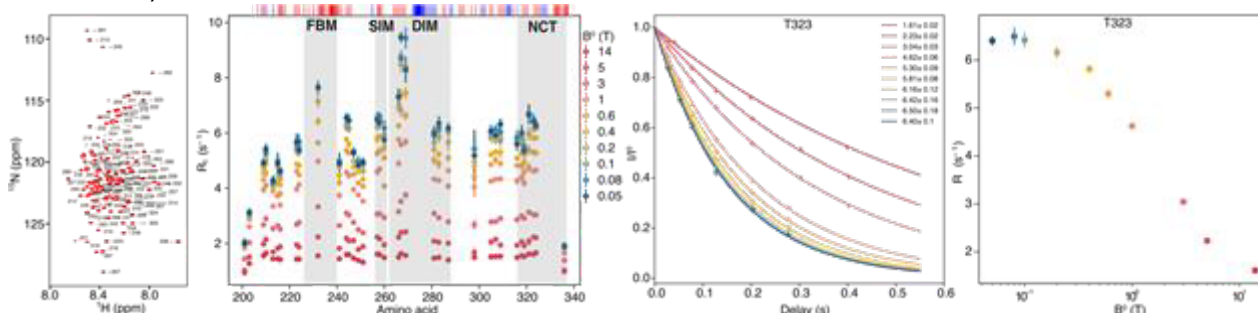
Conclusions:

High-resolution relaxometry allowed us to quantify the changes in nanosecond motions of X4-CTR induced by a crowding agent.

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P-310 - Solution conformational analysis of drug-like molecules

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

X-Ray crystallography and cryoEM can provide vital information on the structure of a ligand whilst it is bound to a protein target. However, they do not provide information on which conformations a compound can adopt in solution. Moreover, they do not assess the relative population of the compound in solution that adopts the protein bound shape, as many other non-binding conformations are typically present.[1] Conformational analysis can provide information about the set of molecular shapes that can exist in solution and can be used to estimate their energy levels compared to the bioactive conformations.[1] An investigation of conformational populations in solution can drive chemical synthesis towards better binding molecules, for example by pre-organising the ligand into the bioactive conformation.

NMR spectroscopy has been successfully employed in certain cases to the conformational analysis of pharmaceutical compounds and elucidation of chemical mechanisms.[1-2] NMR is sensitive to conformational changes and can provide structural information through several parameters such as chemical shift, J-couplings, NOE intensities, residual dipolar couplings and relaxation rates.

Results

Described here is an NMR Analysis of Molecular Flexibility in Solution (NAMFIS)[3] workflow which was employed to study drug-like molecules. This utilised the Maestro Macromodel (Schrodinger) programme to perform a search for potential conformational shapes followed by MNOVA's StereoFitter software for NAMFIS to fit the derived NMR parameters to actual data and to derive the relative populations of the conformers. In addition, geometry optimisations of Maestro conformers and the calculation of their NMR parameters was performed using Gaussian with DFT. The solution conformations obtained by this process are compared to the bound conformation of a T. cruzi (Chagas proteasome) inhibitor acquired from cryoEM (GSK).

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P-182 - Relaxation Interference Effects Leading to Observable Signal in Parahydrogenation of Symmetric Molecules

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Singlet states in NMR are characterised by a relaxation time significantly longer than T_1 . The reduced relaxation rate derives from the immunity of such long-lived states to intra-pair dipolar relaxation, which is a major relaxation mechanism in liquid state NMR. These states extend the use of NMR to study slower timescales e.g., slow chemical exchange or diffusive processes. Furthermore, they have been utilised in the areas of spectral editing and the measurement of protein-ligand interactions. Parahydrogen, the singlet state spin isomer of molecular hydrogen, is an example of such a long-lived state. It is well established that parahydrogen can be used to improve the sensitivity of NMR through hyperpolarisation. If the protons of parahydrogen are transferred to a new spin system in a pair-wise manner, but remain equivalent, the singlet state is retained. Such molecules are an attractive prospect to increase both the timescale and enhance the sensitivity of NMR experiments. Here, the parahydrogenation of a symmetric substrate, producing a symmetric product — where the parahydrogen derived protons remain in a pseudo-A2 spin system — is studied. It is shown that, at high magnetic field, a transient intermediate of the reaction causes a loss of the singlet state. Through cross-correlated chemical shift anisotropy and dipole-dipole relaxation effects, the loss of singlet order manifests as a negatively hyperpolarised signal in NMR experiments. This process is successfully modelled, considering both chemical kinetics and relaxation dynamics, and further corroborated by the study of cross-correlated relaxation in a similar system at thermal equilibrium. This work shows an unexpected route by which the loss of the singlet order of parahydrogen is observed as a hyperpolarised signal, and highlights the difficulties associated with the creation of singlet state molecules derived from parahydrogen.

P-004 - Design of optimal low-frequency NMR detection coils

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

The signal-to-noise ratio (SNR) in NMR measurements notoriously depends on the filling factor and Q-factor of the coil, and on the matching to the low-noise amplifier (LNA). At low frequency, the length of wire in multi-layered coils is not limited and impedance matching to 50Ω is not needed. Instead, noise matching to a high-impedance LNA is important.¹

Aim

We designed PU coils to optimise SNR while achieving sufficiently uniform sensitivity maps. The solenoid-like coils surround ~2-cm³ rectangular cells for diffusion measurements of hyperpolarized ³He gas in ordered Al₂O₃ aerogels² with different constraints on their maximum size.

Methods

The principle of reciprocity conveniently relates the average magnetic field per unit current B/I (computed using the Biot-Savart law) and the emf induced by the sample. At low frequency, the thermal noise is dominated by coil losses and scales as $(R_{AC})^{1/2}$ with the AC coil resistance. A SNR factor $B/I/(R_{AC})^{1/2}$ is therefore computed for arbitrary coil geometry and wire parameters.

Results

SNR factors and sensitivity maps only depend on coil dimensions, not on wire diameter. A broad optimum is found for coil length and winding thickness; moreover, the SNR decrease for more compact coils is easily evaluated (Fig. 1a). The coil sensitivity maps are fairly uniform over a wide range of dimensions (Fig. 1b). Two coils with the same bore and different outer dimensions were made, and tuned near 42 and 84 kHz ($Q \sim 100$). With 1.4-mm-diameter and 0.75-mm-diameter litz wires the tank circuits were suitably noise-matched to a SR560 LNA.¹

Conclusion

The PU coils were used in different set-ups, with measured SNRs consistent with expectations. This generic low-frequency optimisation approach can be used for multi-coil systems as well, e.g. for rejection of interference noise.

¹ V.V. Kuzmin and P.-J. Nacher, J. Magn. Reson. 310 (2020) 106622.

² H. Loutfi et.al., this conference.

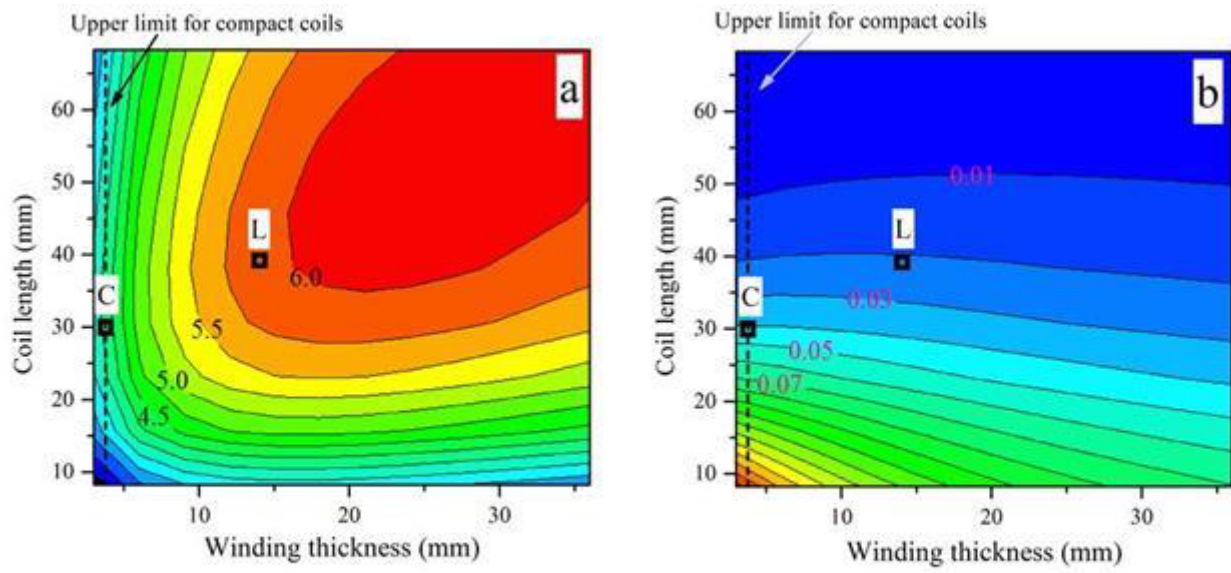


Fig. 1 2D maps of a: the coil SNR factor $B/I/R_{AC}^{1/2}$ and b: the standard deviation of coil sensitivity, for coils with rectangular bore $21.5 \times 24\text{mm}^2$. The symbols mark the parameters of the tested compact (C) and large (L) coils.

P-180 - Low-field relaxation and polarization transfer in solid pyruvic acid doped with trityl

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

A central challenge for the broad application of DNP is the transfer of relevant concentrations of highly polarized nuclear spins into an NMR or MRI magnet. Bullet-DNP solves this by shooting (≈ 100 m/s) the hyperpolarized solid and dissolving it only near the point of use.

To understand potential losses during the bullet-transfer at low field for a prototypical DNP sample, we studied low temperature proton [1] and carbon [2] relaxation in pyruvic acid, doped with trityl, between 5 mT and 2 T using a fast-field-cycling apparatus.

The spin-lattice relaxation times of both species are found to scale approximately linearly with field and to be uncritical for bullet-DNP. The data are modelled using a thermodynamic approach, in which heat is transferred via triple-spin-flips (TSFs) involving two electron spins and one nuclear spin, where the TSF rates are calculated from first principles.

We find that proton relaxation is only affected by the presence of trityl up to about 200 mT, owing to the limited heat capacity of the electron dipolar reservoir. The carbon relaxation is enhanced throughout the investigated field range. At low fields this is attributed to enhanced direct proton-carbon exchange due to radical-induced line broadening. Above 50 mT fast TSF rates mediate indirect heteronuclear exchange via the radical's dipolar reservoir, which accelerates carbon relaxation. The latter is confirmed by additional thermal mixing experiments.

Furthermore, we find cross-polarization effects during the field sweeps, also in absence of the radical, which we attribute to exchange with the tunnelling splitting of rotational states of the methyl group, and we discuss its effects and potential uses for bullet-DNP.

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P-338 - Application of nmr spectroscopy and statistical analysis in characterization of crude oil samples

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Detailed characterization of petroleum samples is of highest priority for quality evaluation of crude oils and crude oil product performances. [1] The crude oil components are usually divided into four main groups: asphaltenes, saturates, aromatics and resins. Asphaltenes are the most complex crude oil components which can aggregate and precipitate during petroleum processing, causing different problems in crude oil industry. [1-3]

Many different analytical techniques and approaches were used to investigate properties and composition of such complex systems as crude oils. [1] In this work it will be demonstrated that NMR spectroscopy is an indispensable tool for investigating crude oil samples. Although 1D NMR spectra of asphaltene samples consist of many overlapping signals, they still provide useful information on composition and aggregation process. It will be demonstrated with several examples that ¹H and DOSY NMR techniques in combination with statistical methods such as principal component analysis and machine learning provide powerful approach to identify and classify crude oil samples of different origin. A model can be proposed for prediction of the crude oil stability as an essential parameter that affect oil properties, thus showing potential for practical applications. [3]

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P-068 - Measuring pseudo contact shifts (PCS) on RNA through covalent lanthanide chelate tagging

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Aims

NMR has proven to be a versatile tool to study the structure and dynamics of nucleic acids. More recently pseudocontact Shift (PCS) measurements have expanded the NMR toolbox giving long distance restraints up to 50Å and beyond setting the stage for powerful experiments in solution structure determination[1]. A prerequisite to this techniques however is the incorporation of paramagnetic spin lables possessing an anisotropic magnetic susceptibility tensor as in various lanthanide metal complexes.

Methods

The use of an solid phase synthesizer for chemical RNA chain elongation in combination with organic chemical development of site specific stable isotope enriched and modified phosphoramidite building blocks enables the synthesis of RNAs up to the length of 70 nucleotides, bearing functional sites for further chemical modification and site specific isotope lables to adress specially tailored NMR experiments.

Results

In this work, we present a reliable new strategy applicable for the site-specific attachment of paramagnetic centers within nucleic acids for NMR spectroscopic investigations. To this end, a 5-trifluoroacetamido-methyl uridine phosphoramidite building block was synthesized and site-specifically incorporated during the RNA chain elongation on the solid phase synthesizer. The subsequent work-up procedure yields a functionally tethered oligonucleotide bearing a primary amine, which was used to attach a paramagnetic center by treating the RNA with a 4-thioisocyanato-DOTA-M8 Tm³⁺ or Dy³⁺ complex. A diamagnetic reference Lu³⁺-DOTA tag was used.

Conclusions

After testing the new methodology on different RNAs we recognized that especially the 5-aminomethyl tether approach in combination with the DOTA-M8 tagging enabled us to generate various pseudo contact shifts in the range up to 1ppm. These shifts are then used as long distance restrains in the RNA structure calculations.

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P-278 - Diffusion-based MRI at 28.2 T to resolve microstructural details and white matter tracts in young adult zebrafish brain

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

This study presents the first Diffusion-based Magnetic Resonance Imaging (dMRI) results of the zebrafish brain at the state-of-the-art magnetic field strength of 28.2 T. Diffusion-based Magnetic Resonance Imaging (dMRI) is a powerful tool for studying the microstructural organization of the brain non-invasively due to its high sensitivity for water movement. In particular, Diffusion Tensor Imaging (DTI) provides increased structural information by exploiting anisotropic diffusion effects. Zebrafish (*Danio rerio*) is an important animal model for neurodegenerative diseases. However, studying the zebrafish brain non-invasively remains challenging as it requires high-spatial resolution and signal-to-noise ratios (SNR), so far limiting the utility of dMRI for neurodegenerative disease research in zebrafish. Here we used 28.2T MRI system to get access to white matter structures in zebrafish brain by optimizing DTI, obtaining axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA) at a high spatial resolution. Short-track track-density imaging by constraint spherical deconvolution (stTDI CSD) was employed for the visualization of white matter structures. Our results show that ultra-high field DTI and tractography provide repeatable and quantitative maps of fiber organization from tiny zebrafish brains. As a proof-of-principle, these techniques were tested on a pathological zebrafish model and compared to control, indicating significant changes in the microstructure of the pathological model.

Acknowledgements: Experiments at the 28.2 T NMR instrument were supported by uNMR-NL. We would like to thank Andrei Gurinov for technical support at Utrecht University, and Volker Lehmann, Antonios Papaioannou, and Thomas Oerther (Bruker Corporation) for the technical help provided.

P-132 - The composite simulation of spin dynamics, chemical kinetics and fluid dynamics in a micro-fluidic chip

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Simulation frameworks for Magnetic Resonance Imaging (MRI) so far have been implemented using Bloch equations and extended to Bloch-Torrey and Bloch-McConnell equations. However, in the interest of more sophisticated and high accuracy simulations, several papers have been published attempting to combine Liouville-space spin dynamics and spatial dynamics. The Fokker-Planck formalism is the crux of the solution for the composite problem described here. However, implementing a simulation framework that is numerically efficient is a separate, complicated task. The dimensions of spatial dynamics generators can be exceptionally large, and when combined with the number of spins in a typical metabolite, the combined evolution generators (which arise from the Kronecker product of two matrices) are far too large to be stored and propagated. This problem was solved by Allami et al., and this project builds on the solutions provided within, whereby large-scale combined evolution generators are propagated by algebraically manipulating the polyadics involved. This is applied in the context of a micro-fluidic chip and the translated medium of simulation is a non-uniform triangular mesh, on which we model the stationary flow of a para-hydrogenation reaction. We implement Voronoi tessellation to compute the fluid dynamics generator. The chemical kinetics is described by a kinetics superoperator and the quantum mechanical treatment of spin is maintained. The combined dynamics are placed in and propagated through the Voronoi cells.

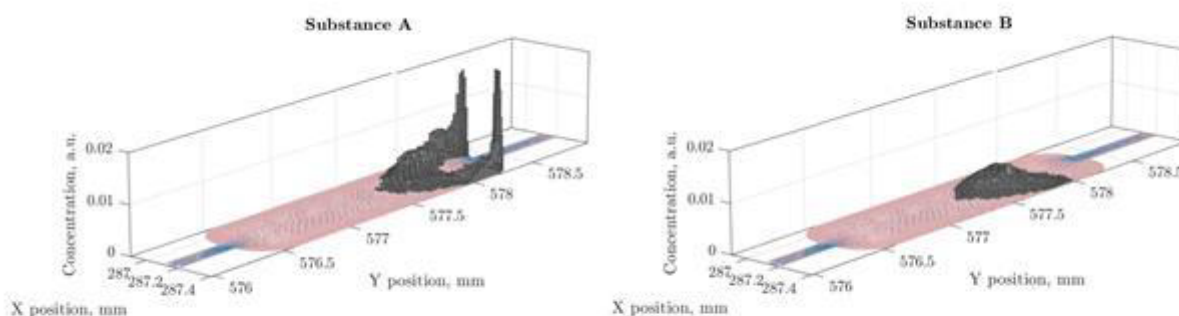


Figure 1: Simulation of flow during an asymmetric exchange reaction of substances A and B in the sample chamber of a micro-chip using the Fokker-Planck equation. The initial concentration of A was set to 1 in arbitrary units with the reaction rate at $10^{-3}/s$.

P-218 - Characterization of Lipidic Cubic Phases by Solution NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Lyotropic lipidic cubic phases (LCPs), a subgroup of liquid-crystalline phases, are formed via the spontaneous self-assembly of certain lipids, such as monoolein (MO), in an aqueous environment.¹ LCPs have been successfully applied to the in meso crystallization of membrane proteins for structural studies by X-ray crystallography,² and have also shown promising potential to serve as matrices for drug and nutrient delivery in vivo.³ The dynamics and structural properties of LCPs are of considerable interest for the application of these materials.

Aims

To characterize LCPs using solution NMR.

Methods

Solution NMR spectroscopy and PGSE NMR measurements.

Results

The following results for LCPs formed by MO will be presented: (i) the quantification of hydration levels,⁴ (ii) the assessment of additives induced chemical shift perturbation,⁴ (iii) the evaluation of chemical exchange of hydroxyl groups,⁵ and (iv) PGSE measurement of molecular translational diffusion,⁵ including the elimination of magnetization exchange in complex systems, such as LCPs, with the use of a chemical shift selective RF sequence.⁶

Conclusions

The structural, dynamics, and translational diffusion properties of LCPs, which are readily accessible by solution NMR, provide additional means for the characterization and optimization of these materials.⁷

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P-282 - Dissecting the Functional Connectivity in Cytoarchitectonic Subdivisions of the Primary Motor Cortex: The Interplay of Handedness and Hemispheric Lateralization

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Objective: Using cytoarchitectonic mapping, Brodmann area (BA) 4 has been identified to contain two separate subregions: an anterior subregion (BA 4a) and a posterior subregion (BA 4p). Limited research demonstrates that these primary motor control subregions perform different functions depending on tasks. It is crucial to examine the functional connectivity of these subregions bilaterally using resting state functional magnetic resonance imaging (rsfMRI) and to explore the impact of handedness on their functional connectivity within major brain networks.

Methods: In total, 48 left- and right-handed participants were evaluated using region-to-region-network rsfMRI analysis of BA 4a and BA 4p, targeting eight major functional brain networks. The Cambridge-Buckner dataset, which is a component of the 1,000 Functional Connectomes Project (an open-access platform without any restrictions; see the IRP statement http://fcon_1000.projects.nitrc.org), was used to collect and download the data.

Results: The findings reveal that BA 4a and BA 4p not only differ in cytoarchitectonic characteristics but also exhibit unique functional connections to specific brain networks. The anterior subregion, BA 4a, displays increased functional integration with distinct networks, particularly in right-handed individuals, while BA 4p, the posterior subregion, demonstrates other functional organizations related to attentional and higher-order complex networks.

Conclusion: The study indicates heterogeneous findings between the two hemispheres of these subregions, especially in right-handed subjects, suggesting enhanced interhemispheric and physiological brain organization. The results support the notion that functional differences exist between the two subregions of the primary motor cortex (M1), which are influenced by cytoarchitectonic properties. These disparities are either hemispheric or handedness-dependent. Future research should take these findings into account when analyzing functional connectivity in healthy individuals and those with neurodegenerative diseases, and should consistently account for hemispheric lateralization and handedness covariates.

P-184 - A targeted dissolution-dynamic nuclear polarization (D-DNP) approach to the study of the cellular metabolism of glutamine

Ms Karen DOS SANTOS

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Glutamine plays a central role in cellular metabolism and in some diseases. It is for instance under scrutiny regarding its metabolic deregulation linked to energetic reprogramming in cancer cells. Starting from the study of L-asparaginase, an enzyme possessing a secondary activity converting glutamine to glutamate to deprive lymphoma cells of energetic supply, our studies thus focus on glutamine, from basic in vitro monitoring of enzymatic reactions to more complex biological samples.

To address the issue of low concentration of metabolites in complex biological samples, we have developed several formulations using highly concentrated glutamine and asparagine for D-DNP in order to work with the inexpensive TEMPOL radical associated to cross polarisation sequences, while literature only describes the use of Trityl for glutamine, and asparagine has not been studied to our knowledge. Concentrations ranging from 300 mM to 1.5 M were reached by varying the proportions of solvents in the sample as well as its pH. ^{13}C polarization levels higher than 30% in solid state were achieved at 6.7 T and 9.4 T and 1.5 K.

This has allowed us to start tackling a variety of applications, the first being in-vitro enzymatic reaction monitoring to compare the action of L-asparaginase and glutaminase on their primary substrates (asparagine and glutamine) and to investigate secondary activity such as the one of L-asparaginase on glutamine. We then demonstrated that our high-concentration sample formulations provide sufficient polarisation to perform basic hyperpolarised MRI using a RARE sequence, and we have moved on to the study of more complex systems such as cellular extracts to study more thoroughly the whole metabolic cascade of glutamine.

P-228 - Gaining Insight into the Mechanochemical Synthesis of [Cu(Cl)(NHC)] Complexes using Solid-State NMR Spectroscopy

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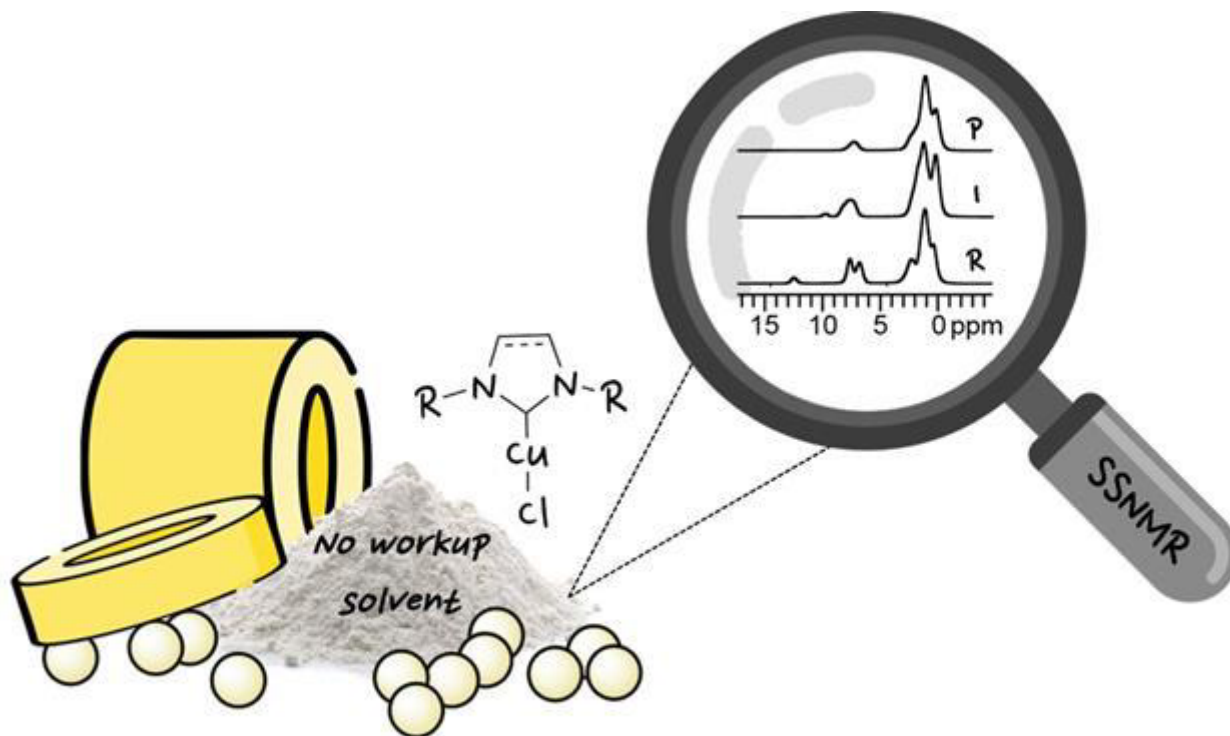
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Solvents are extensively used in large quantities in modern chemical synthesis. In order to reduce the environmental footprint of synthetic methods, their use should be reduced or, ideally, eliminated. Mechanochemistry is one possible route to achieve this goal. However, several reaction mechanisms involved in mechanochemistry remain elusive. As a local characterisation technique capable of atomic-level resolution, solid-state NMR spectroscopy is a promising method to unveil the mechanism of mechanosynthesis. Recently, solid-state NMR has been applied to study the mechanochemical synthesis of transition metal complexes bearing N-heterocyclic carbene (NHC) ligands, with applications in catalysis and anti-infective coatings in medical devices. (1-3) In particular, the effect of the successive work-up protocols for the preparation of a [Cu(Cl)(NHC)] complex was investigated. This compound was prepared under ball milling and solvent-free conditions in two different ways: (i) one-pot synthesis and (ii) step-wise synthesis. Multinuclear (¹H, ¹³C, ¹⁵N, ³⁵Cl, and ⁶³Cu) solid-state NMR experiments at 9.4, 18.8 and 28.2 T were performed to monitor the consumption of the reactants and the formation of intermediate and final products for the different synthetic routes and work-up protocols. Resonances were assigned with the aid of 2D heteronuclear correlations and DFT calculations of NMR parameters. The signals of ¹³C sites bonded to ⁶³Cu/⁶⁵Cu species in [Cu(Cl)(NHC)] complex exhibit multiplets due to J-couplings and quadrupolar-dipolar cross-terms. This presentation will highlight the results of these experiments and the notable differences observed between the one-pot and stepwise processes.

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Mechanochemical synthesis

P-178 - Continuous flow PHIP-RASER allows for study of nonlinear phenomena

Ms Jing Yang

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

RASERs (Radio Amplification by Stimulated Emission of Radiation) allow to measure high-precision NMR spectra as well as study nonlinear phenomena. Moreover, RASER uses spontaneous emission of radiation and therefore does not require external RF excitation. To operate a RASER, a population inversion is required, which can be generated by hyperpolarization methods. With parahydrogen fueled RASERs, various new use-cases were unveiled in the recent years, all based on RASERs with multiple frequencies. These included precision NMR below the mHz regime, the study of nonlinear phenomena, and encoding spatial information with RASER MRI.

Despite all these advantages, parahydrogen fueled RASERs operating at high magnetic fields are burdened by the parahydrogen pumping itself. Parahydrogen bubbles generate susceptibility artifacts that broaden RASER signals. Turning off the pumping during acquisition alleviates this problem, but limits RASER signals to bursts that only last until the polarization is depleted. Additionally, over the course of such a RASER burst, the RASER passes through different operating regimes and features different types of nonlinear phenomena due to the decaying polarization.

Results

In this work, we realized steady-state RASERs operating in different regimes due to the nonlinear interaction between RASER modes. We demonstrate operating regimes dominated by five different scenarios depending on the population inversion: a starting RASER, the “normal NMR” two-mode RASER, frequency combs, period doublings, and chaos (Fig. 1a). The steady state RASERs are acquired on a benchtop NMR (1.45 T) by continuously supplying a highly polarized ethyl acetate (EA) (Fig. 1b). The polarized EA is generated within a tube-in-tube reactor^[4] by hydrogenating vinyl acetate (VA) with parahydrogen at the earth’s magnetic field (Fig. 1c).

P-108 - Mechanistic Impact of Different Ligand Scaffolds on FXR Modulation Suggests Avenues to Selective Modulators

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The Farnesoid-X-Receptor is strongly relevant for treatment of several diseases and is mechanistically interesting, as the conformation of ligand-bound receptor determines which type of cofactor binds and thus results in an enhanced or attenuated activity.¹

Fundamental understanding of the different FXR modulations can consequently pave the way to the development of selective modulators. Using a variety of different assays and NMR spectroscopic data, we have established a workflow to distinguish between full and partial agonists.²

Looking at the protein sequence of FXR, we find that there are only two tryptophans in the entire protein, both located in the binding site region and thus involved in FXR ligand binding and activation. Tryptophan side chains resonate in a unique spectral region when a ¹H,¹⁵N-HSQC spectrum of uniformly ¹⁵N-labeled protein is obtained, so it can be perfectly used as a reporter signal regarding binding events.

Our NMR spectroscopic comparison of the known full agonist GW4064 and the newly synthesized ligand IMU-838 showed significant differences in conformational changes of FXR, so that we are able to identify IMU-838 as partial agonist. This is in line with the data of our biological assays and show up an useful method to determine the agonistic effects of new ligands for FXR.

P-024 - Assembly mechanisms of the plant protein REMORIN into membrane nanodomains

Mr Zeren XU

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Plant-specific REMORINs (REMs) are crucial proteins in plant defense against viral propagation by regulating cell-to-cell connectivity. They are tightly associated with the clustering of nanodomains at the plasma membrane, driven by specific protein-protein and protein-lipid interactions. Despite recent advances, the precise underlying mechanisms of nanodomain clustering by REMs are still unknown. REMs can be classified into 6 groups, containing a membrane-associating C-terminal anchor (REMCA) and a coiled-coil domain followed by an intrinsically disordered N-terminal region (IDR). We have shown that StREM1.3's nanodomain clustering depends on its phosphorylation status in the IDR, oligomerization potential, and interactions with specific lipids. We reveal that the different REM groups rely on diverse sequence motif arrangements and REMCA sequences. We investigate REM's structural and dynamic organization based on domain-specific analysis and consider the context of the three-domain protein. Bioinformatics analysis on multiple sequences of the REM groups reveals diversity in sequences and motif distribution, suggesting their implications in regulating nanodomain clustering. We performed 3D structure determination of REMCAs of different REM groups by NMR, discovering the REMCA motif diversity, further highlighting the role of sequence adaptation and structure modulation to control membrane association. Towards re-contextualization, we have then extended our analysis to investigate the molecular architectures of stable REM multimers, searching for the most favorable energetic coiled-coil structures.

P-334 - Guidelines for the absolute quantification of complex mixture of metabolites in different aqueous solutions by ultra-high resolution nuclear magnetic resonance

Ms Xi Chen¹

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Ultra-high resolution pure shift NMR has recently been shown to constitute an interesting approach to providing quantitative metabolic profiles that can be used in metabolomic studies. In this approach, a library of ¹H pure shift reference spectra recorded on metabolites of interest was created using a pure shift sapphire method combined with water suppression technique¹. These reference spectra have been successfully used to quantify metabolites in extracellular cancer cell medium. However, only a limited range of biological samples has been analyzed using this approach so far.

Aim and methods:

Applying a pure shift approach to quantify different kinds of biological samples (urine, plasma, tissue extracts...) requires evaluating the quantitative of the pure shift library over a broad range of sample conditions and experimental settings. Here, we present an exhaustive protocol that carefully addresses the influence of main experimental conditions: i) sample preparation, and ii) spectrometer parameters, on the robustness of our pure shift library regarding metabolite quantification in different biological samples. To that end, we have designed model metabolites mixtures prepared under different solvent conditions, which mimic biological systems.

Results:

We have successfully applied our pure shift library to quantify metabolites in different model mixtures with good linearity, trueness, and precision, which is due to the high reproducibility of our analytical protocol whatever the physico-chemical conditions in the different samples.

Conclusions:

Our results show that the library of pure shift reference spectra will allow for performing accurate quantification of a broad range of samples as long as they show similar ¹H relaxation properties. We recommend that preliminary control should be made when a new class of biological matrices will be considered.

[1] X. Chen, C. Caradeuc, A. Montagne, V. Baud, G. Bertho, C. Lucas-Torres, N. Giraud, *Anal. Chem.* 2022, 94, 14974–14984

P-240 - Hot liquid phase stability of zeolites produced from waste fly ash

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Heterogeneous catalysis in liquid phase reactions is valuable in fine chemical industries for alkylation, deoxygenation, aldol condensation reactions, amongst others. The performance of catalysts like zeolites is determined by the framework structure, Si/Al ratio, acid density, tuneable tetrahedral coordination, and the pore environment. While zeolites are relatively stable in the vapour phase temperatures of 350 - 500 °C, the tectosilicate structure gradually disintegrates in liquid at temperatures exceeding 150 °C [1].

Aim:

To understand the stability and structural integrity of the synthesised fly ash-based BEA zeolite catalysts in hot liquid environments.

Methods:

The zeolites were synthesised from coal fly ash according to patent procedures [2] with some modification. Stability tests were carried out in an autoclave reactor at different temperatures and times. The zeolite was then recovered and characterised using a plethora of techniques such as SEM and XRD. Nitrogen sorption isotherms and Brunauer-Emmett-Teller (BET) surface areas, and DFT models were utilized to determine the pore size distributions, while ²⁹Si and ²⁷Al solid state NMR (including MQMAS) was acquired at 11.4 T.

Results:

The structural integrity of the coal fly ash-based zeolites was compromised only under prolonged reaction conditions (24 h) at 200 °C. Desilication of the framework structure, caused by hydrolysis of Si-O-Si to terminal Si-OH, influenced the tetrahedral Al framework and the octahedral extra framework. The BET surface area, pore volume and pore size distribution were also affected by prolonged exposure at 200 °C. This zeolite may therefore be used for heterogenous catalytic reactions at temperatures below 200 °C in the liquid phase.

References:

[1] Ameh, et al., Catalysis Today, <https://doi.org/10.1016/j.cattod.2019.08.006>.

[2] Petrik, et al. Process for Production of High Silica Content Zeolite from Fly Ash, WO/2017/221192, (2017).

P-402 - Fast Synthesis of High-Silica Zeolites and their Solid-State NMR Characterisation

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Aluminosilicate zeolites are important heterogeneous catalysts for acid- and metal cation-catalysed reactions. Their catalytic properties derive in large part from the substitution of trivalent aluminium for silicon in their tetrahedrally-coordinated framework, and the associated presence of charge-balancing protons or metal cations but understanding the behaviour of the aluminium in these catalysts remains incomplete. Among zeolite catalysts, zeolite Y is one of the key catalysts in the petrochemical industry, and when in the high silica zeolite form is integral to the petrochemical industry[1]. Increasing the SiO₂/Al₂O₃ ratio (SAR) and overcoming the kinetic limitations to devise a fast synthesis route to obtain high-silica zeolite Y by direct synthesis (rather than via extensive post-synthetic treatments, including steaming) have been long-standing challenges in the zeolite industry[2]. Zhu et al. have recently reported a facile strategy to realize the fast crystallisation of high-silica zeolite Y, which involves the combination of high crystallisation temperature, ultra-stable Y (USY) seeds and efficient organic-structure directing agent (OSDA)[3]. Here we have investigated this new route with a view to understanding the crystal chemistry of this high silica Y. The reported synthesis method is found to be sensitive to synthesis parameters and can also yield high-silica ZSM-11. These materials have been characterised using traditional solid-state techniques; Powder X-ray Diffraction, Scanning Electron Microscopy and most importantly, solid-state NMR spectroscopy, including ²⁷Al, ²⁹Si, ¹⁵N and ¹³C. ¹³C NMR spectra, together with computer modelling of the organic structure directing agent in the zeolites' pores, is found to reveal details of the location and local environment of the OSDA.

[1] D. Verboekend, et al., Chem Soc Rev 2016, 45, 3331.

[2] U. J. Etim, et al., Fuel 2016, 178, 243.

[3] D. Zhu, et al., Angewandte Chemie - International Edition 2022, 61, e202117698

P-356 - Connect NMR UK: A National NMR Network for Physical and Life Sciences

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Connect NMR UK is a EPSRC, BBSRC and MRC funded project that aims at increasing knowledge exchange and maximising NMR capital investment and impact of the UK NMR infrastructure into the broader community. With the objectives of expanding awareness of and facilitating access to the UK NMR infrastructures, the sharing of knowledge and good practice and supporting discussion and exchange of information with non-NMR experts who can benefit from the existing NMR capabilities. The aims of this poster are to highlight how researchers may benefit from working with the network. Through the network we can put you in touch with NMR scientists and hardware, as well as support training for PhD/PDRAs in order to learn more about the NMR techniques that may be beneficial to their research. Connect NMR UK also provides a training mobility grant of up to £600 in order to support travel, accommodation and subsistence for NMR training or workshops within the UK. We have funded many training days at different institutions throughout the UK, covering solution-state, solid-state and biological NMR. We have also hosted workshops on solid-state NMR for solution-state spectroscopists at the UK High-Field Solid-State NMR Facility at the University of Warwick, in March the last two years. Our website connectnmruk.ac.uk provides information on upcoming NMR events, available funds and some highlights of recent NMR research, as well as the NMR research facilities throughout the UK, with details on the field strengths and additional capabilities e.g DNP, Cryoprobes, extended temperature ranges, etc.

Connect NMR UK is a network for the community in order to make it easier to access NMR infrastructure and connect you with specialist NMR communities in the UK, as well as support training for PhD/PDRAs and promote NMR in the wider infrastructure of the UK scientific community.

P-006 - New applications of atomic spin sensors in NMR and MRI

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

INTRODUCTION: Following bursts of activity in (1) the 1980s via field-cycling techniques and (2) the 1990s using superconducting interference magnetometers (SQUIDS^[1]), ultralow-field magnetic resonance (ULF-MR) is, in the present decade, enjoying a lively third renaissance.

We attribute this to two factors: (a) timely advances in inexpensive, easy-to-operate magnetometers with unprecedented sensitivity in the dc-to-MHz band. These are based on vapor-phase atomic ensembles where development is driven by application in medical fields such as magnetoencephalography and magnetocardiography^[2]; (b) development of ULF^[3]C/^[4]N hyperpolarization methodologies, e.g. SABRE-SHEATH and variants. In this presentation, we outline the current state of the field and promising new topics from our own work^[3-5].

METHODS & RESULTS: Our laboratory operates various commercial and home-built^[6]Rb-vapor magnetometers, which have been applied to fast-field-cycling relaxometry^[5] in the dc-10 kHz band, near zero-field NMR and NQR. New developments to be reported include:

-- Our development of the open-source NMR platform “NMRduino” – software and hardware layers designed to slot between the popular ArduinoTM prototyping ecosystem and NMR scientists, substantially simplifying and democratizing access to magnetic resonance experiments worldwide (Figure 1). A low-cost, credit-card-sized pcb provides electronic components for control of magnetic fields from dc up to ~100 kHz, plus interfaces with detectors including atomic magnetometers and conventional inductive pickup coils. Connects to any laptop, desktop or Raspberry Pi via USB. Use in teaching and hyperpolarized NMR will be shown.

-- ULF-MR Imaging of μL fluid volumes in multi-material chip-scale devices made from glass, 3d-printed resin, metals etc. We report one- (Figure 2) and two-dimensional^[7]H images using ~20 mT prepolarization and ~10 μT detection using atomic magnetometers, discussing present limitations and applications such as paramagnetic contrast imaging.

CONCLUSION: Sub-mT NMR detection has been traditionally regarded as “exotica” but is increasingly becoming a convenient and optimal strategy in many use cases.

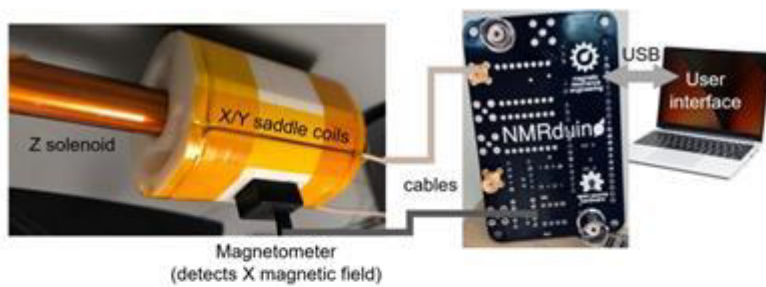


Figure 1. Ultracompact "NMRduino" NMR spectrometer system shown in entirety, with exception of a compact magnetic shield (which houses the coil/magnetometer assembly) and a low-voltage dc power supply.

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- [1] Kraus Jr. et al, Ultra-Low Field Nuclear Magnetic Resonance: A New MRI Regime. ISBN: 9780199347889 (2014)
- [2] Tierney et al., Neurolmage 199, 599-608 (2019)
- [3] Mouloudakis et al. J. Phys. Chem. Lett. 14, 1192-1197 (2023)
- [4] Bodenstedt et al., J. Phys. Chem. Lett. 13, 98-104 (2022)
- [5] Bodenstedt et al., Nat. Commun. 12 4041 (2021)

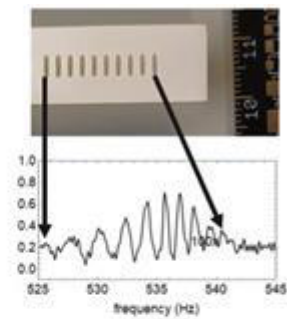


Figure 2. Our first 1D ULF-MR image of a 3d-printed fiberglass phantom. Each fluidic cavity, of 2 mm center-to-center spacing, contains 10 μL H_2O .

P-306 - Intermolecular dynamics and allostery of human BRD4-BD1 and -BD2

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

A deep understanding of the structure-activity relationship between proteins and their ligands is crucial for the development of drugs. A thorough description of the intermolecular interactions requires the characterization of geometry (structure) and dynamics of the molecular complex at atomic resolution, including the internal dynamics of both partners and the intermolecular dynamics taking place at the complex interface. However, the quantitative involvement of dynamics has been rarely studied due to the lack of adequate experimental methods at such resolution.

This project aims to establish a quantitative structure-dynamics-activity relationship for protein-ligand interactions. Proteins BD1 and BD2, which are part of the human bromodomain-containing protein 4, will be probed along with several small binders. First, a structural ensemble of proteins in their free and complex forms will be obtained by NMR. NOE build-up curves will be acquired and converted into distances, from which a multiple-state ensemble of structures will be derived. NOE violations will be analyzed to obtain a map at atomic resolution of allosteric communications through motions in the apo- and holo-proteins, which will allow to follow the effect of ligand binding on the protein dynamics. Afterwards, NOESY experiments at several tmix and temperatures will be acquired to derive population distributions from which thermodynamic parameters ΔH and ΔS will be calculated. This will enable for “calorimetry-like” measurements to be obtained, hence determining at atomic resolution the enthalpy and entropy of the protein-ligand interaction.

ITC experiments have been done to derive the global affinity, ΔH , and ΔS of the binding of BD1 and BD2 with ligand ABBV-774. The backbone and the side-chain assignments of both proteins have been completed, as well as relaxation experiments to derive their τ_c . The next milestone is to acquire and interpret the NOE build-up curves to derive the network of distances covering the whole protein (dynamics-activity relationship).

P-058 - Investigating ligand-induced conformational changes in the tRNA-guanine transglycosylase dimer by ^{19}F NMR

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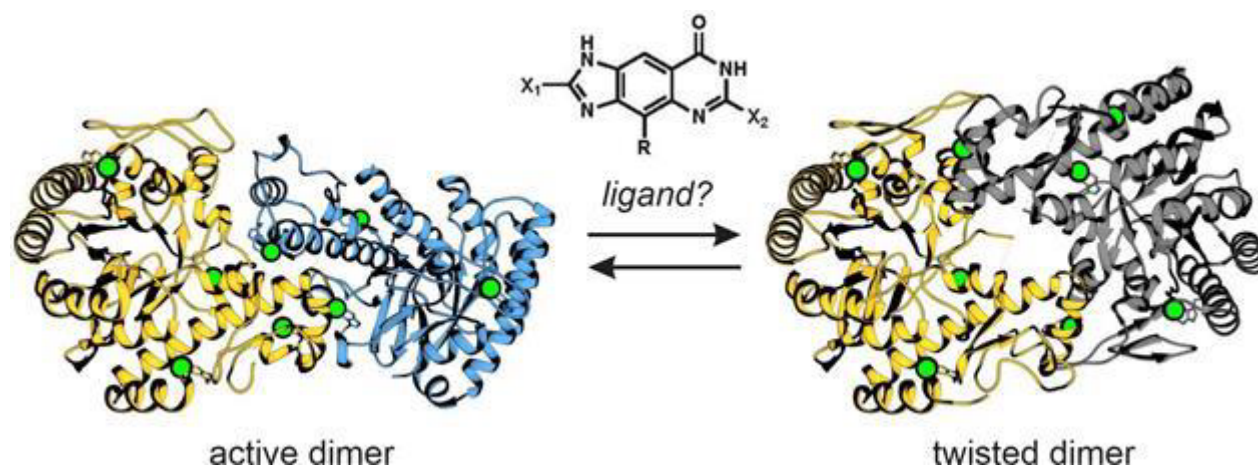
Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Recent crystallographic studies have shown a novel twisted and functionally inactive form of the homodimeric tRNA-guanine transglycosylase (TGT), a putative target against Shigellosis.

In X-ray studies, some active-site ligands are exhibiting a 130° twist between the two monomers, leading to a functional loss of TGT. Additional investigations in solution were needed to verify and quantify a ligand-induced shift of the equilibrium between native and twisted dimer states of TGT. Because of the size of the TGT dimer (86 kDa), we have used ^{19}F -NMR spectroscopy, incorporating 5-fluorotryptophans (5FW) at the four tryptophan positions in wild type TGT.

The inhibitor-induced conformation of 5FW-TGT in solution was assessed from ^{19}F -NMR chemical shift perturbations relative to free 5FW-TGT. In 1D ^{19}F spectra, different perturbation patterns could be observed that can be correlated with either a normal or twist-inducing ligand binding.

It could be shown that ligands known to induce the twisted dimer in crystals will lead to a characteristic ^{19}F shift pattern, with a pronounced signal shift for 5F-W178 and 5F-W326. However, other ligands not known to induce the twisted state in crystals were found to show a ^{19}F pattern that implies the existence of a dynamic equilibrium between normal and twisted state. Comparison of various benzoguanine ligand structures hints to a role of the C4 substituent in inducing the twisted state, by de-stabilizing helix αA and the adjacent loop-helix motif near the ligand-binding site of TGT. The ^{19}F shift pattern of the normal functional 5FW-TGT dimer could only be seen with ligands without any C4 substituent – or with a substituent actually stabilizing helix αA with H-bonds. These findings on the various degrees of twist-induction from different ligands suggest a novel concept for the design of new drug candidates.



P-262 - Classification Models and Metabolite Identification: The two Sides of the same Coin

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

In recent years NMR based metabolomics has become an important tool to establish the authenticity of foods and food products. An advantage is the simultaneous analysis of a multiparametric system, such as an aqueous extract, composed of tens to hundreds of individual metabolites. These chemical fingerprints encode information, e.g., the variety of a food. They are difficult to imitate in their complexity, making life more difficult for food counterfeiters. Truffles are an example with extremely huge price differences. For example, the black Tuber melanosporum achieves prices of 1000-4000 €/kg, while its Asian relative, the morphological similar Tuber indicum, is offered at prices of 60-200 €/kg. While the distinction usually is only possible for few experts, NMR and multivariate data analysis can unequivocally differentiate both varieties.

While the statistical analysis is independent on the knowledge of the chemical composition of the sample, a biological or chemical interpretation requires identification of these markers. If the signals of interest belong to a composition of marker compounds, then the isolation of every single analyte can be extremely time consuming. On the other hand, due to signal overlaps and concentration differences, the reliable identification of individual metabolites from NMR or MS spectra alone is hardly possible. The MATLAB based app SCORE-metabolite-ID enables the semi-automatic correlation of NMR and MS data via the time course of a chromatographic fractionation. This leads to the dissection of NMR signals originating from the same molecule and correlates them with corresponding mass-to-charge ratios from MS spectra. Without the need of the isolation of individual compounds, efficient and reliable identification is possible even for structures not enlisted in the common databases. Extending this concept to the correlation of 2D NMR spectra with MS data facilitates the analysis further, with the advantage of gaining additional spectroscopic information at the same time.

P-174 - Elucidation of MYC:MAX-DNA Molecular Network Behavior through the Combination of NMR, DNP, and EPR

Miss Ludovica Martina Epasto

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Transcription factors (TFs) often feature high conformational plasticity that mediates their physiological functions. The MYC-associated factor X (MAX) is a TF, described as a partially intrinsically disordered (ID) basic helix-loop-helix leucine zipper (bHLH-LZ) TF that homodimerizes in solution to bind to DNAs. In the presence of MYC, it forms the transcriptionally active heterodimer MYC:MAX. pH, temperature, and protein concentrations¹ strongly affect the equilibrium of MAX conformational states.

Interestingly, the ID breast cancer type 1 susceptibility protein (BRCA1) also modulates the MYC:MAX-DNA network by binding DNAs² and MYC. Yet, little is known about the structural dynamics of this highly complex interaction network.

Here we present an approach combining dissolution dynamic nuclear polarization (dDNP), with ¹³C-¹⁵N correlated NMR, real-time experiments, continuous wave EPR, and nanoscale distance measurements to elucidate many structural and dynamic details of MYC:MAX-DNA/BRCA1.

Particularly, we tackled three factors impacting the MAX conformational equilibrium:

1. Low concentrations: these induce a conformational shift of MAX accessible via dDNP.² This shift leads from MAX:MAX to (formerly unnoticed) globularly folded monomeric MAX forms fostering MYC:MAX heterodimerization upon MYC exposure.
2. BRCA1 activity: ¹³C-¹⁵N NMR and EPR experiments revealed that BRCA1 impacts the MAX conformational equilibrium by competing with bound nucleic acids for the N-terminal DNA-binding site.
3. MYC activity: we used real-time NMR to monitor the influence of MYC on the MAX:MAX-DNA conformational space and its transition to MYC:MAX-DNA. To this end, we injected a MAX:MAX-DNA solution into a selectively (Ile and Leu) ¹³CO labeled MYC sample to monitor the conformational transition of the latter by a time series of ¹³C edited 1D spectra.

[1] LM Epasto, K Che, F Kozak, A Selimovic, P Kaderavek, D Kurzbach, Sci.Adv. 2022, 8(31), eabq5179.

[2] K Che, T Kress, M Górka, S Žerko, W Kozminski, D Kurzbach, JMRO 2022, 12-13(ISSN 2666-4410).

P-052 - Translational diffusion of folded proteins and IDPs: empirical D-M relations, behaviour in denaturing media and under crowding conditions

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

As hydrodynamic properties of folded proteins and IDPs differ, systematic PFG NMR studies enable the establishment of empirical translational diffusion coefficient-molecular weight (D-M) relations. With proper validation and corresponding corrections for different media these D-M correlations are applicable at different temperatures. We show, how the choice of reference molecules such as dioxane and water alters the outcome of the evaluations, and how can an improperly chosen reference lead to high errors and false conclusions when calculating molecular size.

Furthermore, we monitor protein unfolding process in different media. We follow the denaturation of three model proteins (lysozyme, ubiquitin and BPTI) and the effect of the disulphide bonds on denaturation. 8M urea and DMSO-water mixtures act in a distinct manner on protein unfolding. A high concentration of DMSO can fully unfold structured proteins to a random coil state, disregarding the presence of disulphide bonds, while in 8 M urea, proteins do not completely unfold, indicating that transient secondary structural elements persist even under strongly acidic conditions. Only complete reduction of disulphide bridges can bring the studied proteins to a disordered state. However, protein investigations are generally conducted in diluted aqueous solutions, and work in cellular environment may question the reliability of these earlier results. Using different concentrations of crowding agents PEG, Ficoll, and BSA to mimic the cellular environment, we characterize the translational diffusion of various components: the crowder, the model protein lysozyme and some small molecular diffusion standards.

An impediment of evaluating the commonly used ¹H-based translational diffusion measurements is the intensity of the protein compared to the intensity crowding agent. For this purpose, using ¹⁵N labeled proteins, we developed the BEST-DOSY approach – that enables investigations in crowded media as well as under in-cell conditions.

P-238 - Combining solution- and solid-state NMR for the structural elucidation of carbohydrate biopolymers and colloidal materials

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

NMR spectroscopy is particularly valuable for carbohydrate materials and colloid systems due to its ability to detect structural and dynamic domains (surface/core, organised/amorphous, rigid/mobile). The heterogeneous nature of these systems requires a combined application of solution- and solid-state NMR to decipher surface and interior environments of different sites. In addition, the combination of NMR with diffraction and computational methodologies is essential for understanding the molecular scale arrangement of such complex systems.

Using examples of different carbohydrate-based polymers (i.e. starches [1, 2], functionalised nanocelluloses [3, 4], mixed-glucan systems [5]) we have studied the organisation of rigid components with different levels of ordering and identified flexible parts in such assemblies. We have correlated these structural features with functional properties of such materials including their potential applications as novel foods and drug delivery vehicles.

We have developed a novel NMR based tool-kit to elucidate the mechanism of partitioning of anti-microbial pharmaceuticals in micellar drug-delivery systems. Molecular level organisation of fluconazole and indomethacin within hydrophilic and hydrophobic domains of micellar aggregates was established using the combination of NMR methods (1D ¹H NMR, 1D ¹⁹F NMR, 2D ¹H–¹H NOESY and 2D ¹H–¹⁹F HOESY, and the multifrequency-STD NMR), corroborated with molecular dynamics (MD) simulations. This is the first application of multifrequency-STD NMR to colloidal systems, enabling the elucidation of intricately detailed patterns of drug/micelle interactions in a single NMR experiment within minutes [6].

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2. T. T. Koev et al. Carbohydrate Polymers, 2022, 289, 119413.
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P-036 - NMR Studies of Hepatitis Delta Virus Antigens

错了 Yang Yang¹, Marie-Laure Fogeron¹, Mrs Morgane Callon^{1,2}, Dr Alexander Malär², Loïc Delcourte¹, Yi Ni³, Stephan Urban³, Prof. Dr. Alexander Barnes², Prof Beat Meier², Dr Lauriane Lecoq¹, Mrs Anja Böckmann¹

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Hepatitis delta virus (HDV) is the dominant cause for liver carcinoma. The small and large hepatitis delta antigens (S-HDAg and L-HDAg) are the two proteins encoded by the hepatitis delta virus (HDV) that form together with the viral genome the ribonucleoprotein (RNP) complex, with L equals S plus a 19 amino-acid C-terminal extension. Despite their importance, little is known about the molecular structure and dynamics of S- and L-HDAg. To address this, we synthesized full-length HDAgs, as well as two subdomains thereof, corresponding to the oligomerization (S1-60) and so-called RNA binding (SΔ60) domains, in milligram amounts using wheat-germ cell-free protein synthesis. S1-60 is completely insoluble and was investigated by fast MAS solid-state NMR (ssNMR). In contrast, SΔ60 is fully soluble and could be analyzed by solution NMR. In the present study, backbone and sidechain resonances of the S1-60 protein were obtained using 3D experiments, including at 1.2 GHz field and 100 kHz MAS. This, combined with selective labelling schemes, facilitated extensive resonance assignments. We also experimentally determined the solution NMR structure of SΔ60. We find that SΔ60 contains two intrinsically disordered protein segments separated by a helix-turn-helix motif spanning residues 96-142 (Yang et al., 2022). This structured domain comprises parts of the two arginine-rich motifs of the protein. We show that the domain binds to different types of RNAs and we identified the residues involved in the binding. Finally, we compare the data of the individual domains to the full-length proteins S-HDAg and L-HDAg in the context of the RNP.

Reference: Yang, Y., Delcourte, L., Fogeron, M.-L., Böckmann, A., & Lecoq, L. (2022). 1H, 15N and 13C backbone and side chain solution NMR assignments of the truncated small hepatitis delta antigen Δ60-S-HDAg. *Biomolecular NMR Assignments*. <https://doi.org/10.1007/s12104-022-10096-1>

P-386 - MAS NMR evidence for plastic crystallinity of chlorosomes of the *C. tepidum* bacteria

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Chlorosomes, the largest and the most efficient light-harvesting antennae comprising up to several hundred thousand bacteriochlorophyll (BChl) pigments, form a supramolecular aggregate independent of proteins to harvest light and subsequently transfer excitation energy to the reaction center via the baseplate and FMO complex. Studying robust systems like chlorosomes helps us design and mimic them in the artificial photosynthetic research field. Magic angle spinning (MAS) nuclear magnetic resonance (NMR) allows us to study the plastic crystallinity of BChl pigments at the atomistic level, making it a powerful tool to study how the bacteriochlorophyll in the self-assembled supramolecular complex is tuned for its antenna function. Here we employed temperature dependence of two complementary types of experiments CP and rINEPT and used them to resolve differential dynamics and to distinguish rigid and dynamic parts of the system. In addition, 2D ¹³C experiments such as Proton-driven spin diffusion (PDSD) at different temperatures gave insight into restricted dynamics seen for the chlorosomes in line with the plastic crystallinity of the chlorosome structure.

P-288 - Not Your Average Coupling: NMR Monitoring of the Ullmann Reaction

Ms Nicole Fohn

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Mechanistic studies of heterogeneous reactions by liquid NMR spectroscopy have long posed a challenge. Because of their inhomogeneity and the lack of stirring options for NMR tubes these reactions could not be monitored inside an NMR spectrometer. The only feasible alternative has been ex-situ monitoring which requires labour intensive sampling procedures for sufficient data density and needs suitable quenching procedures. However, most of these procedures quench active intermediates as well, making their monitoring challenging. In this study we compare classic ex-situ monitoring to a recently developed in-situ monitoring method. This method utilizes a state-of-the-art mixing device for NMR tubes which allows monitoring of heterogeneous and air-sensitive reactions within an NMR tube inside a spectrometer. Therefore, labour intense sampling methods and quenching procedures are no longer necessary. A suitable reaction for this perspective was the nickel-catalysed Ullmann coupling. Collected kinetic data allowed comparison to reported literature and further analyses the dependency of the reaction rate kinetic profile on changes in the reaction conditions.

P-122 - NMR Online - Electro: Yesterday's Software for Tomorrow's Solutions!

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Nuclear Magnetic Resonance (NMR) spectroscopy is used in a multitude of disciplines and, over the past three decades, a plethora of software packages for different NMR analyses has been released; however, these packages can require specific operating systems, compiler versions, or dependency installation, and, consequently, cannot be easily installed on some operating systems or chipsets. Furthermore, transforming data into the correct format for each package in a pipeline can unnecessarily complicate the analysis of a dataset.

Aims

With NMR Online Electro, we aim to provide a comprehensive data analysis platform that is not limited by operating system or device. Our platform is designed to enable users to perform diverse data analyses without the need to learn numerous packages. NMR Online – Electro aims to give users the flexibility to perform analysis as a workflow, by selecting an analysis type, or by selecting specific software.

Methods

NMR Online Electro has been built using a microservice architecture, hosted in the cloud. We have worked closely with numerous partner labs and software authors to develop user-friendly workflows that exemplify each software's features, whilst minimising required user interaction. All services are fully tested using data from our various partners, and they automatically scale out in response to load.

Results and Conclusions

We have taken all the feedback we received from our NMR Online BETA release, and our ever-growing social media audience, to build a solution driven by the needs and wants of the NMR community. We have built services encompassing spectrum processing, lineshape fitting, analysis of exchange, relaxation, and paramagnetic data, along with chemical shift and dihedral angle prediction. Our NMR data manager enables a user to see, at a glance, all the data and results they have inside NMR Online, search for specific data, and visualise it all on the same platform.

P-370 - Resolving atomic details of the donor-strand complementation mechanism in TasA filaments from *Bacillus subtilis* biofilms

Mr Florian Lindemann

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Studying mechanisms of bacterial biofilm generation is of vital importance to understand underlying cell-cell communication, multi-cellular cohabitation principles and the higher resilience of microorganisms in a biofilm against antibiotics.

Biofilms of the non-pathogenic, gram-positive soil bacterium *Bacillus subtilis* serve as a model system with biotechnological potential towards plant protection. Its main protein component in the extracellular matrix is the 234 amino acid polypeptide called TasA. The nature of filaments formed by TasA has been of debate, and several forms have been observed. Solid-state NMR measurements, partially recorded under in vivo conditions, revealed intercalation of the N-terminal segment into subsequent protomers to form a non-amyloid filament composed of β -sandwich subunits. The secondary structure around the intercalated N-terminal strand β_0 is conserved between filamentous TasA and the Fim and Pap proteins which form bacterial type I pili, demonstrating such construction principles in a gram-positive organism for the first time. Intriguingly, the most conserved residues in TasA-like proteins (camelysines) of Bacillaceae are located within the protomer interface offering a mechanism for inter-species biofilms.

This findings are the basis for a deeper, general, understanding of how biofilms form on a molecular level useful for the development of anti-bacterial countermeasures.

P-260 - Liver metabolic landscape of the murine NASH models by quantitative ^{31}P -NMR analysis of the phosphorome

Dr Ganeko Bernardo¹, Jon Bilbao², Dr David Fernandez-Ramos³, Dr Fernando Lopitz-Otsoa², Dr Virginia Gutierrez de Juan², Karen Salua Alarcon-Morales², Sara Martin-Ramos², Dr Marcos F. Fondevila⁴, Dr Ruben Nogueiras⁵, Dr Jose M Mato², Dr Oscar Millet²

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: The liver plays a central role in all metabolic processes in the body. However, precise characterization of liver metabolism is often obscured by its inherent complexity. Phosphorylated metabolites occupy a prominent position in all anabolic and catabolic pathways.

Aims: Here we develop a ^{31}P -NMR-based method to study the liver “phosphorome” through the simultaneous identification and quantification of multiple hydrophilic and hydrophobic phosphorylated metabolites.[1]

Methods: We applied this technique to define the metabolic landscape in livers from well-known dietary murine models of nonalcoholic steatohepatitis: mice fed with a high-fat diet (HFD), and a high-fat choline deficient diet (HFCD), and standard diet (STD). Disease models were metabolically characterized at different periods of time (3-10-20-52-72 weeks) to monitor the development of the liver injury.

Results: We report alterations in the concentrations of phosphorylated metabolites that are readouts of the balance between glycolysis, gluconeogenesis, the pentose phosphate pathway, the tricarboxylic acid cycle, and oxidative phosphorylation, and of phospholipid metabolism and apoptosis. Moreover, these changes correlate with the disease development period and with the main histological features: steatosis, apoptosis, iron deposits and fibrosis.

Conclusions: These findings indicate that NMR-based phosphoromics may be used to unravel metabolic phenotypes of liver injury and to identify the mechanism disease development.

P-090 - Structural studies of d(CACGTG)₂ DNA sequence and baicalein using 2D NMR spectroscopy

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

INTRODUCTION

Targeting nucleic acid is an active research area in the field of medicinal chemistry and therapeutics. Therefore, it is a major target for developing new anticancer agents. Baicalein belongs to the class of flavones widely present in plants, fruits, vegetables, and metabolites. It possesses broad biological activities such as antioxidant, anti-inflammatory, antibacterial, and anticancer activities. Interactions of small molecules with DNA can inhibit the cellular pathways at the molecular level.

AIMS AND METHODS

We report the interactions of hexanucleotide d(CACGTG)₂ with baicalein. The CACGTG motif is extensively present in various promoters, such as humans, rats, and flies, which regulates several transcription factors. 1D proton NMR experiments were carried out at different temperature ranges 288K-328K. The 2D 1H-1H NOESY NMR experiment of DNA and baicalein-DNA complexes were performed at 300K with 250 ms mixing times. The diffusion coefficient of DNA and baicalein-DNA complex was evaluated using 2D DOSY. Molecular dynamic simulations of baicalein-DNA complex were done using AMBER 18 package.

RESULTS

NMR results suggested that the G4NH and T5NH protons were shifted upfield by 0.13 ppm and 0.15 ppm on adding baicalein. The A2H1', C3NH2b, and C3NH2nb protons showed upfield shift by 0.04, 0.10, and 0.04 ppm respectively. Observed intermolecular NOEs between baicalein protons H3 α /H4 α /H5 α and C3NH2nb, C3H6, and G4NH2b DNA protons and H8 proton of ring A of baicalein gave NOE with G4NH2nb. Diffusion coefficient values of baicalein-DNA complex and free DNA are 1.98x10⁻⁹ m²/s and 2.16x10⁻⁹ m²/s, respectively, suggesting the less diffusion in baicalein-d(CACGGTG)₂ complex as compared to alone DNA.

CONCLUSIONS

The NMR findings confirm that baicalein gives the NOEs to DNA via A2, C3, and G4 residues. Intermolecular NOEs validate, baicalein interacts at C3pG4 sites of DNA and forms a stable complex, confirmed by decreased diffusion coefficient. Data from spectroscopic studies corroborate the NMR findings.

P-126 - Post-acquisition correction of NMR spectra distorted by dynamic and static field inhomogeneity of cryogen-free magnets

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Due to relatively low sensitivity and high-resolution demand, NMR spectroscopy requires strong and homogeneous magnetic fields. The latter are normally created by superconducting magnets at liquid helium temperatures. The sources of helium are close to exhaustion, so liquid magnets become more and more expensive. The solution to this problem is the use of dry cryogen-free magnets.

An intrinsic feature of cryogen-free magnets is the mechanical vibration caused by the cold head operation. This dynamic field inhomogeneity is a potential drawback for the use of cryogen-free magnets in high-resolution NMR. Also, the static magnetic field inhomogeneity due to imperfections in the main coils is never fully avoidable.

Aims

In this work we suggest numerical mathematical ways of correction processing of NMR spectra against dynamic and static inhomogeneity typical for cryogen-free magnets.

Methods

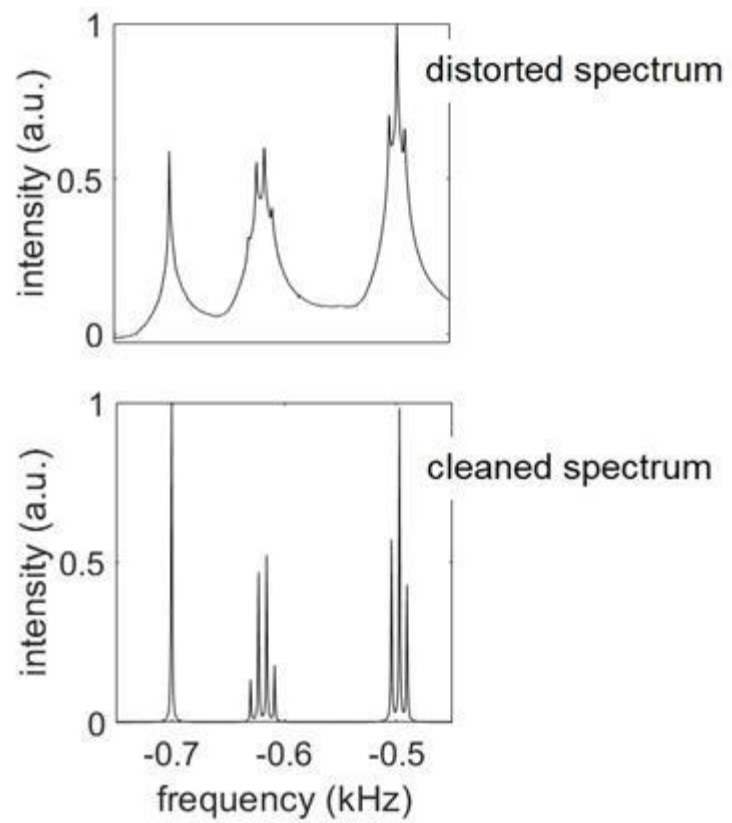
We show that the dynamic distortions can be cleaned off by a variant of reference deconvolution. Here the phase of the FID signal of a reference sample can be used to extract the dynamic field distortion and correct then any spectrum obtained on the same dynamically distorted magnet.

We show also that the static inhomogeneity can be significantly reduced by a delayed Fourier processing. This enables the broadening caused by fast decaying FID signal components to be removed from the spectrum.

Results and Conclusion

We verify the efficiency of the above methods by correction of experimental liquid-state H-1 NMR spectra of water and ethanol and solid-state C-13 spectra of adamantane at high magnetic field, with the use of a home-built (Cryogenic Ltd) magnet with an appreciable dynamic and static field inhomogeneity. Our methodology shows its high efficiency and proves good perspectives for using cryogen-free magnets in high-resolution NMR spectroscopy. Our mathematical methods are universal and can be used in a wider context of post-acquisition refinement of NMR spectra.

Dynamic and static magnetic field inhomogeneities of cryogen-free magnets are mathematically removable from high-resolution NMR spectra by reference deconvolution and delayed Fourier transform



P-030 - Amyloid formation and stability of the tumour suppressor protein p16ink4a is strictly controlled by disulfide bond formation

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The tumour suppressor protein p16ink4a is a small and stable all alpha-helical protein that inhibits cyclin-dependent kinases and thereby controls cell division. We recently discovered that upon exposure to low levels of oxidants, the protein dimerises through formation of an inter-molecular disulfide bond and subsequently converts into amyloid fibres.

Here we present a unique mechanism where the presence of disulfide reducing agents lead to disassembly of the amyloid, thereby rendering the first example where amyloid conversion is fully redox controlled [2]. Physiologically relevant oxidants such as hypothiocyanous acid quickly convert the protein to amyloid, whereas hydrogen peroxide is rather inefficient, suggesting a specific role for this structural conversion.

p16ink4a is frequently mutated in various cancers and we find that single point cancer-variants fold faster into amyloid than the wild-type protein. By studying previously reported mutations that stabilise the monomeric fold, we find that the oxidation-induced amyloid formation is fully removed. We further present cell-based studies where we observe redox-based aggregation and dis-aggregation of fluorescence-labelled p16ink4a. We are currently expanding our efforts to other homologue CDK inhibitors and a zebrafish model system where we study molecular knockouts that lose their ability to fold into amyloid.

[1] Goebel et al., Redox Biology 2020, 28, 101316

[2] in revision

P-392 - NMR crystallography of DOTAM complexes

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

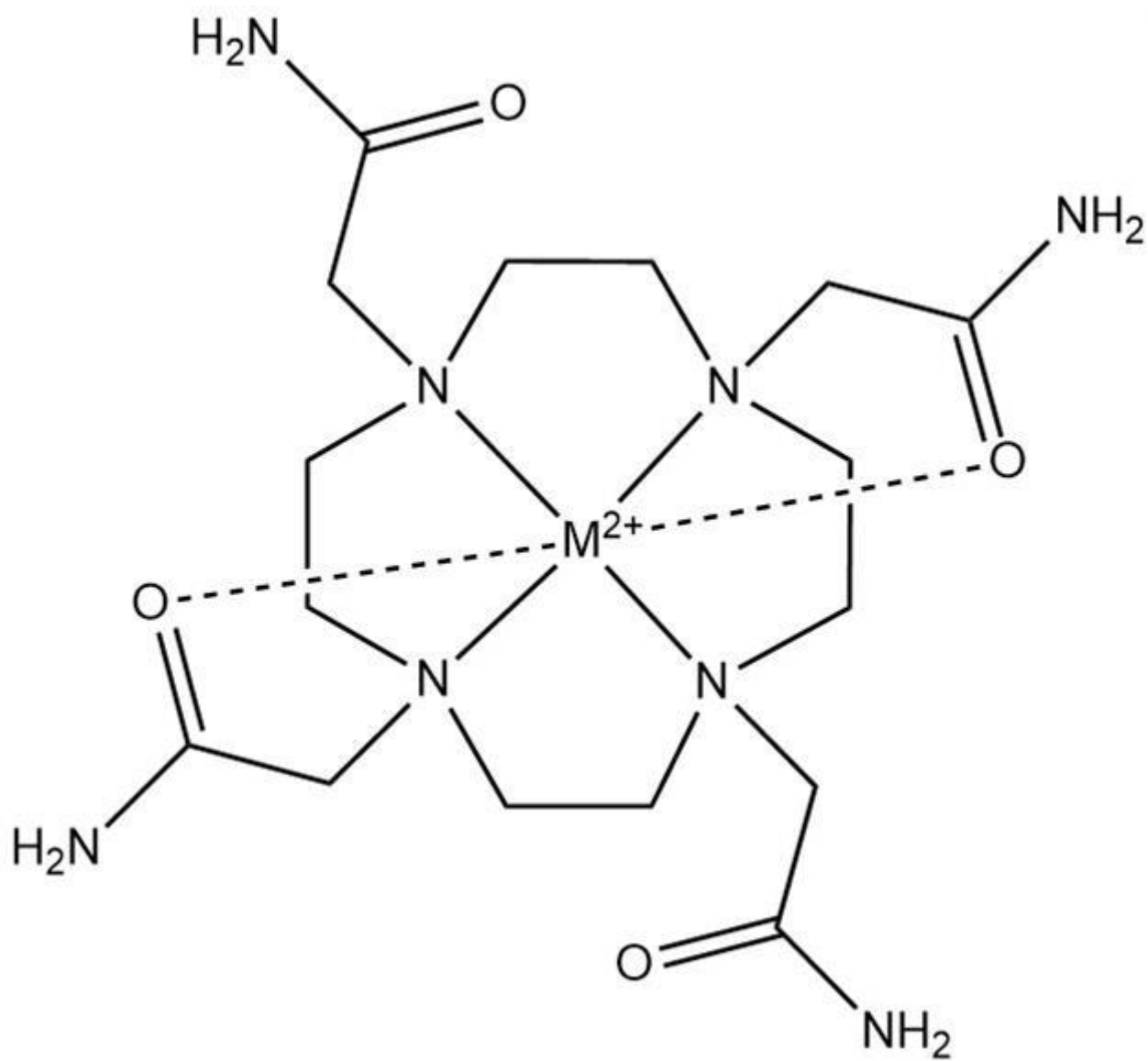
Complexes of macrocyclic ligands have several important uses in medicinal applications, such as diagnostic probes for MRI, SPECT or PET, and radionuclide carriers for radiotherapy.

However, understanding and predicting the properties and behaviour of these complexes in vivo requires thorough knowledge of their molecular structure. Structure determination of such compounds so far depended largely on single crystal X-ray diffraction, which limits the range of complexes with determined molecular structures.

In the present work, we approach the determination of the molecular structure via the use of solid-state NMR spectroscopy, methodology not used to study macrocyclic complexes to this date. Such an approach should expand the scope of compounds with experimentally determined molecular structures, allowing us to also study powder materials, not only single crystals.

A series of transition metal complexes of DOTA-tetraamide with known X-ray structures was chosen as a starting point of our study. (metal ion = Zn, Cd, Hg). Central atoms in these complexes have different coordination numbers (6, 6+2, 8), which is caused by different ionic radii of these metals. The difference in molecular structures in the solid state should also be mirrored in different solution dynamics.

Therefore, these complexes were studied both in solution and in the solid state under various conditions to get as much structural information as possible. Also, these compounds were subjected to variable temperature NMR studies in order to observe their dynamical behaviour. This information, in particular, is inaccessible from X-ray diffraction.



P-158 - Advances of hyperpolarized NMR through the use of robust field cycling setup

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Hyperpolarization techniques are one of the practical solutions to low sensitivity in NMR. One of the least technically demanding techniques is Para-Hydrogen Induced Polarization (PHIP). The source spin-order in such case is provided by para-enriched hydrogen gas that chemically interacts with a substrate on which polarization transfer can be initiated. Such spin-order can be also mimicked and tested using polarization at thermal equilibrium. Such alternative allows to independently assess the performance of various methods later to be applied practically to yield highly polarized molecules.

In this work, we employ a field cycling setup which merges sophisticated high-field NMR equipment and low-field PHIP device together and analyse microTesla fields as an attractive regime for polarization transfer. We demonstrated that both transfer under an effective Hamiltonian during CW-excitation (SLIC) [1] and pulsed coherence transfer (ADAPT, PulsePol) [2,3] can be employed. Using 1-¹³C,^{d6}-dimethyl maleate, these methods can achieve ¹³C nuclear spin polarization of approximately 60%. More excitingly, we show that polarization levels in excess of 30% can be obtained for 1-¹³C-pyruvate esters using PHIP-SAH approach [4] and over 20% polarization of 1-¹³C,^{d3}-pyruvate using SLIC-SABRE. We also demonstrate that spin decoupling during hydrogenation can be essential for achieving high ¹³C polarization in many molecules and discuss the influence of relaxation to polarization transfer efficiency at different magnetic fields. This illustrates how simple, robust and versatile setup can help to develop polarization transfer in hyperpolarized molecules and to pave the way to widespread application of hyperpolarization.

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- [2] G. Stevanato, Journal of Magnetic Resonance 274, 148-162, 2017.
- [3] I. Schwartz et. al., Science Advances 4 (8), 2018.
- [4] F. Reineri et. al., Nature Communications 6 (5858), 2015.

P-284 - Using Metal–Organic Frameworks to Confine Liquid Samples for Nanoscale NV-NMR Spectroscopy

Professor Dominik Bucher¹, Dr. Kristina Liu, Prof. Roland Fischer, Prof. Ian Sharp, Anna Semrau, Dr. Alex Henning, Dr. Roberto Rizzato, Xiaoxun Ma

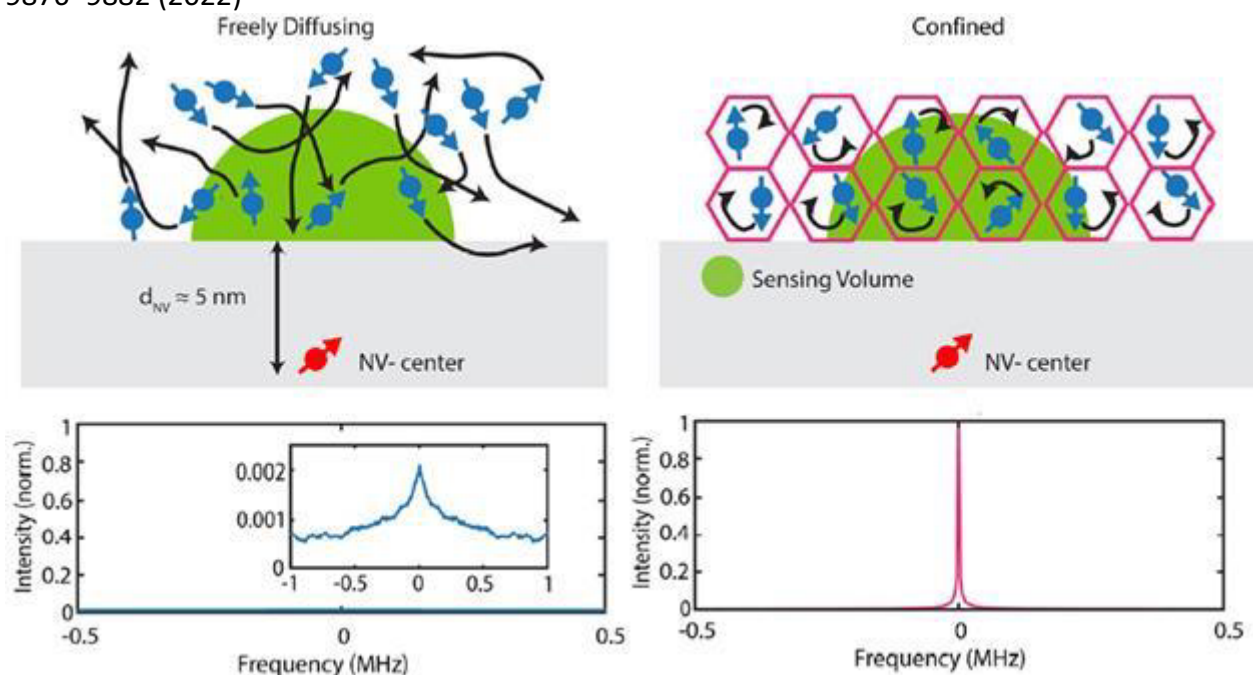
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Nitrogen vacancy (NV) centers have been extensively studied for their ability to detect magnetic fields with unprecedented sensitivity on small length scales. This technique has demonstrated the ability to detect NMR signals from single molecules and nuclei. In typical nanoscale NV-NMR experiments, the NV-center is within a few nanometers from the surface of a diamond chip and detects a few thousand spins from the sample on the diamond. However, the main drawback is the diffusion of liquid samples across the nanoscale detection volume, which limits the sample's interaction time with the NV sensor. This results in broadened signals for viscous samples and undetectable signals for low-viscosity liquid samples. In my talk, I will present our recent study, where we demonstrate that confinement-induced restriction of diffusion enables nanoscale NMR spectroscopy on liquid samples. Our method involves using metal-organic frameworks (MOF) with tiny pores on a diamond chip to confine the sample molecules in close proximity to the NV centers. This confinement enables the detection of NMR signals from liquid samples that would otherwise be undetectable. These findings pave the way for high-resolution liquid-phase NV-NMR spectroscopy on the nanoscale or even on a single molecule.

References:

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- 2) Liu, K., Ma, X., Rizzato R. Semrau A., Henning, A., Sharp I. D., Fischer R A., Bucher, D. B.°, Using metal-organic frameworks to confine liquid samples for nanoscale NV-NMR, Nano Letters, 22, 24, 9876–9882 (2022)



P-344 - Methyl TROSY to Access the Dynamic Structure of Bio-Inorganic Silica Pre-Nucleation Species

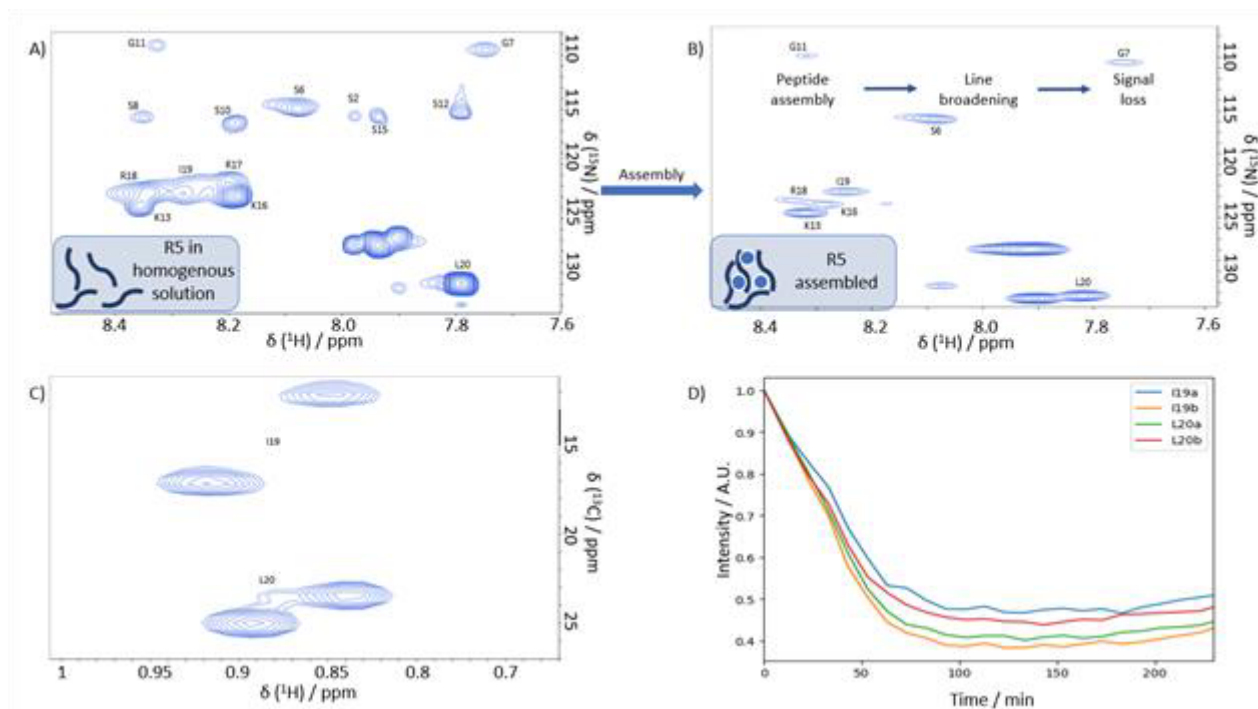
Ms Dörte Brandis^{1,2}, Mrs Fanny Kozak^{1,2}, Professor Christian F.W. Becker¹, Professor Dennis Kurzbach¹

¹Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria, ²Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Vienna, Austria

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The theory of biomineralization was recently challenged by the discovery of so-called pre-nucleation species (PNS), which can act as solution-state precursors for solid mineral phases.¹ However, the investigation of PNS entails many challenges, for example, their dynamic structure and high molecular weight. As a result, the major method to investigate molecules in solution at atomic detail, Nuclear Magnetic Resonance Spectroscopy (NMR), meets its limitations. One of the main challenges of this method is its low sensitivity for assemblies with high molecular weights such as PNS. An approach to overcome this, is transverse relaxation optimized spectroscopy (TROSY), enabling NMR measurements of structures with molecular weights up to 1 MDa. Herein, we develop this method further using TROSY tailored to methyl groups² to access supramolecular structures. Using R5, a silica precipitating peptide,³ which forms PNS upon exposure to phosphate counterions, as a model system for biomineralization we thus obtained high-quality spectra of PNS within short acquisition times (Fig. 1 C). In contrast, in a common HSQC strong line broadening yet impeded high resolution NMR (Fig. 1 A, B). Eventually, the short Methyl-TROSY acquisition times enabled real-time monitoring of the silica precipitation process upon exposure of R5-based PNS to silicate.⁴ Proof-of-concept methyl TROSY-based real-time precipitation assays demonstrate that PNS with molecular weights far above 100 kDa can be monitored at a sampling rate of 1 min⁻¹ (fig. 1 D).

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P-032 - Protein charge modulation and induction of reentrant condensation by ATP

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Aims

Adenosine triphosphate (ATP) is a small polyvalent anion that is crucial for cell biology, acting as a primary energy source driving biochemical reactions in all cells, is signalling molecule in signalling cascades. Recently, it has been established that ATP can have a major impact on protein assembly processes, including protein liquid liquid phase separation, condensation and protein aggregation. However, the molecular insight into non-specific ATP-protein interactions and their effect on protein solubility is still largely unknown.

In this study we sought out structural detail of the ATP binding to surfaces of folded proteins using a combination of NMR and X-ray Crystallography and assessed the impact of such binding to protein solubility and self-assembly

Results

We performed a comprehensive structural study of ATP interactions with a model globular protein. We identified multiple binding sites on the protein surfaces and show that binding occurs through the polyphosphate chain which is in contrast to previous reports. Additionally we determined the binding occurs in the millimolar range, and identified that ATP preferentially binds to arginine compared to lysine residues. We also show that such binding occurs also at cellular concentrations of Mg²⁺ ions with similar affinity.

Conclusions

Our findings suggest that ATP binds non-specifically to folded proteins in physiological conditions, altering their surface properties which in turn drastically impacts their solution behavior, leading to reentrant condensation. This has further implications for maintain the colloidal stability of folded proteins in cellular environments.

Reference: M. Zalar, J. Bye, R. Curtis: Nonspecific Binding of Adenosine Triphosphate and Triphosphate Modulates the Phase Behavior of Lysozyme JACS 2023 145 (2), 929-943 DOI: 10.1021/jacs.2c09615

P-368 - Ionic substitutions in synthetic and biological apatites: a multinuclear solid-state magnetic resonance case study

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction. Bone mineral consists of crystalline apatitic nanometric platelets surrounded with an amorphous calcium phosphate surface layer. Bone elemental analysis has revealed the presence of many ionic substitutions (carbonate, sodium, magnesium, chlorine, etc.) occurring within this material, hence affecting bone properties [1]. However, classical elemental analysis suffers from low spatial resolution [2], leading to remaining uncertainties regarding the substitution sites that could be in the crystalline core and/or the amorphous surface layer.

Aims. Solid-state NMR is a powerful tool to probe the structure of materials at the atomic scale. This study aims to shed light on the substitution sites of many elements using multinuclear (²³Na, ²⁵Mg, ³⁵Cl, ⁶⁷Zn, ⁸⁷Sr, ²⁰⁷Pb) solid-state NMR on bone apatites. Our findings will be helpful to i) better understand bone mineralization, ii) diseases, and iii) for the design of relevant biomaterials.

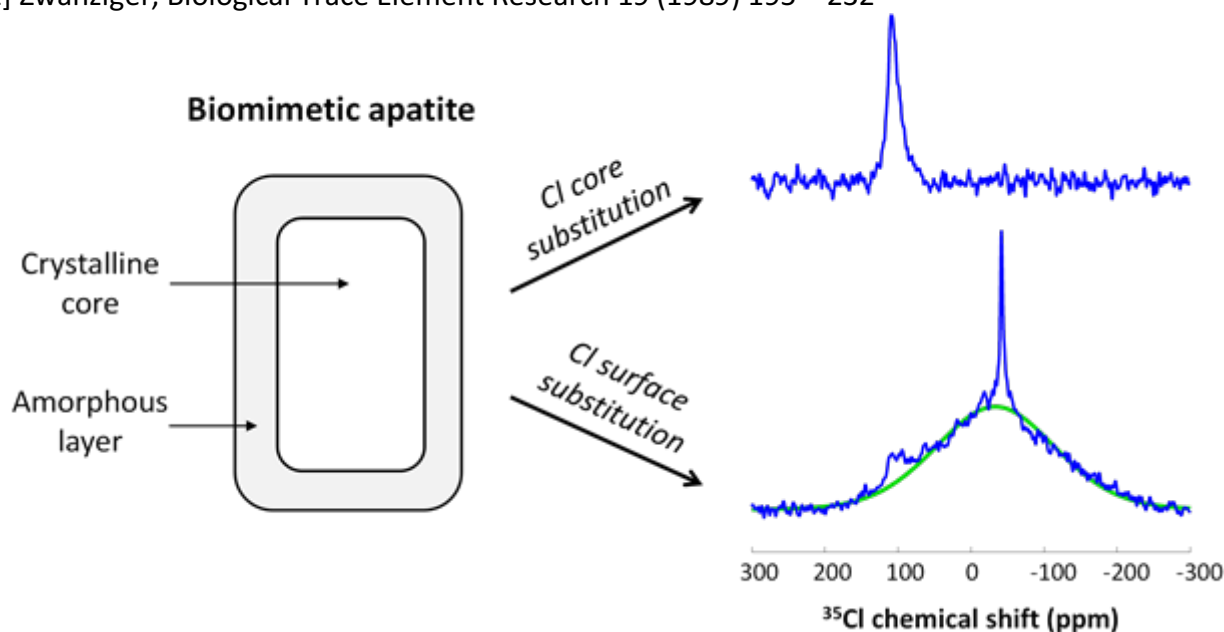
Methods. We selectively induced ionic substitutions in the crystalline core or the amorphous surface layer of synthetic apatites by means of precipitation methods. Thereafter, solid-state NMR was used to characterize these distinct substitutions.

Results. Our ³⁵Cl results clearly show that chlorine can be incorporated both in the apatite lattice (sharp signal) and the amorphous surface layer (broad signal). The same trend was also observed for sodium substitutions through ²³Na NMR. Pb²⁺ and Sr²⁺ were successfully incorporated in the crystalline lattice, while the detection of a ⁶⁷Zn broad signal was attributed to Zn²⁺ surface sites.

Conclusions. Multinuclear solid-state NMR was used to differentiate various core and surface substitutions in synthetic apatites. This approach can be extended to study other common ionic substitutions within the apatite structure. Further analysis of biogenic samples will allow us to better understand the substitution sites for several minor, yet important, elements.

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[2] Zwanziger, Biological Trace Element Research 19 (1989) 195—232



P-100 - Two novel antibodies against cancer and their binding to the carbohydrate antigen 19-9 characterized by STD NMR spectroscopy

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Gastrointestinal cancers, including pancreatic cancer, are among the most common malignancies in humans. Changes in cell surface markers are a hallmark of cancer, and the alteration of cellular glycosylation during malignant transformation leads to the expression of tumour-associated carbohydrate antigens (TACAs). Sialyl Lewis A (CA19-9) is a TACA that is overexpressed in epithelial tumours of the gastrointestinal tract and appears in 75 % of patients with pancreatic cancer. CA19-9 is one of the most reliable cancer markers in the blood serum of pancreatic cancer patients and the only biomarker approved by the Food and Drug Administration (FDA) for the screening and management of pancreatic cancer. Therefore, its detection and monitoring are of interest for diagnosis of pancreatic cancer.

By immunising mice with well-defined synthetic CA19-9 and hybridoma technology, we obtained two novel mAbs that bind to native and synthetic CA19-9. We characterized these antibodies and their binding to CA19-9. Using Saturation Transfer Difference (STD) NMR spectroscopy we analysed the binding epitope of CA19-9 and discovered a different mode of binding for the newly obtained antibodies compared to the commercially available antibodies. In addition to the different binding epitope the new mAbs also showed significantly different binding kinetics. Through a careful repeated STD NMR measurement series, we also performed a statistical evaluation to show the significance and reproducibility of the results.

From the results, we conclude improved antigen recognition for one of the new mAbs compared to the commercially available one. This knowledge could be used to develop a more advanced and sensitive diagnostic method for pancreatic cancer.

P-304 - NMR-Challenge.com: Enhancing NMR Spectral Interpretation Skills through Interactive Exercises

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Elucidation of molecular structure from NMR spectra is considered to be a part of organic chemists' core knowledge. Resolving NMR spectra is no trivial task but rather a complex process. It requires prior knowledge such as understanding splitting patterns and approximate chemical shift values for different functional groups. But more than applying memorized rules, the whole process resembles puzzle solving. For many students, acquiring this skill can be challenging because the only way to become proficient in NMR structure determination is through practice and exposure to various patterns in spectra.

We present an interactive website nmr-challenge.com which allows students to gain proficiency in NMR structure solving. The website features over 150 tasks which are categorized by difficulty to engage both beginners and advanced students. Users can submit their solutions using a drawing tool which prevents potential nomenclature errors and provides immediate feedback on their proposed structures. The assignments use real recorded spectra and various types of NMR experiments including one-dimensional ¹H and ¹³C NMR spectra as well as two-dimensional correlation spectra. This provides users with a realistic experience of working with NMR data and helps them develop the skills they need to solve complex structures. This tool is meant not only for students but also for experienced chemists looking to sharpen their skills.

Reference:

Socha O., Osifová Z., Dračínský M., J. Chem. Educ., 2023, 100 (2), 962-968



P-200 - Improving XiX DNP with optimal control

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¹University of Konstanz, Konstanz, Germany, ²University of Southampton, Southampton, United Kingdom

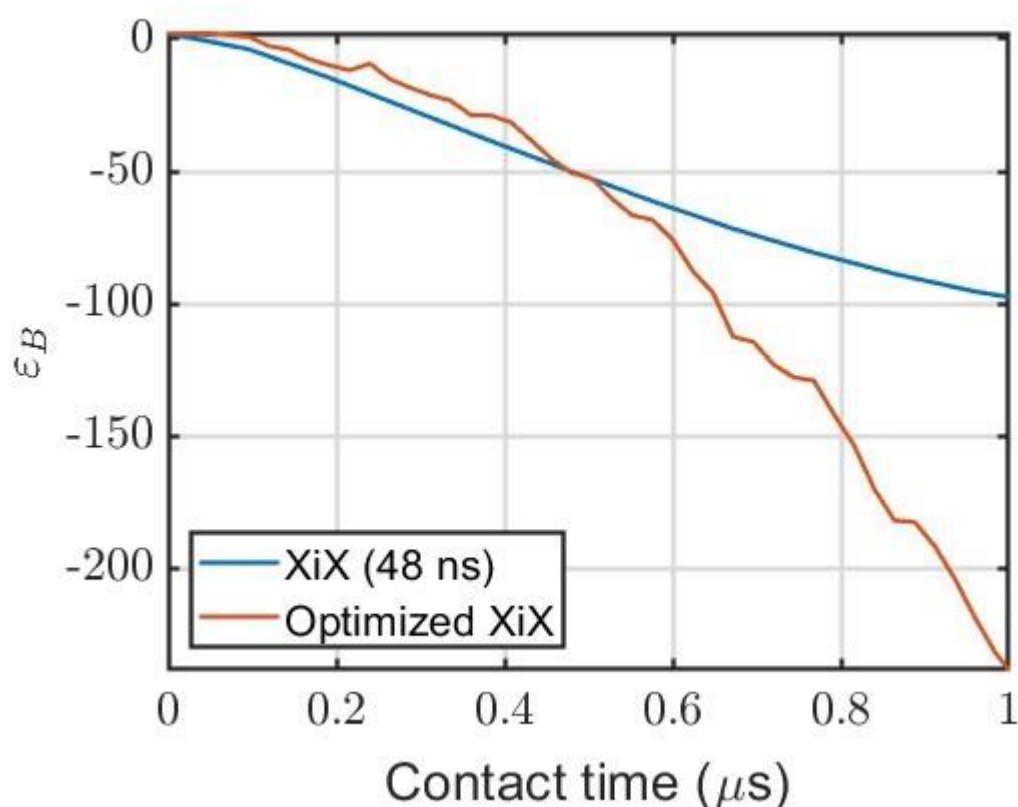
Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Pulsed dynamic nuclear polarization (DNP) is a promising strategy to improve the sensitivity of high-resolution magic-angle spinning (MAS) nuclear magnetic resonance (NMR). In the X-inverse-X (XiX) DNP pulse sequence, two equal pulses of opposite phases are repeated over a fixed contact time (t_c) [1]. This sequence generates a two times faster polarization transfer than the time optimized pulsed (TOP) DNP sequence. XiX DNP experiments performed at Q band (34 GHz/1.2 T/51 MHz) with trityl concentration of 6.4 mM resulted in an enhancement factor (ϵ_B) of -144 at a microwave resonance offset of -60 MHz and an electron nutation frequency (ν_{1S}) of 19 MHz for a pulse duration of 48 ns.

We previously carried out numerical simulations of pulsed DNP using the Spinach simulation library [2] and found outcomes helpful in predicting experiments. Here, we combine these simulations with gradient ascent pulse engineering (GRAPE), a quantum optimal control algorithm [3], to further improve XiX DNP. Using a modified version of GRAPE, we optimize the duration of the individual pulses while alternating the phases by 180°. We call the new sequence optimized XiX DNP.

At $\nu_{1S} = 20$ MHz and $t_c = 1$ μ s, numerical simulation of optimized XiX DNP yields an ϵ_B of -241 for a combination of 84 pulses, where the pulse lengths range up to 24 ns. The figure shows the contact curves of XiX (48 ns) and optimized XiX DNP pulse sequences. Experiments with optimized XiX DNP will be performed at Q band in the coming months. Meanwhile, we pursue better XiX sequences using optimal control, also including a preparation pulse.

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P-286 - Vector detection of microwave magnetic fields by a single orientation of nitrogen-vacancy centres in diamond.

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Nitrogen-Vacancy (NV) centres in diamond have interesting properties for sensitive detection of AC and DC magnetic fields. Sensitive detection of microwave magnetic fields is important for microwave technology and related applications. Vector detection of microwave magnetic field can be achieved by using three different orientations of NV centres. Here, we propose and experimentally demonstrate a method to measure both strength and orientation of microwave magnetic fields by using only one orientation of NV centres in diamond. This method can also be implemented by using single NV centres. The nanoscale spatial resolution offered by single NV centres combined with high-sensitivity detection of microwave magnetic fields at room temperature potentially leads to interesting applications in condensed matter physics and microwave technology.

P-298 - From screening to structure - Boosting NMR-based drug discovery with hyperpolarisation and label-free structure determination

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¹ETH Zürich, Zürich, Switzerland, ²NexMR GmbH, Schlieren, Switzerland, ³University of Vienna, Vienna, Austria

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Nuclear Magnetic Resonance (NMR) is the gold standard for fragment-based drug discovery (FBDD). However, due to its low sensitivity and tedious analysis, it needs high sample concentrations and has a low throughput.

We present the use of photo-Chemically Induced Nuclear Polarisation (photo-CIDNP) [1] and NMR Molecular Replacement (NMR²) [2] to establish a workflow from screening to structure-activity relationship for FBDD, overcoming the above-mentioned limitations. Photo-CIDNP drastically reduces the time and amount of material needed and NMR² makes protein-fragment structures accessible within a few days without the need for isotope labelling.

A photo-CIDNP fragment library is screened against the cancer target PIN1. Photo-CIDNP enables accelerated measurements of a few seconds. Furthermore, we demonstrate the possibility to screen at nanomolar concentrations and cryogen-free benchtop NMR spectrometers within a few minutes. Hyperpolarisation also enables to determine the affinity of the hits within minutes. We present a new method analogous to STD-NMR to measure the affinity of protein-ligand interactions with photo-CIDNP. Several examples are presented.

Finally, we show the implementation of a T₁, T₂-filtered 2D-NOESY pulse sequence to measure fragment-protein distance restraints. NMR² does not need a protein assignment and the distance restraints are directly converted to a protein-fragment complex structure. More than 10 complex structures of the oncogenic protein K-Ras have been elucidated this way.

This study shows how new NMR methods are implemented into a FBDD workflow. Lowering the target concentration for screening and KD measurements into the nanomolar regime, moving away from cryogenic magnets, and elucidating structures without isotope labelling could help to tackle new targets that were up to now out of reach for NMR and medicinal chemists.

[1] Torres, Bütikofer, et. al., Ultrafast NMR fragment screening using photo-hyperpolarized (CIDNP) NMR. (Under review)

[2] Torres, et. al., Protein-fragment complex structures derived by NMR molecular replacement. RSC Med Chem. 2020

P-390 - Solid-state NMR as a tool for the characterization a protein-drug conjugate

Mr Luis Padilla

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

The development of a suitable drug delivery system is a crucial step in drug design, and the conjugation of drugs to natural or synthetic large polymers is often used to increase the half-life by reducing the renal excretion. Human transthyretin (TTR), a physiological protein, has already been identified as a possible carrier protein for the delivery of cytotoxic drugs.

Aims

Here we describe the characterization of the interaction of TTR with a paclitaxel derivative conjugated to the tafamidis molecule by a long linker.

Methods and results

A combination of x-ray crystallography, solution NMR and solid-state NMR allowed us to get a global insight of the binding of the paclitaxel-tafamidis derivative to the TTR, with rehydrated freeze-dried samples yielding high quality solid-state NMR spectra.

Conclusions

These results demonstrate that the high sensitivity of solid-state NMR to the effects of ligand binding and to small conformational heterogeneities, make this technique extremely helpful to characterize the interaction of drug candidates with large carrier proteins as a complementary technique to X-ray and solution NMR, as well as a technique on its own when conventional biophysical techniques are not suitable.

P-112 - Biomolecular NMR and highly selective isotope labeling at the MAG-LAB

Dr Roman Lichtenecker², Univ.-Prof. Robert Konrat³, PhD Gerald Platzer¹, PhD Sven Brüscheweiler¹, Dr. Matus Hlavac¹, MSc. Thomas Kalina¹, Dr. Katharina Siess¹, BSc Sarah Kratzwald¹

¹MAG-LAB, Vienna, Austria, ²MAG-LAB and Institute of Organic Chemistry, University of Vienna, Vienna, Austria, ³MAG-LAB and Department of Computational and Structural Biology, Max Perutz Labs, University of Vienna, Vienna, Austria

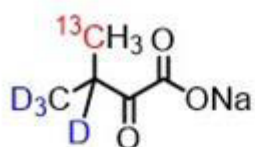
Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Understanding the conformational properties and interaction networks of biomolecules is the key to unravel the principles of life at a molecular level. Analyzing the processes at the nodes of cell signal cascades is the precondition for targeted drug design. NMR spectroscopy offers the ability to investigate protein properties with atomic precision under conditions closely resembling physiological environments. One remarkable advantage of protein NMR is the extensive repertoire of pulse sequences available for studying structure, dynamics, and interactions. This methodological toolkit continues to expand alongside advancements in isotope labeling technology. Customized isotope patterns incorporating carbon-13, nitrogen-15 and deuterium have paved the way for applying NMR methods to analyze large biomolecular complexes, study extensively unstructured proteins and probe intact cellular systems.

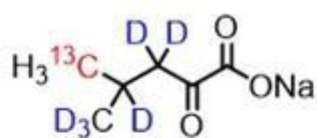
In our research at MAG-LAB, we employ multistep organic synthesis to generate metabolic amino acid precursors starting from readily available heavy isotope-containing compounds. The resulting target molecules serve as nutrients in minimal media expression systems, which leads to highly selective isotope patterns in the corresponding target proteins. The precise distribution of isotopes meets the specific demands of cutting-edge NMR experiments, which we, in collaboration with our partners, utilize to further explore the methodological potential of protein NMR.

We established different methods for Ala, Leu, Val, Ile, Met, Phe, Tyr, Trp, Arg and His labeling. The corresponding protein samples exhibit well-resolved signal resonances that can be applied in the straightforward mapping of binding sites by recording chemical shift perturbation. Employing various labeling approaches, we directly measure the affinities and geometric properties of ligand-protein complexes. Our straightforward NMR data analysis does not only improve lead structure optimization processes but also provides a novel perspective on how molecular therapeutics are designed.

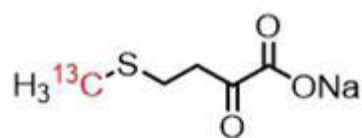
Selected precursors for isotope labeling at specific target residues



Leucine / Valine



Leucine



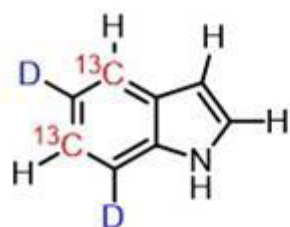
Methionine



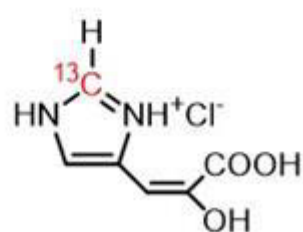
Phenylalanine



Tyrosine



Tryptophan



Histidine

P-220 - Hydrogen bonds in bulk and nanoconfined ionic liquid crystals

Professor Sergey Dvinskikh¹¹KTH Royal Institute of Technology, STOCKHOLM, Sweden

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The formation of liquid-crystalline phases in ionic liquids is driven by a balance between dispersion forces, electrostatic interactions, and hydrogen bonding [1-3]. A minor shift of the force balance can lead to discernible changes in phase behaviour and local molecular ordering and dynamics. In the present work, we discuss cation–anion hydrogen-bonding interactions in imidazolium-based ionic liquid crystals in bulk and nanoconfined states [3-5]. Nanoconfined ionic liquids present a class of hybrid composites combining the functional properties of ionic liquids and porous solids. Due to non-covalent interactions with the solid interface, confined ionic liquids exhibit distinct structural, orientational, and dynamic preferences different from the bulk.

We apply solid-state ^1H NMR and heteronuclear ^1H - ^{13}C spectroscopy (HETCOR) to access proton chemical shift interactions in hydrogen-bonding centres [5]. Hydrogen bonding effects are compared in isotropic, liquid crystalline, and solid phases of bulk ionic liquid crystals as well as in the nanoconfined state. The influence of anion properties, cation structures, ion dynamics, and orientational order on hydrogen bond strength is discussed.

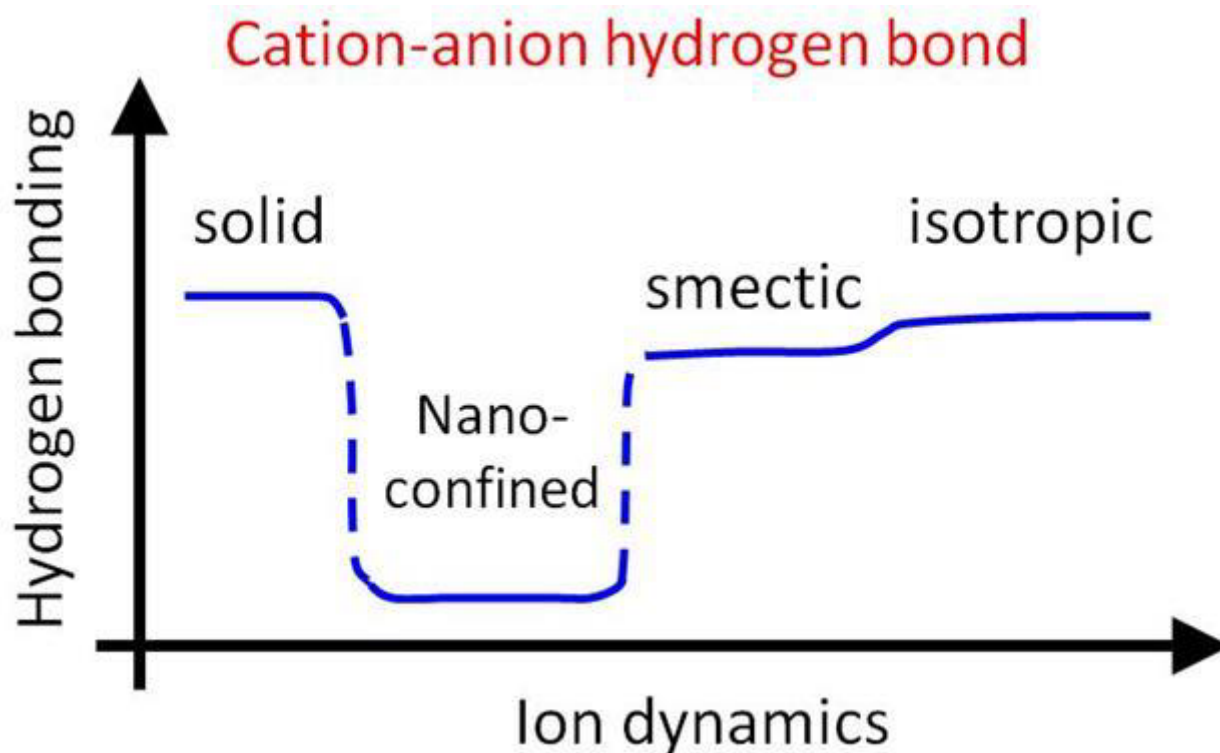
[1] J. Dai, D. Majhi, B. B. Kharkov, S. V. Dvinskikh. *Crystals* (2019), 9, 495.

[2] D. Majhi, J. Dai, A. V. Komolkin, S. V. Dvinskikh. *Phys. Chem. Chem. Phys.* (2020), 22, 13408.

[3] S. V. Dvinskikh. *Liq. Cryst.* (2020), 47, 1975.

[4] D. Majhi, S. V. Dvinskikh. *Sci. Rep.* (2021), 11, 5985.

[5] D. Majhi, J. Dai, S. V. Dvinskikh. *Phys. Chem. Chem. Phys.* (2022), 24, 23532.



P-172 - Parahydrogen-enhanced NMR reveals elusive, transient intermediates of [Fe]-hydrogenase catalysis

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¹Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany, ²Center for Biostructural Imaging of Neurodegeneration, Göttingen, Germany, ³Molecular Imaging North Competence Center (MOIN CC), Kiel, Germany, ⁴Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, ⁵Biogas Institute of Ministry of Agriculture and Rural Affairs, Chengdu, China, ⁶Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany, ⁷FAcTs GmbH, Köln, Germany

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Hydrogenases are nature's ingenious tools for efficient hydrogen catalysis. These enzymes are capable of catalyzing H₂ splitting or evolution at high turnover frequencies, using nickel and iron complexes as catalytically active centers. To exploit or mimic hydrogenase activity for H₂ production or conversion is an attractive research aim, which could facilitate the transition into an economy that uses H₂ as primary energy carrier [1,2].

Hydrogenases have therefore extensively been studied to elucidate their H₂ catalysis mechanism, with major contributions from X-ray crystallography, FT-IR, XAS, Mössbauer spectroscopy and EPR [3,4]. The characterization of the relevant hydrogen atoms at the active sites hereby poses particular challenges [5-7]. Here we demonstrate, that hyperpolarized NMR can contribute to mechanistic studies of hydrogenases, by offering a way of characterizing these bound hydrogen species during catalytic turnover.

We show that splitting of parahydrogen by the wild-type [Fe]-hydrogenase produces PHIP signals. We used these signals to characterize intermediates of the [Fe]-hydrogenase catalytic cycle, which could previously not be studied experimentally and by that describe the reaction path of the hydrogens correcting the earlier proposal [8] and the kinetics involved. CEST profiles collected using these PHIP signals [9] yield chemical shifts of bound hydrogen species in the active site. This work illustrates the potential of PHIP experiments for the mechanistic investigation of hydrogenases.

[1] Appel, Hueren, Boehm, Gutekunst, Nat. Energy 2020, 5, 458-467.

[2] Schilter, et al., Chem. Rev. 2016, 116, 8693-8749.

[3] Volbeda et al., Nature 1995, 373, 580-587.

[4] Lubitz, Reiherse, Gastel, Chem. Rev. 2007, 107, 4331-4365.

[5] Fan et al., J. Am. Chem. Soc. 1991, 113, 20-24.

[6] Ogata, Nishikawa, Lubitz, Nature 2015, 520, 571-574.

[7] Ogata et al., Nat. Commun. 2015, 6, 7890.

[8] Kiryutin et al., J. Phys. Chem. C 2017, 121, 9879-9888.

[9] Huang et al., Nat. Catal. 2019, 2, 537-543.

P-166 - An all-in-one, high-SNR spectrometer solution for NMR applications

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¹Tabor Electronics Ltd., San Francisco, United States, ²University of California, Berkeley, Berkeley, United States

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

In the field of magnetic resonance, the signal-to-noise ratio (SNR) is a key figure of merit for any spectrometer, determining parameters such as the acquisition time, the number of averages, and the minimum sample size. As quantum sensing applications place higher requirements upon experimental hardware, it is vital that the quality of the signal generation and acquisition electronics grow in proportion alongside the advances in magnet technology.

In this talk, we introduce a compact, FPGA-based commercial solution for such applications. The device has a small physical footprint, with a fast and high-resolution DAC for arbitrary pulse generation in the microwave or RF regimes. On the receiver side, the device has a large waveform memory and can perform real-time digital signal processing. We then demonstrate the use of this device in a real lab setting, for experiments involving time-crystals in NV centers [1].

[1] Beatrez, W., Fleckenstein, C., et al. Nat. Phys. 19, 407–413 (2023).

P-110 - Rhomboid-Catalyzed Intramembrane Proteolysis Requires Hydrophobic Matching with the Surrounding Lipid Bilayer

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¹University of Leipzig, Leipzig, Germany, ²University of Cologne, Cologne, Germany

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Membrane thinning of the rhomboid GlpG has been proposed to reduce the hydrophobic mismatch between the enzyme and its surrounding lipid environment.

Aims

We aimed to directly show that the membrane environment of the rhomboid influences the velocity of substrate cleavage.

Methods

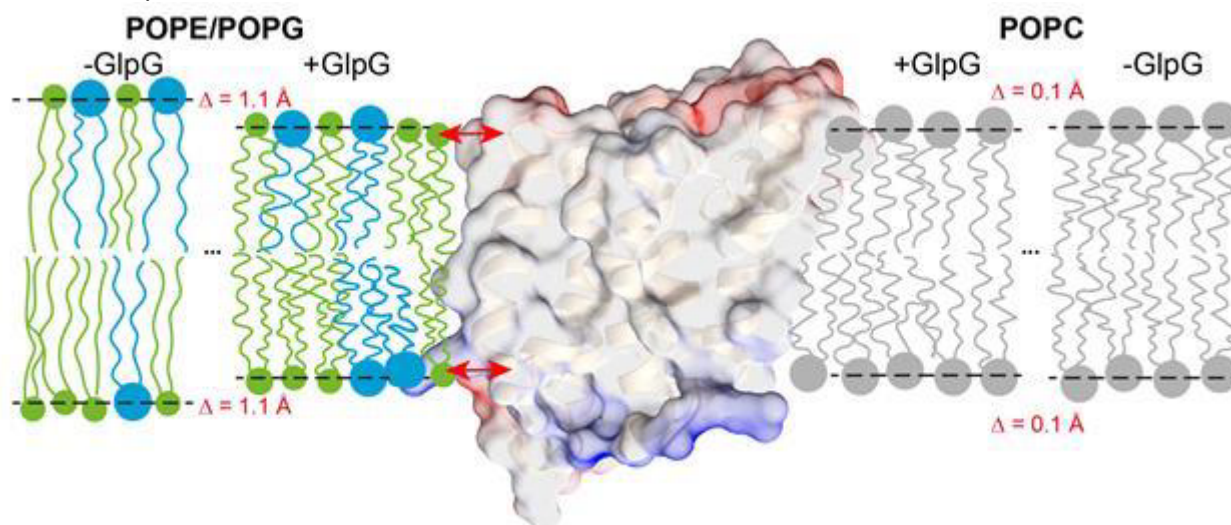
We used stationary solid-state and solution state ²H and ³¹P NMR spectroscopy

Results

We first measure the impact of GlpG on the hydrophobic thickness in phosphatidyl-choline membranes of varying thickness, where the rhomboid only marginally alters the surrounding membrane. However, in an *E. coli* relevant lipid mix of phosphatidyl-ethanolamine and phosphatidylglycerol, a decrease in hydrophobic thickness of -1.1 \AA per leaflet is observed. The cleavage velocity of GlpG is highest in DMPC followed by POPC, POPE/POPG and DLPC, while in the thickest membranes (DPPC/cholesterol) enzyme function is abolished. This suggests that an optimal window of membrane thickness (between $\sim 24 - 26 \text{ \AA}$) exists while headgroup specificity does not seem to be decisive for protein function.

Conclusions

We infer from these results that the lipid environment can fine-tune GlpG function. By adjusting membrane thickness, for instance through dynamic domain formation, the cell can regulate membrane protein function.



P-130 - Multiple quantum nuclear magnetic resonance of $^{23}\text{Na}^+$ ions in uniformly stretched and compressed hydrogels

Dr Stuart Elliott

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Uniformly stretched and compressed hydrogels yield an anisotropic environment that leads to a motionally averaged alignment of embedded quadrupolar nuclear spins such as guest $^{23}\text{Na}^+$ ions. These hydrogels typically elicit a residual quadrupolar coupling (RQC) that is not evident in conventional nuclear magnetic resonance (NMR) spectra in isotropic solution. In these systems, a sufficiently pronounced RQC is observed from dissolved $^{23}\text{Na}^+$ ions which splits the single quantum ^{23}Na NMR spectrum into a characteristic triplet and gives an oscillation in the evolution trajectories of multiple (single, double and triple) quantum filtered spectra as a function of the evolution time during a spin-echo. We derived complete equations of motion by using a Liouvillian superoperator approach including the coherent quadrupolar interaction and effects of incoherent relaxation to give full analytical expressions for the evolution of rank-1, 2 and 3 single quantum trajectories. We performed simultaneous numerical and analytical fits to the experimental single quantum NMR spectrum and to the rank-2 and 3 single quantum trajectories for varying degrees of stretching, compression or in the relaxed state, to derive estimated values of the RQC, rotational correlation time and alignment tensor given by a 3×3 Saupe matrix. We extracted the residual RQC and corresponding spherical order parameter, which showed a linear dependence on the degree of extension. The sign of the evolution trajectories observed experimentally were invariant under uniform hydrogel stretching or compression despite an inversion in the sign of the residual RQC. The analytical expressions are completely concordant with the numerical approach, which allows a more complete understanding of these experiments in oriented hydrogel model systems. This will grant extension to more complicated biological systems such as ^{23}Na bound to proteins or located inside or outside live cells in high-field NMR experiments and the anisotropic environments found in vivo by ^{23}Na magnetic resonance imaging.

P-398 - Slice and Dice: Improved Throughput of Relaxation Experiments by Interleaved Experiments

Dr Trent Franks¹, Dr Jacqueline Tognetti¹, Prof Józef Lewandowski¹

¹University Of Warwick, Coventry, United Kingdom

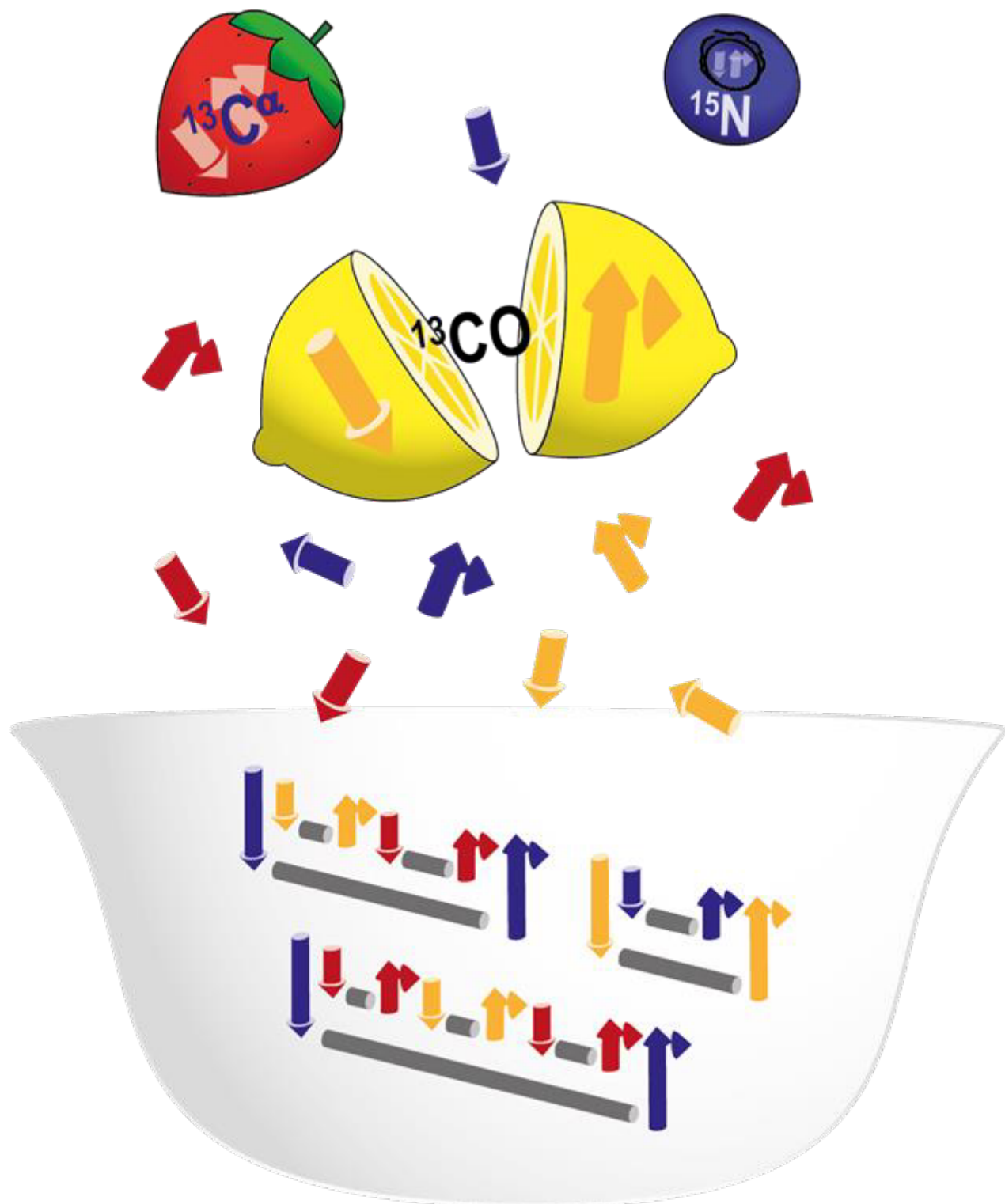
Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Quantifying molecular motion on multiple timescales in the solid state requires many measurements, at many different field strengths. These experiments can become quite expensive in terms of instrument time.

R1 measurements for microcrystalline proteins routinely require up to 40 seconds for amides and 20 seconds for carbonyls to adequately sample the timescale of the relaxation. For the long time points, nothing happens on the spectrometer for an excruciatingly long time, and it is not clear whether the time point can even be used in the final analysis due to poor sensitivity.

In this work we present an approach to use this time spent waiting for the relaxation to occur to collect similar data on separate polarization transfer pathways. For example, we excite Nitrogen polarization, and store it for relaxation. In the 40 seconds before converting the stored polarization for detection, we acquire several points of the relaxation curves of the carbonyl and alpha carbon. And during the longest time points of the carbonyl, we acquire several points of the nitrogen relaxation curve.

We show that R1 data can be collected for all backbone heavy atoms 2 to 2.5 times faster, with no loss in sensitivity, precision, or accuracy using this technique.



P-134 - NMR transverse relaxation induced by cubic nanoparticles: a Monte Carlo simulation study

Mr Florent Fritsche¹, Dr. Gilles Rosolen², Dr Yves Gossuin¹, Prof. Bjorn Maes², Dr Quoc Lam Vuong¹

¹UMONS - Biomedical Physics Unit, Mons, Belgium, ²UMONS - Micro- and Nanophotonic Materials Unit, Mons, Belgium

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Superparamagnetic nanoparticles in aqueous solution decrease the proton relaxation times: their great magnetic moment produces a dipolar magnetic field which interacts with the diffusing water proton spins. Usual theories that predict the influence of the nanoparticles on the relaxation times often suppose that the nanoparticle shape is perfectly spherical. However, for the last 15 years, several experimental studies have focused on the synthesis of exotic shapes of nanoparticles and observe an increasing effect on the relaxation rates which are attributed to the specific shape of the nanoparticles.

Aims

This study aims at theoretically evaluating the impact of cubic nanoparticles on the transverse relaxation rate at high static magnetic field (secular term) and compare the predictions to previous experimental results published in the scientific literature.

Methods

The magnetic field produced by a magnetic cube is computed by the COMSOL software using a finite element method. The obtained magnetic field is then used to compute the proton spin dephasing through a well-known Monte Carlo simulation in which the proton movement is modeled by a random walk.

Results

Simulation results show that the relaxation rates associated to cubic nanoparticles follow two regimes, also observed in the spherical case: motional averaging (small particle) and static regime (large particle). No difference is observed between sphere and cube in the static regime. A small deviation is observed in the motional averaging regime.

Conclusions

Cube and sphere do not seem to produce very different relaxation rates if equal nanoparticle volumes are considered. This work constitutes a proof of concept for the simulation of exotic-shaped nanoparticles and will be applied to more complex shapes in future work.

Reference: Vuong, Q. L.; Gillis, P.; Roch, A.; Gossuin, Y., WIREs Nanomedicine Nanobiotechnology 2017, 9 (6).

P-146 - Constant-time ESEEM experiments exploiting 2π -pulses

Mr Sergei Kuzin¹, Dr Mikhail Agrachev¹, Dr. Daniel Klose¹, Professor Gunnar Jeschke¹

¹ETH Zurich, Zurich, Switzerland

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

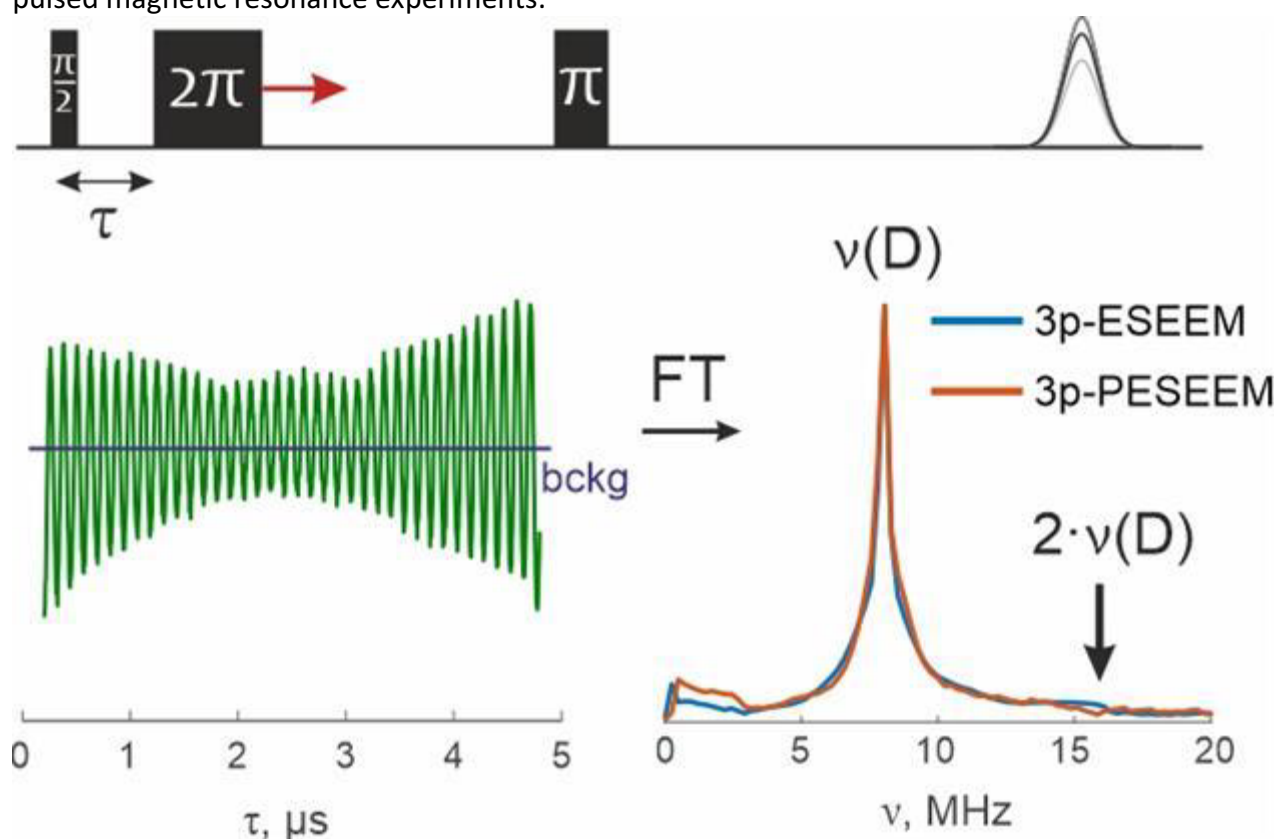
Methods of EPR hyperfine spectroscopy based on the ESEEM effect, such as 2p-, 3p-ESEEM, HYSCORE and many more, have become a standard and widely-used tool for characterizing magnetic interactions of an unpaired electron with close nuclei. The essence of such experiments is an excitation of electron-nuclear forbidden transitions and partial transfer of electron coherence to electron-nuclear coherences (ENC).

Methods and Results

We report an effect of nuclear modulation of an electron echo in the presence of 2π -microwave pulses (Figure 1). This pulse both creates forbidden ENCs and affects the existing ones. A remarkable feature of such pulse sequences consists in a modulation of a standing echo resulting in a constant-time experiment. Additionally, the role of the 2π -pulse's parameters was studied experimentally and theoretically. Two constant-time 2D-experiments similar to HYSCORE were suggested and investigated. The new experiments were tested on nitroxide radicals in a mixture of protonated and deuterated solvents both in X- (vMW 9.5 GHz) and Q-band (vMW 34 GHz) at cryogenic temperatures. Besides, they were tested on a set of transition metal complexes (Gd³⁺, Cu²⁺, Mn²⁺). With the help of numerical computations, two mechanisms for the origin of ESEEM were revealed. Overall, the observed effect of the 2π -pulse leads to designs of new ESEEM experiments that uniquely combine advantages of different traditional techniques, such as being dead-time free, showing a constant background, absence of combination peaks from a single nucleus and absence of modulation blindspots.

Conclusions

The new set of methods can be useful to study magnetic interactions of electron spin with low- γ nuclei and may lead to a broader conceptual and applied understanding of non-ideal pulses in pulsed magnetic resonance experiments.



P-168 - Sustainable and cost-effective MAS DNP at 30 K with cryogenic sample exchange

Dr. Subhradip Paul¹, Mr Eric Bouleau¹, Mr Quentin Reynard-Feytis¹, Mr Jean-Pierre Arnaud¹, Dr Frank Engelke², Dr Armin Pürea², Dr Sabine Hediger¹, Dr Gael De Paepe¹

¹Univ. Grenoble Alpes / Cea / Cnrs, Grenoble, France, ²Bruker Biospin, Ettlingen, Germany

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Progress in MAS-DNP instrumentation and polarizing agents have provided orders of magnitude sensitivity enhancements to ssNMR. However, the theoretical limit of sensitivity is still far from what is routinely obtained experimentally. An answer to improve the sensitivity lies in the ability to perform DNP at lower temperature and faster MAS than commercially available.

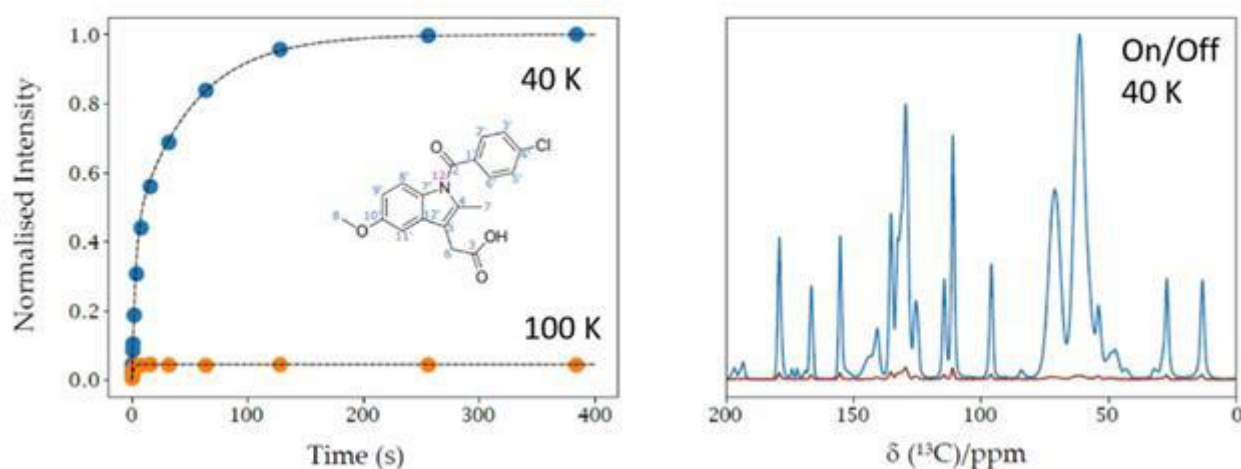
We will discuss the Grenoble approach towards sustainable ultra-low temperature MAS-DNP and progress achieved in the last years. Our setup relies on the use of a closed Helium (He) loop system to spin and cool the sample. We reported in 2015 faster sample spinning and one to two orders of magnitude in experimental time savings compared to 100 K DNP,[1] despite high thermal losses from this first-generation He DNP probe. Instead of adding more cryo-coolers to compensate for the losses, we redesigned the entire system, including a new He DNP probe with improved thermal insulation, robust tuning/matching and cryogenic sample exchange.

Our new setup is sustainable (no LHe consumption) and cost-effective. It relies on the use of a single compressor and a single two-stage cryo-cooler for bearing, drive and sample cooling together, reaching 30 K at the sample. The sample can be exchanged in few minutes using an innovative insert/eject device preventing moisture to enter the probe. Triple channel experiments with ¹H decoupling at 100 kHz (64 W, 25 ms) can be performed routinely with stable sample spinning over days (tested for 72 h at 30 K), as illustrated by 2D ¹³C-¹³C SR26 and ¹⁵N-¹³C TEDOR experiments performed on U-¹³C, ¹⁵N f-MLF. Combined with cAsymPol-POK,[2] we can improve the signal-to-noise for proton-dense methyl-containing organic powdered sample by up to a factor 20 when going from 100 K to 40 K.

[1] Bouleau et al., Chem. Sci. 2015, 6, 6806.

[2] Harrabi et al., Angewandte, 2022, 61, e202114103.

Indomethacin microcrystals impregnated with 10 mM cAsymPol-POK



P-226 - Unraveling the Local Structure in Mixed Halide Double Perovskites by means of ^{125}Te SSNMR and Density Functional Theory.

Mr Andrea Scarperi

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Perovskite materials have aroused enormous interest in the materials science community over the past decade due to their surprising optoelectronic properties, making them promising candidates in the production of new generation solar cells and light emitting diodes. In particular, metal halide perovskites such methylammonium lead iodide ($\text{CH}_3\text{NH}_3\text{PbI}_3$) and formamidinium lead iodide ($\text{CH}(\text{NH}_2)_2\text{PbI}_3$) have shown excellent power conversion efficiencies values. Nevertheless, concerns about lead toxicity and instability issues have limited their commercial scale deployment. Alternative compositions and structure of perovskites are therefore under investigation in order to overcome these problems.

Vacancy-ordered double perovskites (A_2BX_6) are a structural defect-ordered derivate from the archetypical ABX_3 perovskite structure, characterized by an antiferro arrangement of isolated octahedral units connected by A-site cations; when B-site cations consist of a Group 16 metal, these materials provide improved stability against air and moisture, while maintaining similar properties to the ABX_3 perovskites.

Aim:

The aim of this study is to determine halide distribution in clustering in the vacancy-ordered mixed-halide perovskites $\text{MA}_2\text{Te}(\text{Br}_x\text{I}_{1-x})_6$, $\text{Cs}_2\text{Te}(\text{Br}_x\text{Cl}_{1-x})_6$ and $\text{Cs}_2\text{Te}(\text{Br}_x\text{I}_{1-x})_6$.

Methods:

We used ^{125}Te Solid State NMR Spectroscopy (SSNMR) operating at 20T and NMR Crystallography methods.

Results and Conclusions:

In this work, high resolution ^{125}Te SSNMR experiments were carried out to study different compositions of the mixed-halide double perovskites $\text{MA}_2\text{Te}(\text{Br}_x\text{I}_{1-x})_6$, $\text{Cs}_2\text{Te}(\text{Br}_x\text{Cl}_{1-x})_6$ and $\text{Cs}_2\text{Te}(\text{Br}_x\text{I}_{1-x})_6$. The combination of this technique with chemical shift calculations by density functional theory, along with statistical analysis, allowed for a comprehensive understanding of the systems composition. Specifically, it was possible to quantify the different octahedral coordination environments, enabling us to draw conclusions on halide mixing uniformity within the mixed-halide materials.

P-308 - Structure and Dynamics of Crystalline Carbimazole: combination of Solid State NMR, Quasielastic Neutron Scattering, Molecular Dynamics, and DFT

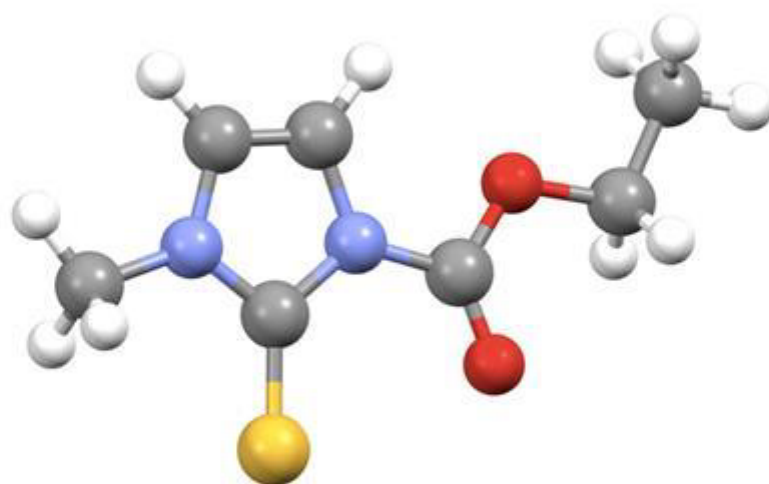
Mr Andrea Scarperi¹, Ms Elisa Carignani^{2,3}, Mr Giovanni Barcaro⁴, Ms Aleksandra Pajzderska⁵, Dr Francesca Martini^{1,3}, Mr Marco Geppi^{1,2,3}

¹Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy, ²Institute for the Chemistry of OrganoMetallic Compounds, Italian National Council for Research, CNR/ICCOM, Pisa, Italy, ³Center for Instrument Sharing, University of Pisa (CISUP)), Pisa, Italy, ⁴Institute for Chemical and Physical Processes, Italian National Council for Research, CNR/IPCF, Pisa, Italy, ⁵Department of Radiospectroscopy, Faculty of Physics, Adam Mickiewicz University, Poznan, Poland

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

In the last two decades, the combination of Solid State NMR (SSNMR), diffractometric techniques and computational methods has been recognized as a powerful tool in the investigation of the structure of crystalline solids, and “NMR Crystallography” is seen as a rapidly maturing subject area in the crystallographic community. Furthermore, Solid State NMR is a very powerful tool for the characterization of dynamic properties in solid phases on a broad time scale (from seconds to picoseconds), and in combination with Quasielastic Neutron Scattering (QENS) the range can be extended to shorter times. Even in the case of dynamics, computational methods add a precious tool to achieve a deeper understanding.

In this work, the dynamic and structural properties of the crystalline form of carbimazole, a prodrug used in the treatment of hyperthyroidism, have been investigated in detail. The combination of DFT calculation with ¹H and ¹³C 1D and 2D NMR experiments has allowed the elucidation of the drug crystal structure, resolving ambiguities in diffraction-derived structures previously reported. The measurement of spin-lattice relaxation times of ¹H and ¹³C nuclei at variable temperatures, QENS and Molecular Dynamic simulations enabled the detailed characterization of the dynamic processes that the carbimazole molecule undergoes in the crystal lattice.



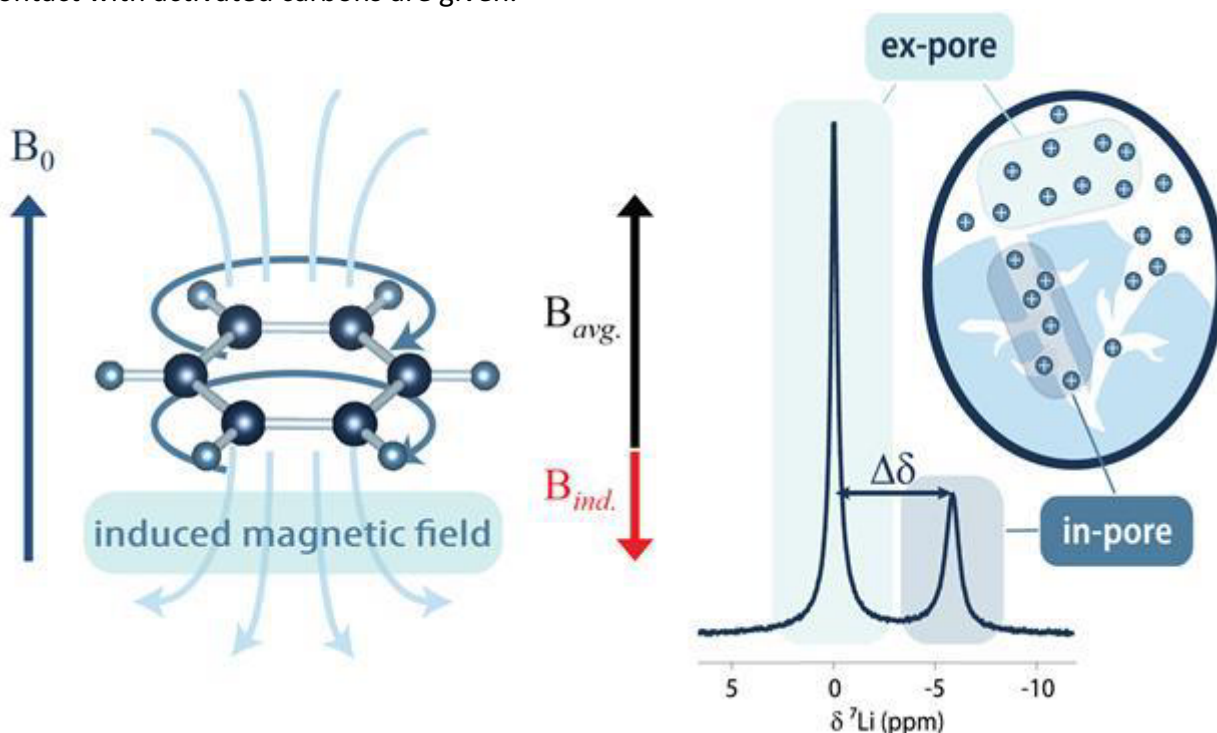
P-242 - Observing aqueous ion behaviour within microporous carbon electrode materials using NMR spectroscopy

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The distribution of ions and the mechanisms controlling their exchange directly impact the performance of numerous adsorption-based applications such as water desalination and electrical double-layer capacitance. Currently, a large degree of ambiguity surrounds these mechanisms and ion behaviour within microporous environments (≤ 2 nm) as conventional spectroscopic techniques are unable to observe interactions within these complex biphasic and disordered environments. Progress towards understanding these mechanisms has been made in the observation of organic systems, but a limited understanding of aqueous systems persists. Here, a quantitative nuclear magnetic resonance (NMR) approach to investigate aqueous electrolytes within microporous carbon materials is detailed. Exploitation of ring-current induced magnetic fields created near the surface of aromatic carbon structures under an applied magnetic field enables the quantifiable determination of in-pore and ex-pore guest species populations. Preliminary data on the effects of microporous carbon structure and ion-specific properties on the ion distribution within uncharged microporous carbon networks is presented for aqueous lithium-, sodium-, and caesium- based electrolytes. Quantitative NMR titration measurements reveal that concentration of the electrolyte, and the nature of the anionic and cationic species, has a measurable effect on the occupation of in-pore and ex-pore environments leading to different ion: solvent ratios in each case. Insights into the relationship between ionic properties and spontaneous exchange rates within the complex carbon structure obtained using two-dimensional exchange (2D-EXSY) NMR spectroscopy are also presented. Lastly, preliminary data and comments on pH variations of the studied aqueous electrolytes in contact with activated carbons are given.



P-002 - Zero-Field J-spectroscopy of Quadrupolar Nuclei

Mr Román Picazo-Frutos

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Zero- to ultralow-field (ZULF) nuclear magnetic resonance (NMR) is a version of NMR in which couplings to magnetic fields originating from the sample itself (such as dipole-dipole interactions and J-couplings) are stronger than couplings to magnetic fields generated by the experimental environment. Non-inductive sensors e.g., optically-pumped magnetometers (OPMs) can measure J-spectra directly at zero field, however, up to date no pure J-spectra of molecules featuring the coupling to quadrupolar nuclei were reported. In this work, we demonstrate J-spectra of ammonia cations ($^{14}\text{NH}_4^+$, $^{15}\text{NH}_4^+$, $^{15}\text{ND}_x\text{H}_{(4-x)}^+$ where $x=0,1,2,3$), taking advantage of the unique tetrahedral symmetry of the molecule which effectively switches off quadrupolar interactions.

Methods:

To observe the signals, we built a setup containing a 2T Halbach magnet for prepolarization, a pneumatic shuttling system, Helmholtz coils for DC pulsing, and two OPMs for detection in a low-frequency range (<1 kHz). To extract J-coupling frequencies with high precision and reduce uncertainty, we acquired 36000 scans and performed statistical analysis by constructing cumulative distribution functions (CDFs). This allowed us to extract the relevant peak frequencies with precision of up to 1 mHz.

Results:

We report zero-field NMR measurements of ammonium cations that feature J-couplings to quadrupolar nuclei. Since measurements are performed on both isotopologues in the same solution, systematic errors (due to temperature, concentration, etc.) are minimized. The measured J-coupling ratios $|J(^{15}\text{N})/J(^{14}\text{N})| = 1.40129(3)$ (calculated from the ratio of the highest frequency peaks in the ZULF NMR spectra) differ from the expected ratio of gyromagnetic ratios, $|\gamma(^{15}\text{N})/\gamma(^{14}\text{N})| = 1.4027548(5)$, and we attribute this deviation of 0.1 % to the secondary isotope effect.

Outlook:

Simple symmetric cations such as ammonium do not require expensive isotopic labeling for the observation of J-spectra and, thus, may expand the applicability of ZULF NMR spectroscopy in biomedicine and energy storage.

P-348 - Investigating the impact of water-soluble polymers on the metabolic profiles of aquatic organisms and microbes

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

This research investigates the impacts of water-soluble polymers on the metabolic profiles of aquatic organisms and microbes. Liquid plastics, also known as water-soluble polymers (WSPs) are polymers that dissolve in water and are commonly used in industrial, commercial, agricultural and pharmaceutical products with varying molecular weights and concentrations. WSPs' environmental impacts do not get as much limelight as microplastics, where there is hardly any media coverage of them or strict production monitoring. Therefore, WSPs can enter the aquatic environment through direct disposal, landfill sites, water treatment processing stages and agricultural soil-run offs.

Aims:

This project aims to characterise and quantify the WSPs present in the aquatic environment by the use of Nuclear Magnetic Resonance (NMR) and to also determine the beneficial and detrimental environmental impact of WSPs.

Method:

Fourteen river sites situated near wastewater treatment sites, recycling centres, industrial sites and public parks have been sampled. Samples were then concentrated via high vacuum and analysed using 1D 1H and 2D DOSY NMR spectroscopy. WSPs in river water and sediment samples were observed through diffusion coefficient comparison against commercial WSP.

Results:

Majority of the sampled river sites have shown contamination of polyethylene glycol (PEG) situated at 3.715 ppm – 3.726 ppm with a molecular weight of around 100 - 1000 g/mol with a diffusion coefficient of ranging from 2.62e-10 – 8.42e-10 m²/s and concentrations varying from 2.10 – 33.5 ug/L.

Conclusion:

This project is at the cusp of investigating the impacts surrounding WSPs and the aquatic environment with sampled field work achieving characterisation and realistic concentration values. Future research will be looking at the observation of environmental assays, metabolic profiles and LD50 studies will be undertaken. The project will therefore create a multivariate analysis of metabolic profiles for different aquatic microorganisms and microbes at varying concentrations of WSPs.

P-144 - Pulsed Electron Electron Double Resonance (PELDOR) on Spin Labeled SARS-CoV-2 3'-UTR Δ HVR

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PELDOR (pulsed electron-electron double resonance) also called DEER (double electron-electron resonance) is a magnetic resonance method to determine the distance and the distance distribution in double spin-labeled macromolecules like proteins, RNA, or DNA as well as polymers [1,2]. The maximum accessible distance r_{max} is limited by the dipolar observation time window t_{dip} spanned by the pulse settings $r_{\text{max}} [\text{nm}] \approx 3 \sqrt[3]{(t_{\text{dip}} [\mu\text{s}])}$ [3]. This time window depends strongly on the phase memory time of the nitroxide spin labels. The phase memory time at the experimental temperature of 50 K is mainly determined by the coupling to other spins in the sample and depends on the gyro magnetic ratio of such spins. Therefore, protons and other electrons are the main contributors. Basic techniques to get rid of such spins are substituting protons with deuterons and reducing the radical (and therefore sample) concentration [4].

In this contribution, we investigate the formation of a pseudoknot from SL2 towards SL1 stem-base on the SARS-CoV-2 3'UTR Δ HVR construct [5]. The influence of the Mg(II) concentration is evaluated by different spin labeled RNAs and a mutation where the pseudoknot contact sequence is altered.

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Acknowledgements: Sino-German Center for Research Promotion (SGC) Fund.

P-366 - Employment of 5D non-uniform sampling NMR assignment strategy to assign disordered protein regions with repetitive motifs

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Nuclear Magnetic resonance (NMR) is often the method of choice when characterizing intrinsically disordered regions (IDRs). However, when using standard triple resonance assignment experiments the successful IDR assignment often becomes unfeasible, as IDRs frequently contain repetitive motifs and numerous prolines resulting in strong signal overlaps in spectra.

In order to overcome these challenges in assigning IDRs, ¹³C-detected 5D non-uniformly sampled (NUS) experiments which were developed in cooperation with our research group and successfully utilized in the past. The 5D NUS experiments CACONCACO and HC(CC-TOCSY)CACON were complementarily used for both the backbone and aliphatic side-chain assignment of 2N4R human variant of Tau protein (441 residues) which is linked with neurodegenerative diseases. Over 99% of Tau residues were successfully assigned when employing the 5D NUS experiments. The assignment efficiency is noteworthy as the 2N4R Tau variant contains 18 aa stretches of the motifs VXSK to PGGG and a proline-rich domain. The assignment provided information about secondary structure propensities and proline-conformation analysis. The obtained assignment will be used to determine amino acids essential for interaction with binding partners e.g., 14-3-3 proteins.

Moreover, this approach was recently employed for the IDR (residues 38-137) of the extracellular surface variant of carbonic anhydrase IX (CA IXsv, residues 38-391) which is associated with aggressive tumor growth and metastasis. As CA IX's activity is increased with the presence of IDR and lower extracellular pH typical for solid tumors, the obtained assignment will be subsequently utilized to investigate the influence of various pH conditions on its local structure features and involvement in the activity of CA IX.

P-074 - Investigations of Fusidic acid resistance conferred by structural rearrangements in domain III of EF-G applying CPMG Relaxation Dispersion

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Fusidic acid (FA) is one of the few remaining drugs used against methicillin-resistant *Staphylococcus aureus* infections. It stalls protein synthesis by binding to elongation factor G (EF-G) after translocation and preventing its release from the ribosome. Different types of resistance to FA treatment have emerged in *S. aureus* strains: FusA, FusB/C/D/F (FusB-type), and FusE. While FusA and FusE constitute mutations on the target, FusB-type resistance is conferred through the expression of an accessory protein which binds to EF-G re-instating its release from the stalled complex. Binding of FusB causes a significant allosterically induced change in the dynamics of domain III of EF-G C3, a truncated version, consisting of domains III, IV and V, which were shown to be sufficient for FusB binding. It has been shown previously that introducing disulphide bonds within domain III restrains the FusB induced dynamics and reduce its ability to confer fusidic acid resistance. In order to identify residues or regions within domain III that confer these allosteric rearrangements we introduce disulphide bonds at different regions within domain III. We use methyl relaxation dispersion NMR, NMR chemical shift mapping and BODIPY-FL-GDP fluorescence assays to follow changes in FusB-induced dynamics and their effect on fusidic acid resistance. We observe that introduction of disulphide bonds to any of the secondary structural elements within domain III prevents FusB from inducing the same dynamics as WT and there appears to be no single key location. The native set of dynamic movements within EFG domain III is required to enable FusB resistance. Effects of FusB on disulphide-bridge constrained DIII dynamics are weakened, but not entirely abolished.

P-378 - CPMAS cryoprobe technology enables multidimensional solid-state NMR studies of the stratum corneum at natural isotopic abundance

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The excellent barrier properties of the human skin originate from its outermost 10-20 micrometers—the stratum corneum—comprising keratin-filled dead cells and lipids organized in a structure resembling brickwork. Through a series of ¹³C MAS studies relying on spectral editing by CP and INEPT for selective detection of solid and liquid molecular segments, we have previously shed light on the molecular-scale underpinnings of the macroscopic barrier and mechanical properties, revealing co-existence of solid and liquid lipid phases as well as solid and dissolved protein filaments with proportions determined by hydration, temperature, and the presence of additives acting as “moisturizers” in cosmetics applications or “penetration enhancers” in drug delivery. Since the raw material for these studies is stratum corneum from pig or human skin, isotopic labelling is not an option, and even basic 1D ¹³C spectroscopy may require a day of measurement time to reach sufficient signal-to-noise to determine the dynamic state of the major protein and lipid components. Here we present a pilot study of natural abundance stratum corneum using a 3.2 mm CPMAS cryoprobe at 600 MHz. Compared to conventional CPMAS equipment, the signal boost offered by the cryoprobe enables not only dynamics characterization of minor protein and lipid components and small amounts of additives along the lines of previous studies, but also CP- and INEPT-based 2D ¹H-¹³C HETCOR with ¹H-¹H spin diffusion to study the nanometer-scale organization of liquid and solid constituents, as well as 1D ¹⁵N spectroscopy for monitoring amide groups on keratin and ceramide lipids. Exploring the limits of sensitivity, we note that even with the cryoprobe technology, potentially valuable 2D ¹³C-¹³C INADEQUATE measurements are not yet feasible at 600 MHz. Still, the 2D ¹H-¹³C measurements may give answers to long-standing questions about partitioning of additives between protein- and lipid-rich regions and solid and liquid phases.

P-330 - Overhauser transfer from long-lived coherences in protein lysozyme and stroboscopic detection of long-lived states in glutathione

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Aims

Singlet-based proton spin order¹ can offer new information for biological molecules: i) Overhauser transfer enhancement based on long-lived coherences² (LLC-ROE) is demonstrated for lysozyme, a globular 18kDa protein³; ii) long-lived states (LLS) in glutathione, one of the main cellular responders to oxidative stress, can be used for on-the-fly detection of redox reactions on time-scales of tens of seconds.

Methods and Results

We have predicted that LLC-ROE transfer⁴ from pairs of J-coupled protons I and S in LLC configurations to a third proton, (Ix-Sx)->Kx, overcomes classical ROE (Ix+Sx)->Kx at high molecular rotational correlation times in strong magnetic fields. This is confirmed for lysozyme Gly-H α dipolar interactions at 950 MHz. LLC-ROEs signals were observed for protons neighboring GLYs-4,49,54,126, which feature LLC relaxation time constants more than twice as long as the classical counterparts. LLC-ROEs are stereospecific, as predicted by theory⁴: the position of interacting spins with respect to the C₂ axis of symmetry of Gly-H α 2,3 determines their sign.

We demonstrate on-the-fly ¹H-LLS detection in glutathione-Cys,Gly residues with relaxation time constants T_{LLS} of up to 16 s. Signals extracted from the initial GSH-Gly-H α polarization stored in LLS using a series of small-flip-angle pulses⁵ follow GSH/GSSG conversions at various catalyzer concentrations.

Conclusions

Long-lived spin order becomes useful for the characterization of proteins in high magnetic fields and following biochemical reactions.

Acknowledgements

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- DOI:10.1103/PhysRevLett.104.053001
- DOI:10.1007/s12104-010-9219-8
- DOI:10.3390/sym13091685
- DOI:10.1021/acs.jpcclett.2c01749

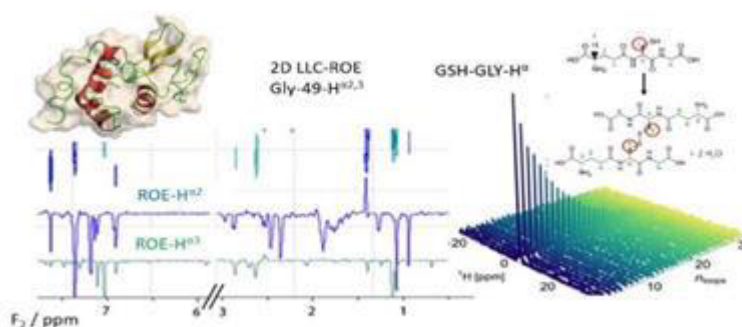


Figure.(Left) 2D LLC-ROEs for Gly-49 yielding positive (blue) and negative (green) signals matching classical 2D-ROE stripes at the frequency of G49-H α 2 (blue) and G49-H α 3 (green). (Right) Time-course of on-the-fly detection of GSH→GSSG conversion using initial GSH-Gly polarization via successive partial excitations of LLS-stored spin-order during 30 s.

P-012 - In-line Low Field Magnetic Resonance Reveals Inversion State of Syrups

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Low-field magnetic resonance has many attractive features for industrial use, from lower costs and maintenance in comparison to high-field analytical systems to the ability to create bespoke systems which can be fitted in-line for measurements at the point of manufacture. A key ingredient in many food products is sugar syrup which comes in many forms.

Simple sugar syrups are prone to dehydration and crystallization and thus present challenges in use in commercial products where long shelf lives and product consistency are essential.

The primary methods to produce invert syrups commercially are heating in the presence of an acid or using an enzyme such as invertase [1]. In this work, we monitor the inversion of syrups undergoing enzymatic and thermal inversion using a custom-built MR system based on a Halbach magnet arrangement shown in Figure 1a. Syrups flow through a pipe in the center of the bore while T2 relaxation spectra are acquired. In Figure 1b, we demonstrate a promising preliminary result for the ability to track the progress of the inversion for both, in real time, thus permitting optimization of the process regardless of the variability of incoming ingredients.

This system will be incorporated into a process line at a confectionary factory as a useful tool to ensure consistency of output.

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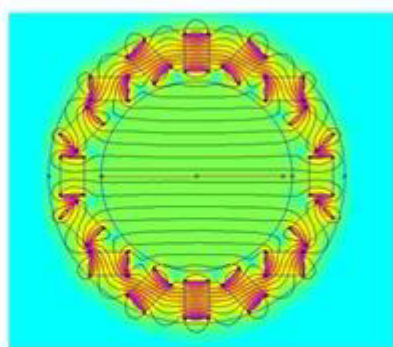


Figure 1a. Custom built magnetic resonance system based on a Halbach magnet arrangement

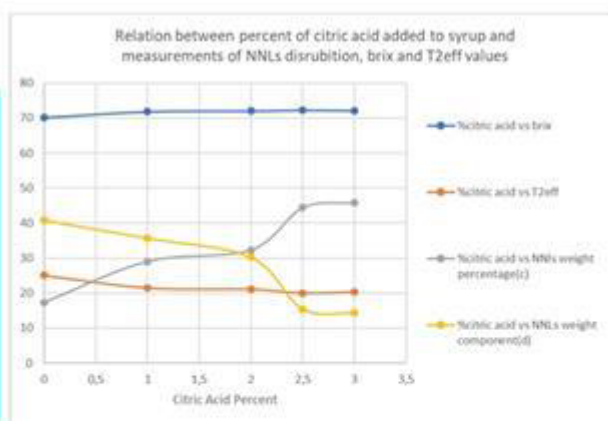


Figure 1b. For the citric acid catalysed inversion of sugar syrup, relation between citric acid added to syrup and measurements of NNLs distribution (%weight c and d components), brix(°), T2eff(ms)

P-020 - Transient structural properties of the Rho GDP-dissociation inhibitor.

Mrs Sara Medina Gómez

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The Rho family of ras-related GTPases is a family of small signaling G proteins. These proteins work as molecular switches in a broad range of critical cellular functions. They are well known for their action in reorganizing the actin cytoskeleton and for modulating a broad range of cellular processes. Alterations of these processes can lead to neurological and immunological problems. The activation of the Rho GTPases is regulated by other proteins, such as Guanine nucleotide dissociation inhibitors (GDIs). The inhibitory activity of GDIs comes from the ability to bind the carboxy-terminal isoprene and extract them from the membranes and the inhibition of GTPase cycling between the GTP- and GDP-bound states. The N-terminal domain of RhoGDI plays a crucial role in these inhibition processes. The formation of this interface has been thought to emerge from an intrinsically disordered state of RhoGDI in its free, apo form, as suggested by previous X-ray crystallography and NMR studies. Here, we used solution NMR to characterize the secondary structural propensities in the N-terminal domain when bound to Cdc42 and in the free state. Opposing the current mechanistic understanding, a diverse set of NMR data unequivocally show that structural properties of the GDI N-terminus characteristic for complex formation with GTPases already exist as largely performed features in free, apo GDI. Moreover, upon Cdc42 binding, the flexibility of the N-terminus is not largely abrogated. These observations suggest an active role of a pre-structured N-terminus guiding the complicated and highly selective complex formation.

P-066 - Aromatic [19F, 13C]-adenosine labeling for TROSY-NMR spectroscopy of RNA

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Aims

While ¹H is the nuclei with the highest NMR sensitivity, the limited shift dispersion of ~15 ppm and the thus resulting chemical shift overlap in bio-macromolecules makes it mandatory to use stable isotope labeling with ¹³C, ¹⁵N or ²H to facilitate NMR experiments in large systems.

As an alternative to ¹H, the ¹⁹F nucleus offers several favorable NMR spectroscopic properties, such as 100 % natural abundance, an NMR sensitivity almost as high as protons, and a large chemical shift dispersion. Despite these advantages the fast transverse relaxation by the chemical shift anisotropy (CSA) mechanism makes its applicability in large macromolecules difficult. Calculations and the subsequent experimental investigations of proteins and nucleic acids showed, that in aromatic ¹⁹F-¹³C spin pairs the mutual cancellation of the transverse relaxation by the chemical shift anisotropy and the dipole-dipole interaction gives very favorable TROSY properties [1].

Methods

I present a synthetic route for both a [2-¹⁹F, 2,8-¹³C]-adenosine phosphoramidite and a [2-¹⁹F, 2-¹³C]-adenosine triphosphate. Subsequently, this [2-¹⁹F, 2,8-¹³C]-rA was incorporated into the 27 nt HIV-1 TAR to investigate its stability during solid phase synthesis and into a 61 nt human hepatitis B virus epsilon RNA. Furthermore the [2-¹⁹F, 2-¹³C]-adenosine triphosphate was used to label a 124 nt long pre-miR-17 RNA. In this sizeable RNA (molecular weight 40 kD) the superior TROSY properties of ¹³C component became obvious.

Results and Conclusion

The experimental data collected on both hHBVε and pre-miR-17 RNA confirms the favorable ¹⁹F-¹³C-TROSY effect in high molecular weight systems.

This novel stable isotope labeling scheme in combination with the TROSY experiment is very promising for investigations of large RNAs and high weight protein RNA complexes.

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<https://doi.org/10.1038/s41592-019-0334-x>

P-118 - Characterization of structurally heterogeneous proteins with NMR: the case of CBP

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

CREB-binding protein is a transcriptional coactivator involved in the transcription of several human genes as well as many signalling pathways¹. This protein counts 2442 residues: more than 60% form disordered regions, while the others are organized in seven globular domains. So far, characterization of both folded domains and disordered regions of CBP has been carried out by isolating them individually. Although this approach simplifies the in-vitro application of biophysical techniques such as NMR spectroscopy, it permits the single domain to be studied in a condition that may differ significantly from the actual one. In this work, we propose a different approach to study two contiguous CBP domains with NMR spectroscopy. We started using this new approach on the CBP-TAZ4 construct, formed by the zinc-binding domain TAZ2 and the ID4 disordered region. NMR, especially ¹³C-direct detected NMR², has been proven to provide clean information about highly flexible disordered regions also when part of complex multidomain proteins³.

Aims

This work aims to enlarge our knowledge of the disordered regions of CBP multidomain protein using NMR.

Results and methods

Sequence-specific resonance assignment of the TAZ4 construct was carried out using triple-resonance NMR experiments. The ¹⁵N relaxation rates (R₁, R₂ and NOE) were measured at different temperatures to obtain information on the dynamics of the two domains. These data show that the structural features of the TAZ2 domain, as well as ID4's dynamic properties, are altered when the two domains are bound together or in isolation.

Conclusions

We show that NMR is an excellent technique for studying structurally heterogeneous constructs such as TAZ4. Furthermore, our findings provide information on the structural and dynamic properties of TAZ4.

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P-342 - Proton-enhanced spectroscopy of ^{103}Rh spin pairs

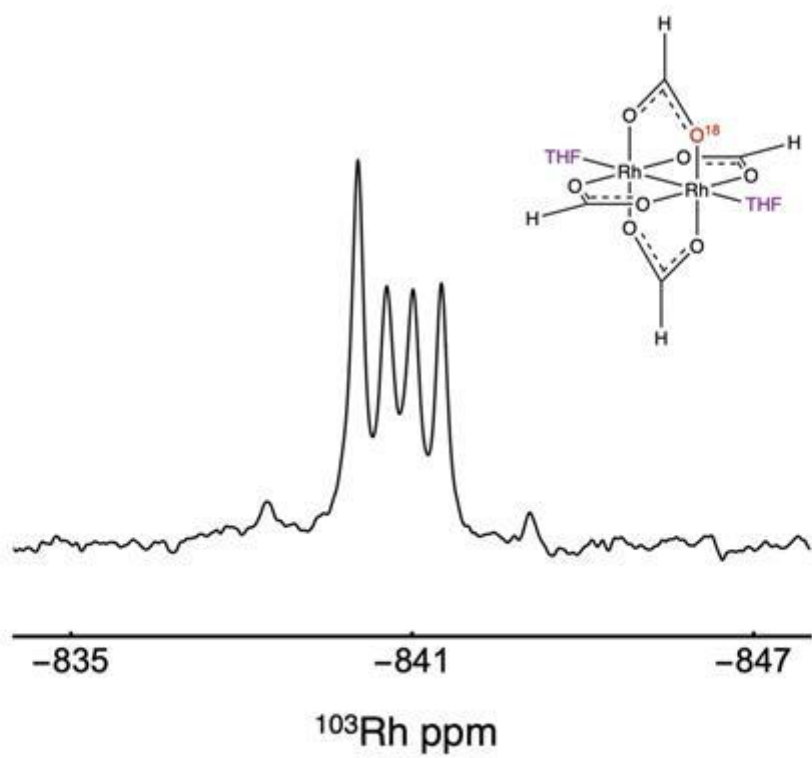
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

A chronic insensitivity issue has long plagued the NMR spectroscopy of ^{103}Rh nuclei; indeed, it is the main culprit for the relative scarcity of ^{103}Rh parameters in the NMR literature. We present proton and rhodium NMR parameters for the rhodium formate paddlewheel complex, making use of indirect detection on protons, which has previously been shown to dramatically improve the sensitivity of rhodium measurements. (1) We enhance the ^{103}Rh signals by polarization transfer from protons, using a novel cross polarization pulse sequence, called DualPol. This is based on the PulsePol sequence, which was originally developed for the spectroscopy of nitrogen vacancy centers in diamond, and which has been interpreted using symmetry-based recoupling concepts from solid-state NMR. (2,3) We utilize oxygen-18 enrichment to measure the rhodium secondary isotope effect and rhodium-rhodium homonuclear J-coupling. We have been successful in generating rhodium singlet order across the rhodium spin pair and report the singlet decay constant T_s across a range of magnetic fields.

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Rhodium formate $^{103}\text{Rh}\{^1\text{H}\}$ spectrum enhanced by DualPol magnetization transfer from protons.

P-156 - Trapping in a Hole for Optical Magic Angle Spinning Magnetic Resonance

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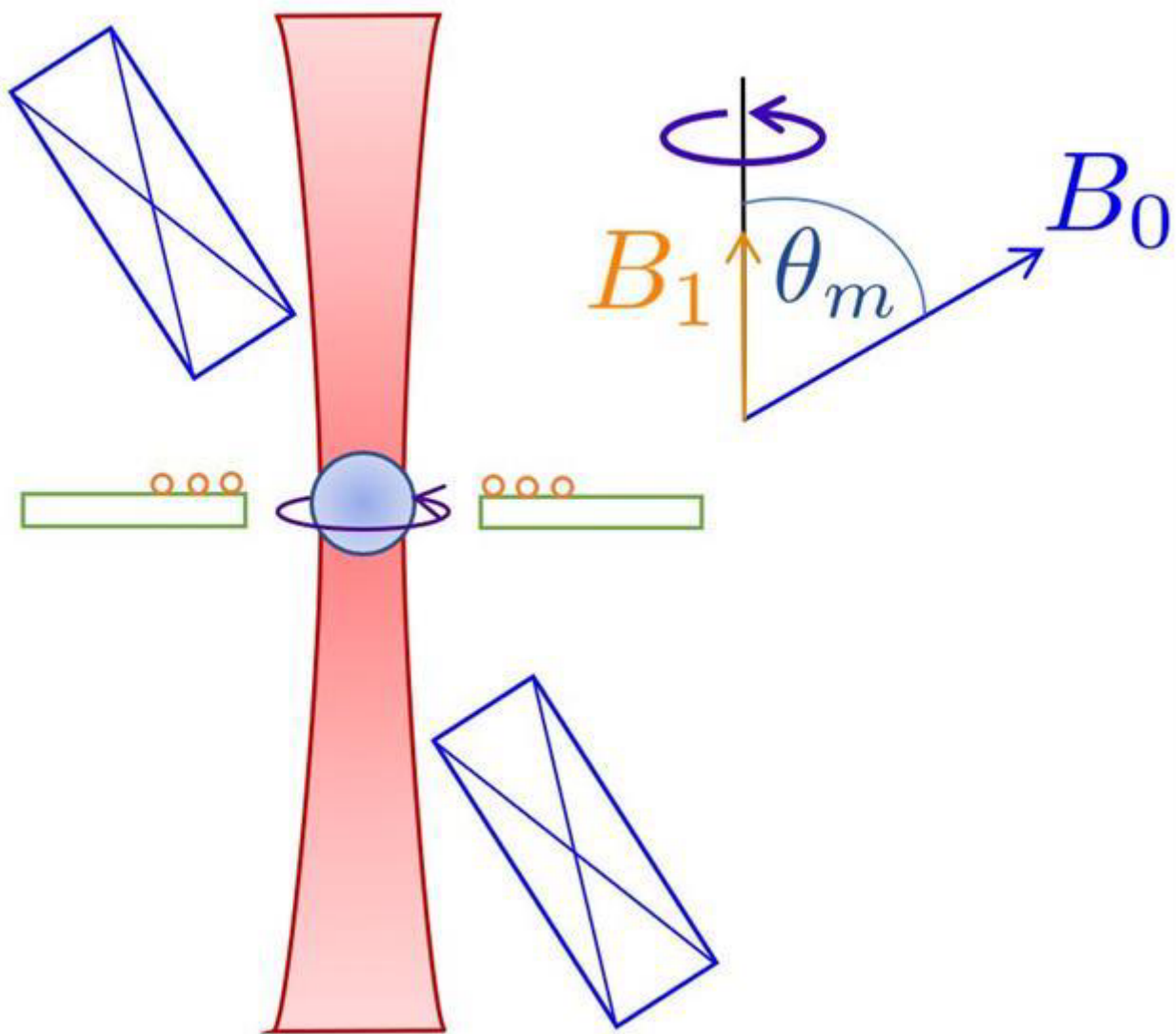
Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Solid-state magnetic resonance suffers from line broadening, which can be mitigated by magic angle spinning (MAS). The maximal achieved rotation frequency of 200 kHz cannot fully average all anisotropic interactions present in nuclear magnetic resonance (NMR), let alone electron paramagnetic resonance (EPR). Meanwhile, in the field of optics, particles have been rotated at up to GHz frequencies.

Our goal is to implement a novel spinning technology dependent on optically trapped and rotated microparticles to reach MHz rotation frequencies. Optical MAS would further allow magnetic resonance on-a-chip technology to benefit from MAS, as conventional MAS technology is not optimized for microscopic radiofrequency coils. In this step, we aim to determine the feasibility of EPR detection on an optically trapped particle and therefore study the optimal placement of the detection coil.

Samples of up to 40 μm diameter are optically trapped at ambient pressure in a vacuum chamber using a 1064 nm CW laser. The laser beam is adjusted in diameter and power, circularly polarized by a quarter-wave plate, and focused by an aspherical lens. The sample particle is trapped in the laser focus. The forward scattered light is focused onto a quadrant position detector for center-of-mass motion detection and onto a polarization sensitive detection system for particle rotation detection. A 3D microstage is used to place a plate with a hole close to an optically trapped particle.

We demonstrate optical trapping of a range of particles in holes of sub-millimeter size. Furthermore, we establish a system to prepare microparticles from macroscopic samples. Finally, we characterize a permanent magnet that will be used for detection in a proof-of principle experiment. We conclude that drilling a hole into the center of a planar microcoil for EPR detection is a feasible approach to maximize the sensitivity in a proof-of-concept optical MAS experiment.



P-186 - Next-generation Enabling Hyperpolarized Clinical Technologies

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

INTRODUCTION: The vast majority of emerging hyperpolarized MRI contrast agents employ heteronuclei (e.g. ¹³C or ¹²⁹Xe) for transient storage of hyperpolarization and detection due to much longer lifetimes of the HP state and the lack of background signal. However, clinical MRI scanners are often poorly suited for excitation and detection of heteronuclei as they typically lack the corresponding RF hardware and software. While multi-nuclear detection capability exists on clinical research MRI scanners, to date their number (<0.5%) is inconsequential for the purpose of achieving widespread implementation of HP MRI.

METHODS/RESULTS: We report on our progress in developing three distinct approaches to enable detection of HP contrast media on clinical MRI scanners equipped with proton-only hardware and software. The first strategy employs ¹³C-¹³C singlet states created in HP [1,2-¹³C₂]pyruvate and its downstream metabolites, which can be detected using proton-only excitation. The second approach relies on the use of ultrafast electronics to “translate” excitation pulses at proton frequency to a given heteronuclear frequency. In this approach, a transmit-receive RF coil is required to transmit excitation pulses and detect the heteronuclear signal. The detected signal is then “translated” back to the proton frequency of the MRI scanner, and can be “fed” to the MRI scanner for image visualization or processed off-line. Radiofrequency Amplification by Stimulated Emission of Radiation (RASER) is the third most promising approach. RASER signals are emitted spontaneously by negatively hyperpolarized spins without external radio-frequency excitation pulses (and thus not requiring the RF excitation coil and the pulse-sequence-synchronization) and without any background signal. The recently demonstrated feasibility of RASER MRI and ¹³C RASER, and the tracking of chemical transformation and chemical exchange, will be discussed. Together, these advances suggest the feasibility of ¹³C and ¹²⁹Xe RASER imaging in vivo, using heteronuclear purpose-built high Q detection electronics for clinical MRI scanners.

P-372 - Analytical descriptions of (multiple-contact) cross-polarization kinetics and spin-lattice relaxation in solid alanine

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¹CNRS, Strasbourg, France

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The kinetics of Hartmann-Hahn (HH) cross-polarization (CP) and multiple-contact CP (MC-CP) are usually treated by a simplified thermodynamic theory, the so-called classical model denoted I-S [1]. However, heteronuclear interactions often lead to coherent energy transfer. In this case, the non-classical I-I*-S model is more appropriate [1,2,3,4].

In this work, exact (non-secular) and approximate (semi-non-secular) analytical solutions for HHCP and MC-CP kinetics under MAS are derived using the I-I*-S model in the presence of T1 ρ relaxation. Moreover, the secular solution originally derived by Naito and McDowell [2] is shown to be incorrect. In the fast spin-diffusion regime, the magnetization decays with the T1 ρ relaxation time constant during the second stage of the HHCP kinetics. On the other hand, when spin diffusion is slow relative to T1 ρ relaxation, the decay time constant of the HHCP signal is determined by the spin diffusion process. These two regimes are readily distinguished by simulation of the HHCP and MC-CP kinetics.

All these theoretical results are verified in practice for the particular case of L-alanine. The analysis of the ¹H-¹³C HHCP and MC-CP kinetics together with (Lee-Goldburg) ¹H T1 ρ relaxation experimental data provides a consistent picture of spin dynamics. T1 ρ relaxation of the CH and CH₃ protons occurs via spin diffusion towards the NH₃ protons. Furthermore, the assumption of common proton spin temperature that is generally valid for T1 relaxation [5] breaks down and leads to non-exponential T1 ρ relaxation.

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P-064 - Structure, dynamics and interactions of kinetochore proteins from *Trypanosoma brucei*

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

The kinetochore is the macromolecular machinery that drives chromosome segregation by interacting with spindle microtubules. Kinetoplastids (such as *Trypanosoma brucei*) are a group of evolutionarily divergent eukaryotes. In *T. brucei*, 24 kinetoplastid kinetochore proteins (KKT1–20, KKT22–25) and 12 KKT-interacting proteins (KKIP1–12) have been identified. These proteins do not appear orthologous to canonical kinetochore proteins, suggesting that kinetoplastids use a distinct set of proteins to build up unique kinetochores.

Aims

We are using NMR spectroscopy, as part of an integrative structural biology approach, to study both structured and disordered domains from selected kinetoplastid kinetochore proteins.

Methods

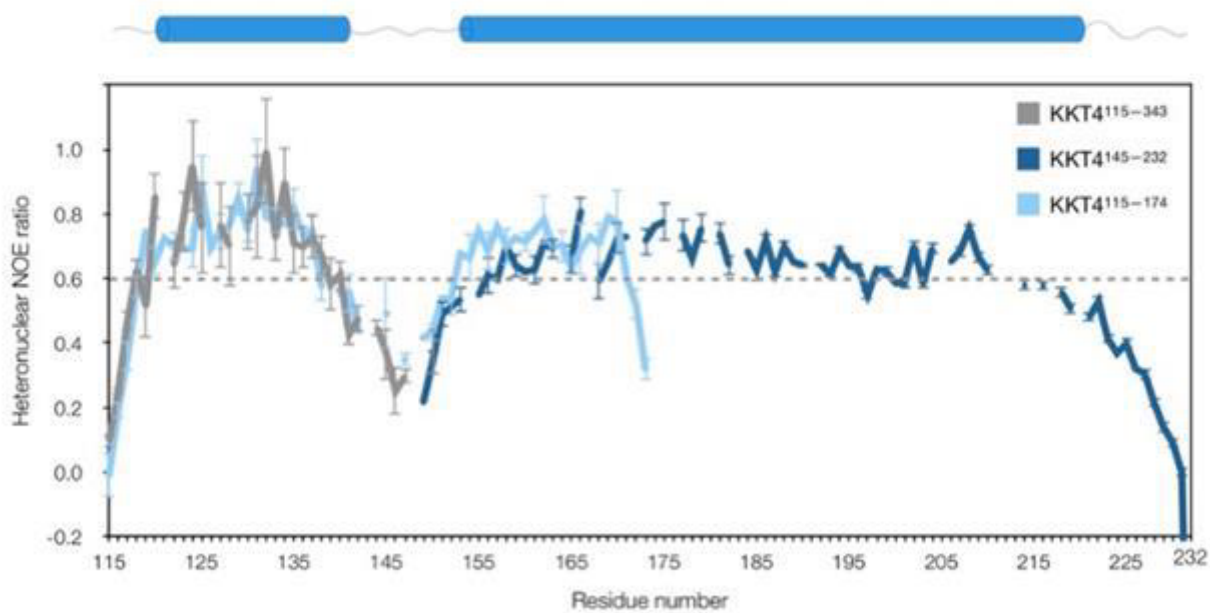
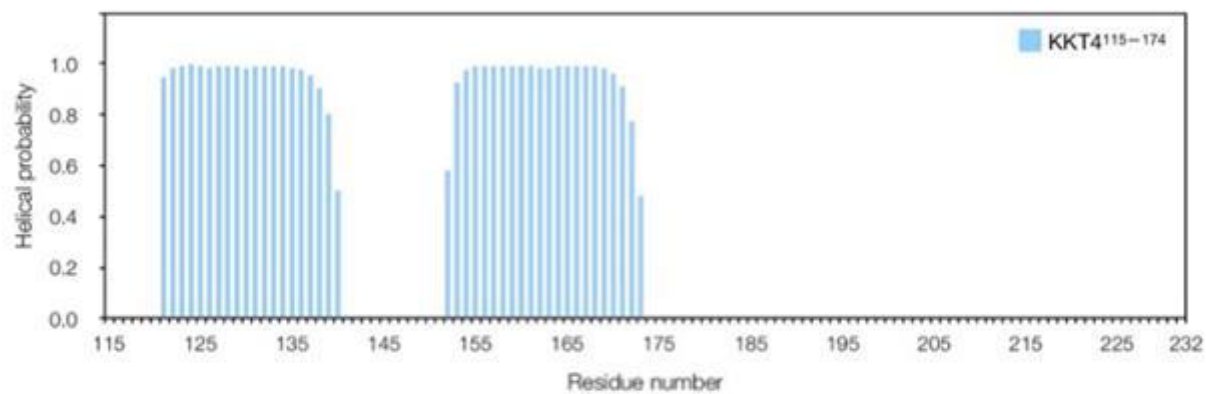
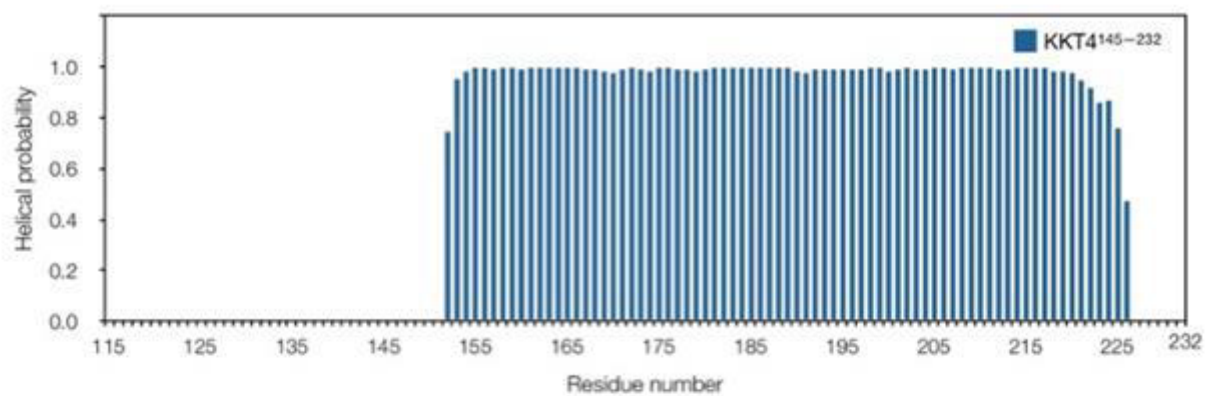
NMR measurements including residual dipolar couplings and (1H-15N) heteronuclear NOE and used in combination with chemical shift analysis and modelling to understand the structure and dynamics of kinetochore proteins in solution.

Results

KKT4 has been identified as the first microtubule-binding kinetochore protein in *T. brucei*. Using microtubule co-sedimentation assays, the KKT4 115–343 region has been identified as the microtubule-binding domain in *T. brucei*. We have shown that this domain consists of a coiled-coil structure followed by a positively charged disordered tail. Interestingly, KKT4 has a putative BRCA1 (BRCT) domain at its C-terminus (KKT4 463-645), with phospho-peptide binding affinity; this type of domain is not present in any known kinetochore protein in other eukaryotes. KKT23 is predicted to have a helical N-terminal domain (KKT23 2-70), a disordered central domain and a C-terminal domain with a Gcn5-related acetyltransferase (GNAT) domain (KKT23 125–348). Interestingly, none of the known structural kinetochore proteins in other eukaryotes has been found to have a GNAT domain.

Conclusions

NMR spectroscopy has already provided important insights into the structure, dynamics and interactions of both the structured and disordered domains within KKT4 and KKT23 and promises to be an important tool for future characterization of other KKT proteins from *T. brucei*.



P-148 - EPR with cryogenic amplifiers independent of sample temperature

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Inspired by the success of NMR cryoprobes, we recently reported a leap in X-band EPR sensitivity by equipping an ordinary EPR probehead with a cryogenic low-noise microwave amplifier placed close to the sample in the same cryostat [1]. Here, we explore, theoretically and experimentally, a more general approach, where the amplifier temperature is independent of the sample temperature. This approach brings a number of important advantages, enabling sensitivity improvement irrespective of sample temperature, as well as making it more practical to combine with ENDOR and Q-band resonators, where space in the sample cryostat is often limited. Our experimental realisation places the cryogenic preamplifier within an external closed-cycle cryostat, and we show CW and pulsed EPR and ENDOR sensitivity improvements at both X- and Q-bands with negligible dependence on sample temperature. The cryoprobe delivers signal-to-noise ratio enhancements that reduce the equivalent pulsed EPR measurement time by 16x at X-band and close to 5x at Q-band. Using the theoretical framework we discuss further improvements of this approach which could be used to achieve even greater sensitivity [2].

The presented research was supported by funding from the European Union HORIZON-MSCA-2021-PF-01 Marie Skłodowska-Curie Fellowship (Project ID: 101064200), and by the Research Council of Lithuania (LMTLT) (agreement No. S-LLT-23-1).

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P-106 - Structural biology-based targeting of viral and human macrodomains

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Macro domains (MDs) constitute a structural family of modules with a distinctive $\beta\beta\beta\beta$ sandwich fold, found in all kingdoms of life and viruses as standalone entities or parts of large proteins. Despite their architectural similarities, they display functional variations, and their role as "readers" and/or "erasers" is indicative of their significance to the ADP-ribosylation process. In viruses, MDs are parts of large non-structural proteins. Recently, their biological function, linked to their de-MARylation capacity and thus to the inhibition of PARP-mediated antiviral activity, has rendered them potential drug targets. MacroPARPs refers to PARP9, PARP14, and PARP15 due to the tandem MDs that are embedded in their sequence, which are responsible for macroPARPs subcellular localization and their interaction with other cellular components.

Herein, we present a comparative study of viral and human macroPARPs MDs with the objective of characterizing the structure, the dynamic properties, and the biochemical characteristics of previously unexplored members. Also, we aim to unravel subtle differences that can lead to the discovery and development of selective binders through targeted drug design, overcoming the sequence identity challenge between the MD family members. Using in silico screening, NMR-driven approaches, biophysical techniques, and biochemical assays, we discovered GS-441524 as a de-MARylation inhibitor that binds selectively to the SARS-CoV-2 MD in comparison to the other viral MDs and elucidated sequence elements that modify the binding affinity. Furthermore, our research expanded to the quest for small molecules as MDs' ligands, spanning a wide range of organic scaffolds, with the aim of identifying new compounds as MDs' inhibitors.

We acknowledge support the project "INSPIRED" (MIS 5002550), under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), and by the European Union's HE programs (2021-2027), "REMOTE-NMR" (GA 101058595) and "ESPERANCE" ERA Chair (GA 101087215).

P-104 - Structural investigation of DTX3L domains

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Human Deltex (DTX) proteins belong to the family of RING-type E3 ligases. The DTX family is comprised of five members, which are characterized by a C-terminal region containing a RING and a Deltex C-terminus (DTC) domain. The latter is a feature that groups DTX proteins into two evolutionary clades, with DTX1, 2, and 4 belonging to the first and DTX3 and Deltex3-like (DTX3L) to the second. The RING domain is responsible for mediating the interaction with E2 enzymes and the subsequent transfer of ubiquitin to the target proteins. Regarding their N-termini, DTX1, 2, and 4 contain WWE domains, that facilitate the interaction with PARylated substrates. Instead, at the respective region of DTX3L are found two domains called D1 and D2, responsible for oligomerization of the protein, while the proline-rich central region of DTXs is replaced by a distinctive D3 domain, which is the interaction region with its intracellular partner PARP9. Taken all the above into consideration, DTX3L is the most divergent member of the DTX family. There is only one available experimental structure submitted in PDB concerning its DTC domain.

Herein we present the preliminary structural and dynamical characterization of human DTX3L polypeptides through high-resolution NMR spectroscopy at the atomic level. The results of our study are important for the further biochemical characterization of this molecule, since it provides the basis to map and define the crucial domain regions and residues for interaction with PARP9. Note that these data can contribute to the design of molecules that can alter the specific complex formation.

We acknowledge support the project "INSPIRED" (MIS 5002550), under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020), and by the European Union's HE programs (2021-2027), "REMOTE-NMR" (GA 101058595) and "ESPERANCE" ERA Chair (GA 101087215).

P-320 - Fluorine NMR, a tool to characterize and quantify per and poly fluorinated products.

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Fluorine is widely used in industrial and consumer products, such as Teflon, refrigerants, and firefighting foams. It is also present in a large number of active molecules used in phytosanitary products or in the pharmacopoeia. This widespread use of fluorine has also led to a significant environmental problem due to the persistence of per- and polyfluoroalkyl substances (PFAS) and other fluorinated compounds in the environment. These persistent pollutants are known to have harmful effects on human health and the ecosystem.

Aims:

This paper aims to explore the possibilities of using ¹⁹F NMR spectroscopy as a general tool for structural and quantitative analysis of fluorinated compounds.

Methods:

Fluorine NMR spectroscopy is highly sensitive, making it a powerful tool for the analysis of fluorinated compounds and polluted samples. The experimental conditions and classical pulse sequences are adapted to tackle the peculiarities of ¹⁹F NMR spectroscopy, such as wide spectral widths. Classical experiments including 1D and 2D homonuclear and heteronuclear experiments are explored.

Results:

The sensitivity of ¹⁹F NMR spectroscopy is explored with several examples of LoD on the order of ng/g for compound concentrations below 1.0 μM.

The perfluoro compounds present a special challenge due to their very wide spectral widths, large homonuclear and heteronuclear J-couplings, and strong ¹⁹F-¹⁹F couplings. Their spectra are analysed, and the strong coupling artefacts interpreted, demonstrating its usefulness in characterizing and identifying fluorinated compounds.

Conclusions:

In conclusion, ¹⁹F NMR spectroscopy is a powerful technique for the analysis of fluorinated compounds. It can be used for quality control or to analyze environmental samples in detail. The ability to detect and quantify fluorinated compounds is critical for understanding their impact on human health and the environment. The use of ¹⁹F NMR spectroscopy can aid in the development of effective remediation strategies for these persistent pollutants.

P-276 - Magnetic Resonance Fingerprinting for spinal cord T1 and T2 mapping

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Magnetic Resonance Fingerprinting (MRF) simultaneously measures multiple tissue properties through a more time-efficient acquisition routine than standard mapping techniques (Ma et al. 2013). The MRF key idea is to apply a train of radiofrequency pulses varying in a pseudo-random way some MR acquisition parameters, e.g. flip-angle and repetition-time, to provide unique fingerprint-like signal evolutions for combinations of desired tissue properties. In recent years, many applications of MRF to different anatomical districts have been proposed, but the extension to the spinal region is still an open topic both in the preclinical and clinical fields.

Aims

We propose a preclinical MRF method to acquire and reconstruct T_1 and T_2 maps of an ex-vivo spinal cord rat phantom. We specifically optimize the MRF acquisition scheme for the spinal cord anatomical region.

Methods

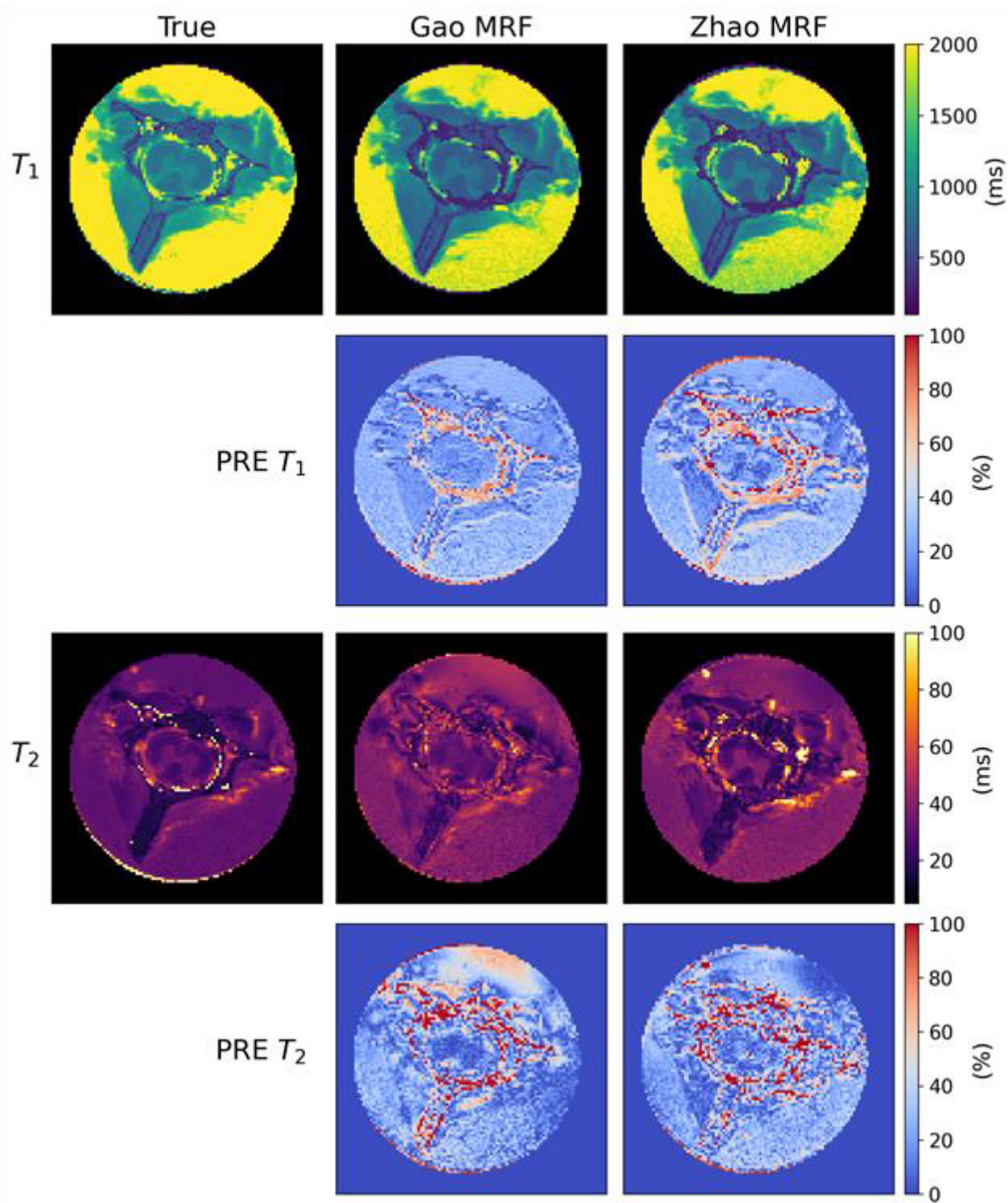
To obtain the relaxation times maps, we have implemented the Gao (Gao et al. 2015) and Zhao (Zhao et al. 2018) MRF-FISP routines and the reference T_1 and T_2 mapping sequences (Inversion-Recovery and Spin-Echo) on a 7T Bruker Scanner (Acquisition-Matrix=128x128, pixel-size=0.16x0.16mm²). Gao and Zhao dictionaries are generated with the Extended-Phase-Graph formalism (Weigel 2015).

Results

The results show that the T_1 and T_2 maps of both MRF sequences have correctly reconstructed the anatomical structures of the spinal cord, preserving the visual contrast between tissues. The mean percentage relative error (PRE) between the MRF maps and the reference ones is equal for T_1 to 21%±11% for the Gao dataset and 21%±19% for the Zhao dataset and for T_2 to 16%±17% for the Gao dataset and to 20%±19% for the Zhao dataset, Figure1.

Conclusion

We propose a MRF framework for the acquisition of parametric maps of the rat spinal cord. In the upcoming months, we plan to apply spinal cord mapping in preclinical models of neurodegeneration, e.g. Amyotrophic Lateral Sclerosis.



P-114 - Characterisation of the alpha-1-antitrypsin pathological polymer by solution-state NMR spectroscopy

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

The glycoprotein alpha1-antitrypsin (AAT) is a 52 kDa serine protease inhibitor found at high concentrations in human plasma. The Z mutation (E342K) occurs in 1 in 1700 Northern Europeans and promotes ordered aggregation ('polymerisation') leading to liver cirrhosis and early-onset emphysema. Solution NMR investigations of the monomeric states of the wild-type and Z variants, using 2D ¹H,¹³C experiments at natural abundance on AAT samples purified from patient donors have allowed us to probe structural and dynamic features at the earliest stages of misfolding and polymerisation (Jagger, Nat Commun 2020). However, the structure of the polymer itself is currently unknown, yet critical to a full understanding of the polymerisation mechanism and application to ongoing drug development efforts.

Results

Polymerisation of isotopically enriched, methyl-labelled AAT was induced artificially at elevated temperature and followed in real-time by 1D ¹H NMR, translational diffusion and ¹H, ¹³C HMQC experiments. This analysis yielded the first high-resolution ¹H, ¹³C spectrum of AAT polymers. These spectra overlaid closely with a proteolytically cleaved, monomeric form of the protein, from which we could obtain and transfer methyl resonance assignments. As it is not certain that recombinant samples accurately recapitulate what is found within patients, we purified pathological polymers from liver explant for comparison. These were fractionated into varying polymer chain lengths for characterisation by ¹H NMR. Using a methyl-selective Ernst angle excitation to improve sensitivity, we compared the high-field methyl resonances of ex vivo polymers with the artificial polymer standards. Differences were found in the 1D spectra that suggest that the polymers may have different structures in solution.

Conclusions

Characterisation of isotopically labelled and natural abundance samples by methyl-viewed 1D and 2D NMR revealed points of commonality and difference between the samples. Further work is being completed on 2D CH solid state spectrum of the liver-derived AAT polymers at natural isotopic abundance.

P-294 - Investigating the binding properties of minor groove binders to an oligonucleotide nucleic acid duplex

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

1D ¹H-NMR and 1D ³¹P-NMR spectra are used for new drug discovery to find the interaction between target and ligand. The complex of drug-target can give spectrum from itself, and it depends on the mechanism of binding and saturated concentration of the stable complex. To show the physicochemical changes on complex, one-dimensional proton and phosphorus NMR spectrum can be employed as the earlier sight for drug-target interaction. Additionally, these methods are effectively why they give quick and precise data about the binding complex, compared with the other approaches (1). Minor groove binders (MGBs) are potential drug-target for anti-infective and anti-cancer therapy (2, 3, 4).

Aims

MGBs are defined that bind DNA minor grooves to inhibit DNA processing enzymes and affect DNA vital functionality. In this study, N-(3-(dimethylamino)propyl)-5-isopropyl-2-(1-methyl-4-(1-methyl-4-(1-methyl-1H-imidazole-2-carboxamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)thiazole-4-carboxamide compound, which was synthesized based on distamycin (5), aimed to bind short oligonucleotides and provide a drug-target complex in concentration gradient.

Methods

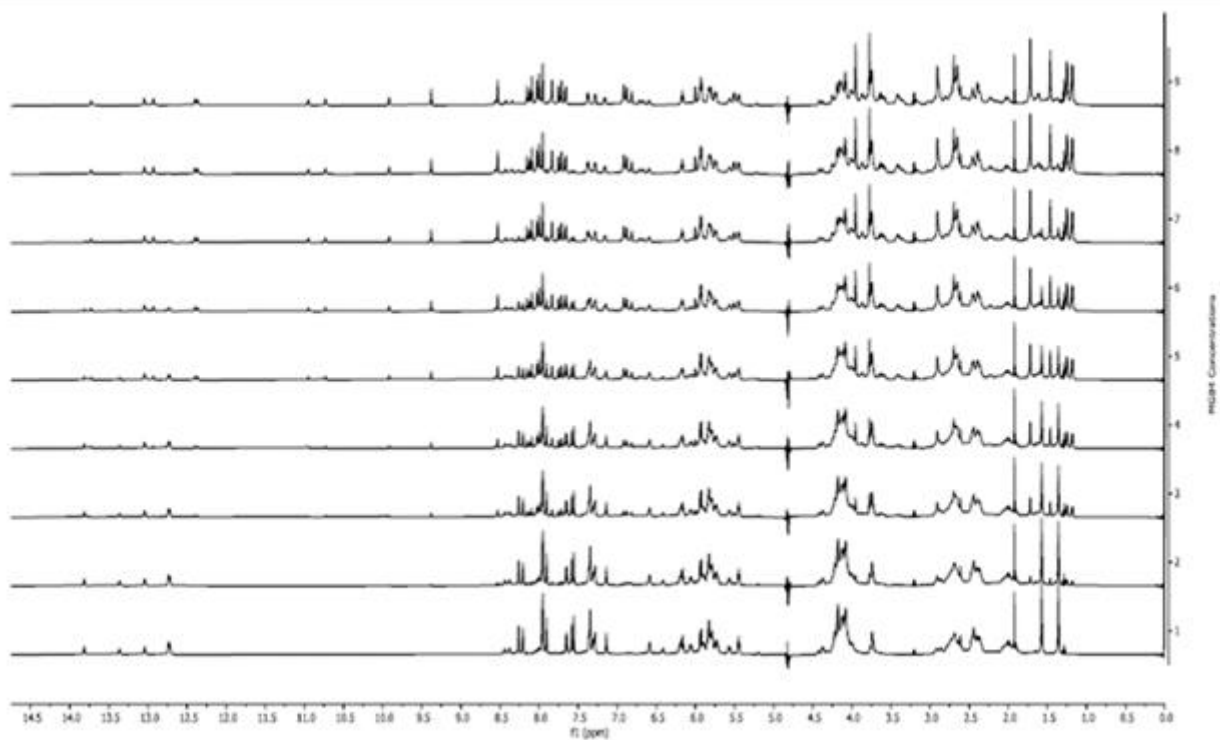
1D ¹H-NMR and 1D ³¹P-NMR datasets used to check the changes inter- and intrabase bounds and phosphorus backbone of the oligonucleotide. This MGB candidate compound was interacted with a 12-bp oligonucleotide (5' – GCGACTAGTCGC – 3') that comprises a model of DNA for ligand. The ligand-target complex was investigated on each 38 µg/5 µL using the same parameters on 600 MHz NMR instrument.

Results

As the result, the 1D ¹H-NMR titration graphs indicated the final concentration of the ligand to comprise the saturated complex is needed to 1.045 mg of MGB ligand. Additionally, 1D ³¹P-NMR peaks were obviously transformed wider form than the baseline spectrum of oligonucleotide.

Conclusion

In conclusion, as the signal shifting and altering signed, MGB ligand has bind to target oligonucleotide. Using the final concentration data from titration study, next 2D spectrum investigations will be made in the same complex.



P-170 - Helium Recovery in NMR Laboratories

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Helium is found in the earth's crust and it can be extracted and purified usually as a by-product of natural gas extraction. Helium, amongst other applications, is commonly used as an extremely efficient cooling agent in superconducting magnets when in its liquid state. However, being the second lightest element, most of the helium currently used is eventually lost to the atmosphere if not captured. This makes it a non-renewable resource and some estimates suggest that global reserves could run out around 2070.

NMR superconducting magnets use liquid helium to cool superconducting coils in order to keep the magnets on field. This helium is steadily lost through boil-off and has to be refilled on a regular basis. Helium losses are significantly increased during helium refills. Helium recovery systems can either capture just the boil-off, or also the helium evaporated during refills. In these systems, helium is delivered through copper pipes to buffer gas bags or metal containers, and subsequently compressed to medium or high pressure for storage in gas cylinders. Compressed helium gas can be either purified and liquefied locally, or sold back to a national supplier (e.g. BOC) for re-liquefaction. Recent natural gas shortages have seen the price of helium rocketing, which has a significant effect on the research cost. The helium recovery not only improves cash flow, but – most importantly – sustainably helps to keep helium in circulation.

In this poster we will demonstrate and discuss an implementation of a helium recovery system in the NMR facility of the School of Chemistry at the University of Edinburgh.

P-326 - Shedding light on dearomatization of transformable electron-rich arenes with LED-NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

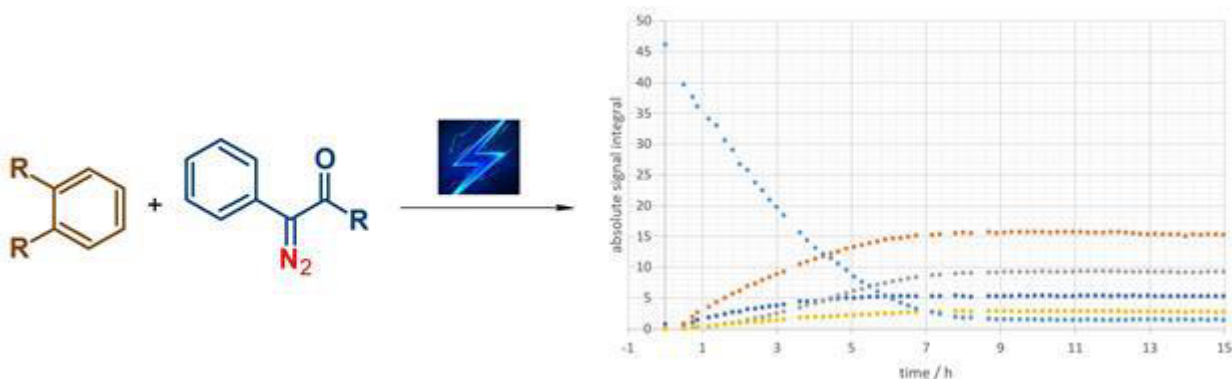
Organic photochemistry underwent a revival in last decade consequentially creating a demand for quick, non-destructive and non-invasive analytical methodology that would simplify study of photoreactions. In situ LED-NMR meets these criteria by exploiting the insensitivity of optical fibres to strong magnetic fields and introducing a light source directly into the centre of the magnet. This allows the structural characterization of reactive short-lived chemical species as well as monitoring of photoreaction kinetics, ultimately leading to the understanding of photoreaction mechanism.

Aim: to establish LED-NMR setup for in situ monitoring of photochemical dearomatization of electron-rich arenes bearing transformable functional groups.

Visible light-mediated dearomatization of electron-rich arenes represents a complementary method to traditional strategies leading to 1,3-cyclohexadienes (norcaradienes). Initially, we identified all reaction components for subsequent in situ ¹H-LED-NMR analysis. Then, a set of specifically designed experiments were performed with aim to: i) determine reaction kinetics, ii) track any background reactions and iii) get insight into reaction mechanism. In addition, these experiments served as basis for comparative analysis of previously reported data in similar transformations.

Five different products of the photoreaction were identified and their structures elucidated. The LED-NMR experiments results shed light onto effectiveness of the analysed dearomatization reaction: i) the consumption of the starting materials and the formation of all products occurred simultaneously, ii) the products were formed exclusively under the blue light irradiation, and iii) the product of dearomatization is more reactive than the arene from which it was obtained. Moreover, the presence of electron-rich groups accelerates subsequent cyclopropanation of the formed norcaradiene.

We have successfully established a reliable protocol for in situ monitoring of photochemical dearomatization of electron-rich arenes using LED-NMR technique. Besides synthetic utility of the formed products in architecting highly complex structures, synergistic integration of the LED-NMR gave unprecedented insight into reactivity of the dearomatization products.



P-352 - Design and application of lanthanoid chelating tags for pseudocontact chemical shift nuclear magnetic resonance spectroscopy on ribonucleic acids

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The understanding of the biological function of ribonucleic acids (RNA) is highly important for future drug development and research. Hence, the investigation of structure, dynamics, interactions and kinetics of biologically relevant RNA molecules is required. Pseudocontact shifts (PCS) in nuclear magnetic resonance (NMR) spectroscopy offer a valuable tool to gain information about functional dynamics of a wide range of biomolecules by attaching lanthanoid chelating tags (LCTs). Numerous LCTs are known for measuring PCSs in proteins. The design of new LCTs applicable for PCS measurements in RNA is greatly desirable and opens new opportunities for structural characterization of RNA molecules. Aim of this research work is a synthetic access to new LCTs suitable for lanthanoid chelate tagging of modified RNA building blocks as well as their application for PCS NMR spectroscopy in RNA. We will present the synthetic approach to new LCTs and a proof of concept solution NMR study. The new LCTs generate sizeable PCSs and deliver precise structural restraints in RNA. They can, therefore, provide information about the structure and function of RNA and RNA protein complexes and hence offer important data access, e.g. for drug screening processes.

P-194 - Hyperpolarization Transfer Experiments for Liquid-state Overhauser Dynamic Nuclear Polarization

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Liquid-state Overhauser DNP at high fields is emerging as viable tool to enhance ¹³C NMR spectra in liquids,[1],[2] however DNP enhancements are not uniform over the molecule. In particular, non-protonated sp and sp² hybridized carbons, as well as hydrogens, typically show a decrease in signal intensity under DNP conditions, due to an inefficient scalar interaction with the radical polarizing agent. Current research is thus aimed at the development of NMR experiments that allow transfer of (hyper)polarization from those nuclei in a molecule that experience large DNP enhancements, to those experiencing little enhancement or even signal attenuation. Pulse sequences that transfer polarization via different mechanisms (i.e., INEPT, NOE, and isotropic mixing-based) are being investigated. From a practical application point-of-view, indirect enhancements of one order of magnitude can now be achieved in both heteronuclear and homonuclear polarization transfer experiments, compared to routine 1D NMR experiments, corresponding to a two order of magnitude reduction in experiment time. Moreover, we found that, at natural ¹³C abundance, a 1D isotropic mixing experiment under DNP conditions allows one-bond carbon–carbon scalar coupling constants (1JCC) to be resolved in only a tenth of the time, and requiring only one tenth of the amount of material, compared to a conventional 1D INADEQUATE experiment at thermal Boltzmann population.

P-076 - RNA chaperoning in RocC-RocR studied by paramagnetic relaxation

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

The 14 kDa protein RocC is an RNA chaperone, binding the small RNA (sRNA) RocR, which is involved in the regulation of bacterial competence. RNA binding is facilitated by a FinO domain in RocC (24 - 126), and additionally by the N-terminal region (1 - 24) that is suspected to be crucial for chaperoning. However, the exact molecular mechanism of the chaperoning process is so far unknown.

Methods

To probe the interaction between the N-terminal region of RocC with the target RNA RocR, paramagnetic relaxation enhancement (PRE) measurements are employed. For this purpose, a paramagnetic tag is located at the N-terminus of RocC in order to shed light on whether or not this region participates in RNA binding. Mutagenesis is used in combination with MTSL tagging. Complementary experiments are performed using paramagnetic labelling of the 5'-end (TEMPO) of the RNA. Methyl-TROSY and backbone amide TROSY experiments are used for detection in the 35 kDa RocC-RocR protein-RNA complex.

Results

Paramagnetic relaxation data, with the appropriate TEMPO tag located on the RNA side have shown significant PRE effects on the methyl groups of RocC. In addition, a limited number of backbone amide signals in RocC also display PRE effects. These signals are suspected to correspond to the N-terminal region of RocC, for which resonance assignments are currently being attempted. First results regarding the paramagnetic labelled N-terminus of the chaperone will be presented.

Conclusion

The experimental data are indicative for a direct involvement of the N-terminus of the chaperone protein. In the complex, the N-terminal region of the chaperone RocC appears to directly interact with the part of RocR that is known to be involved in the regulation of bacterial competence. This mechanistic picture is reminiscent of the flyfishing mechanism of chaperoning that has been described in literature.

P-248 - Structure and Surface Properties of Size-tuneable CsPbBr₃ Nanocrystals

Dr Thomas Hooper¹

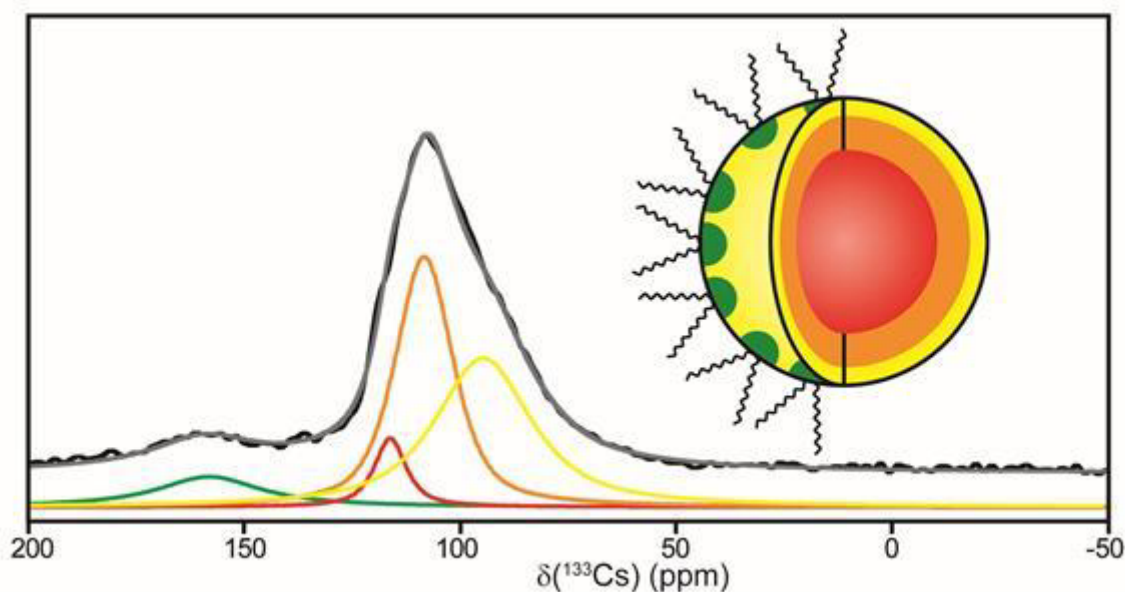
¹KU Leuven, Leuven, Belgium

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Cesium lead halide perovskite nanocrystals (NCs) have exhibited excellent light-emitting properties such as near unity photoluminescence quantum yields and extremely narrow emission bandwidths, while demonstrating greater environmental stability than its organic-inorganic lead perovskite competitors. Light emitting NCs with dimensions smaller than their exciton Bohr radii (5 – 12 nm in CsPbX₃) benefit from quantum confinement effects, resulting in higher energy emission in smaller nanocrystals, providing a controlling mechanism for the emission wavelength. Characterisation of these nano-materials is key, as structural variations (i.e. defects and lattice terminations) can play a significant role in their photoluminescent efficiencies and environmental stabilities.

This investigation has characterised the structure and surface chemistry of CsPbBr₃ nanocrystals with controlled diameters between 6.4 to 12.8 nm. The nanocrystals were investigated via a thorough ¹³³Cs solid state NMR and nuclear relaxation study, identifying and mapping radially-increasing nanoscale disorder. This work has formalised ¹³³Cs NMR as a highly sensitive probe of nanocrystal size, which can conveniently analyse nanocrystals in solid forms, as they would be utilized in optoelectronic devices.

A combined multinuclear solid state NMR and XPS approach, including ¹³³Cs-¹H heteronuclear correlation 2D (HETCOR) NMR, was utilised to study the nanocrystal surface and ligands, demonstrating that the surface is Cs-Br rich with vacancies passivated by didodecyldimethylammonium bromide (DDAB) ligands. Furthermore, it is shown that a negligible amount of phosphonate ligands remain on the powder nanocrystal surface, despite the key role of octylphosphonic acid (OPA) in controlling the colloidal nanocrystal growth. The CsPbBr₃ NCs were shown to be structurally stable under ambient conditions for up to 6 months, albeit with some particle agglomeration.



P-014 - Paws for thought: The use of benchtop NMR for veterinary diagnostics of cerebrospinal fluid

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Cerebrospinal fluid (CSF) analysis is routinely used for identifying and monitoring a variety of neurological disorders such as meningitis, encephalitis, and spinal cord injuries. However, traditional CSF diagnostic methods such as cytology and immunology can be time-consuming, costly, and require specialised equipment. In addition, these methods can lack in their diagnostic ability to classify diseases, potentially leading to misdiagnosis and suboptimal treatment outcomes. Benchtop NMR (bNMR) has the potential to address these issues by providing a rapid, cost effective, and accurate diagnostic tool for CSF analysis.

This study aims to compare the performance of a 60MHz bNMR spectrometer and an 800MHz high field NMR (HF-NMR) spectrometer for the identification and quantification of biomolecules in CSF samples, as well as comparing results achieved with existing clinical technology. Additionally, machine learning (ML) algorithms will be applied to find if bNMR and/or HF-NMR data can help with clinical diagnoses.

CSF samples were obtained from 200+ canines presenting with various neurological disorders. Biomolecules in the CSF samples were identified and quantified using a 60MHz Oxford Instrument bNMR spectrometer and an 800MHz Bruker HF-NMR spectrometer. ML algorithms were applied to the data to visualise the clustering of clinical diagnoses with the NMR results.

The results show that both 60MHz bNMR and 800MHz HF-NMR spectroscopy are effective in identifying and quantifying biomolecules in CSF samples, such as isopropanol, glutamine, and valine. The applied ML algorithms display clustering of diagnoses to help support the clinical findings. The effect of factors such as breed and age on spectra are also presented.

The study demonstrates the potential of bNMR spectroscopy as a rapid and cost-effective diagnostic method for CSF analysis in veterinary medicine. By providing accurate and timely diagnoses, bNMR could help improve the management of neurological disorders in animals.

P-290 - Mechanical insulation and symmetry-breaking in spin-1/2 pairs.

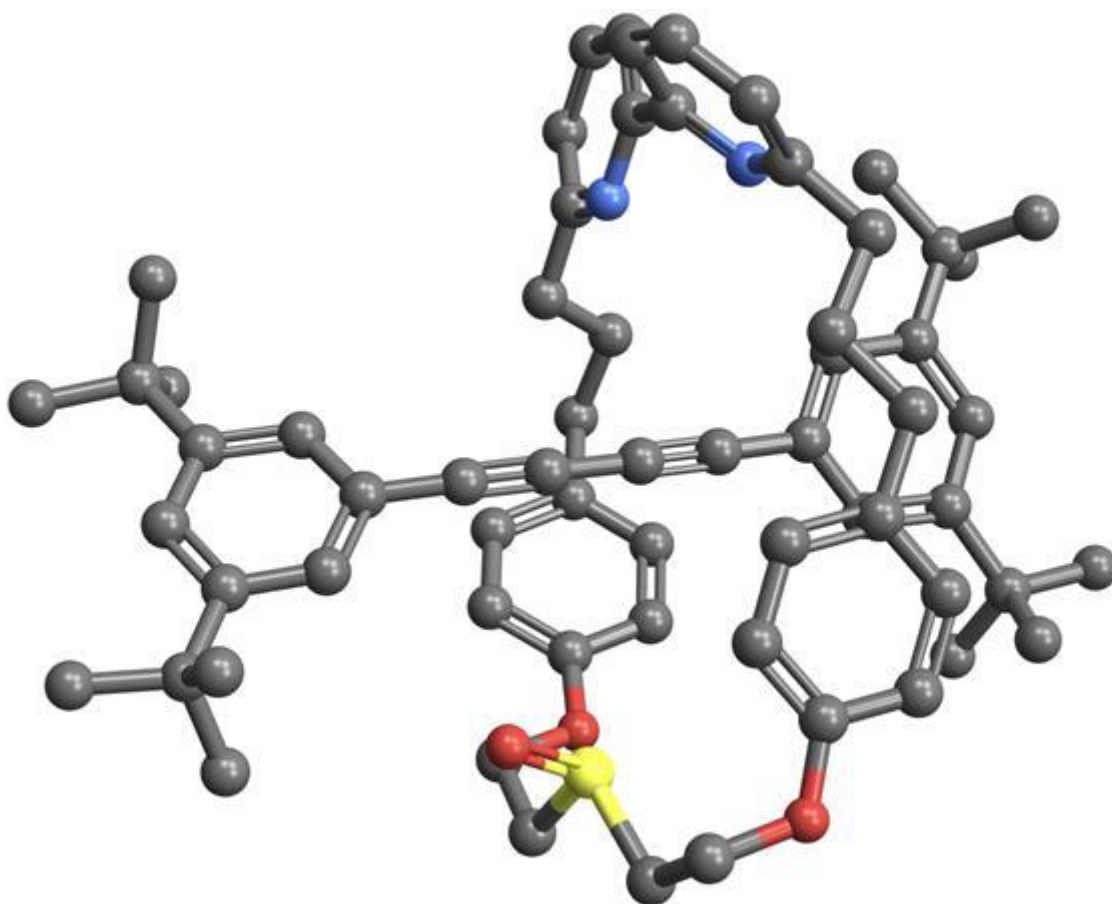
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Singlet-order (SO) is subject to relaxation by various mechanisms; one of which is relaxation by paramagnetic agents, such as dissolved oxygen in solution NMR [1]. One option to protect SO from these agents is to offer mechanical insulation [2]. Here, the spin-1/2 nuclei are hosted by a rotaxane; a linear molecule surrounded by a large ring structure [3]. We show that SO is protected from paramagnetic agents by the ring structure by considering two examples of rotaxane complexes. Further, a symmetry-breaking mechanism is required to access SO. In this case, the linear molecule is completely symmetrical, and the magnetic equivalence is broken by the ring structure. The ratio of T_s/T_1 is approximately 10-fold, and these results are compared to a bare linear molecule in which the symmetry is broken by different constituents in close proximity with the spin-1/2 pair. The unsymmetrical linear molecule shows a much greater T_s/T_1 of approximately 90-fold, attributed to the abundance of protons on the ring-structure of the rotaxane, producing fluctuating fields in the vicinity of the spin-1/2 pair.

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P-246 - Dynamics of adsorbed CO₂ related to ligand rotation in a metal-organic framework

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Metal-organic frameworks (MOFs) are porous solid materials composed of metal cations and bridging ligands and are attractive candidates for CO₂ capture and separation. Understanding the adsorption and diffusion dynamics of CO₂ in MOF pores is essential to improving the separation performance and utilization in the industrial process. In this work, we examined the dynamics of adsorbed CO₂ in narrow pores of a MOF possessing rotational ligands using solid-state NMR. To perform NMR measurements under 0-1 MPa CO₂, we used a homemade MAS NMR rotor having a screw cap with O-rings and a gas-loading chamber to seal ¹³C-enriched CO₂ gas into the rotor. Analysis of ¹³C CSA sideband patterns of adsorbed CO₂ showed that the tilting angle of the local wobbling motion of adsorbed CO₂ became smaller with increasing CO₂ pressure, i.e., with an increase in the amount of adsorbed CO₂. This result indicates that CO₂ mobility is restricted by steric hindrance of adjacent CO₂ and the ligands in the narrow pore. Line-shape simulation analysis of ²H quadrupolar-echo spectra revealed that the rotational rate of the ligands also reduced with increasing pressure. To examine the influence of the steric hindrance of the mobile ligands on CO₂ diffusion in the narrow pore, we observed the exchange between adsorbed CO₂ and gaseous CO₂ outside the MOF crystalline particles, which is accompanied by the CO₂ diffusion in the pore, by ¹³C MAS NMR. The diffusion rate estimated from the exchange rate concurrently decreased with the mobility of the rotational ligand at high pressure, which suggests the effect of the ligand dynamics on CO₂ diffusion. The results of our study propose that the diffusion rate of gas molecules could be controlled by the mobile steric hindrance of the rotating ligands, which would enhance the separation efficiency of the gas permeation method.

P-138 - Chirality-sensitive coherences induced by the perturbation of the interference between dipolar relaxation mechanisms by an electric field

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Although NMR provides much information about the structure of molecules, it does not allow us to determine chirality without creating (pseudo)diastereoisomers. In particular, no effect is known to determine the absolute configuration of a molecule directly. However, this statement may no longer be valid if one considers a perturbation of three spin dynamics by an external electric field in a liquid sample containing chiral molecules possessing permanent electric dipole moments. In this case, an interference exists between (i) dipolar interaction between spins I1 and I3, (ii) dipolar interaction between spins I1 and I2 perturbed by the partial alignment due to the electric field.

The coherences induced by the electric field are located in a sub-block of the relaxation matrix containing spins states $(I1xI2y-I1yI2x)I3z$. Consequently, the chirality-sensitive signals may be excited by an electric field, oriented as the magnetic field, and oscillating at the differential frequency of spins I1 and I2. The induced coherence phase depends entirely on the relative orientation of the triangle, whose apexes are spins I1, I2, and I3, relative to the molecular electric moment. Therefore, the effect allows determining the absolute configuration of a chiral molecule without the need for alternating the sample structure.

An estimation of the effect magnitude gives the chirality-sensitive signal about 1% of the amplitude of the unperturbed dipole-dipole cross-correlation by the electric field, assuming that the dipolar relaxation time of each pair of nuclei is one second, the electric field is 5 kV/mm, and the molecule has the permanent electric dipole moment of two debyes. Thus, the expected signal is a fraction of the standard cross-correlated dipolar relaxation, and it would presumably be measurable by a sensitive NMR detector.

PG acknowledges the National Science Centre, Poland, for the financial support through OPUS 16 Grant No. 2018/31/B/ST4/02570.

P-244 - Using NMR Crystallography to probe structure in chemically-reduced organic anode materials for Lithium-Ion Batteries

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

As demand for lithium-ion batteries increases, so too does the demand for all of the components within, including the graphite anode. Graphite mining and refinement are associated with high energy requirements and significant environmental, ethical and geopolitical concerns. Organic anode materials could offer a more sustainable alternative to graphite anodes, with their single step synthetic process and abundant elemental makeup. Despite their respectable electrochemical performance,[1] questions remain regarding the structure of these materials when they are reduced to their charged phase.

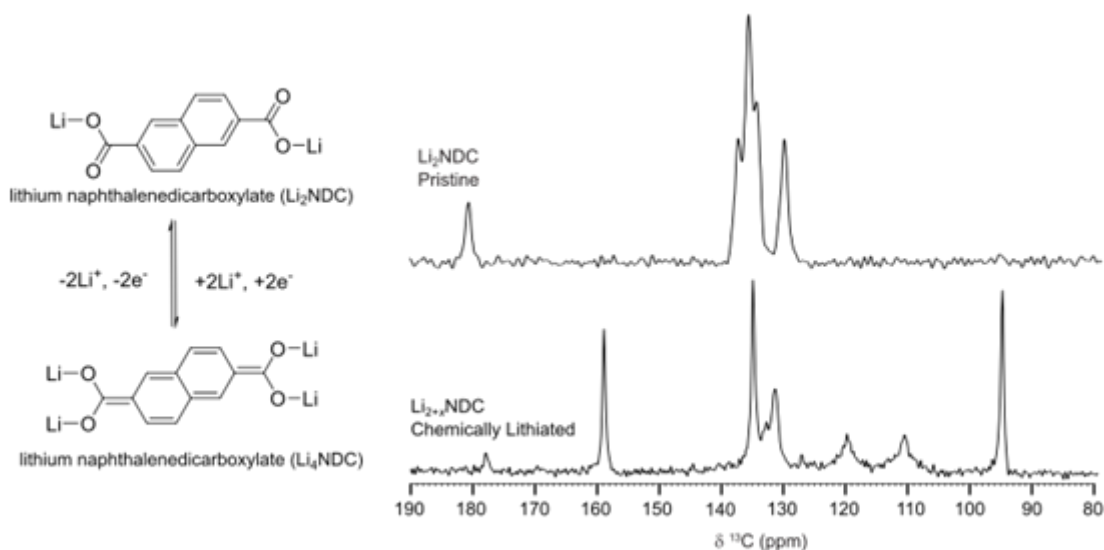
Aims and Methods:

While the pristine phases are now much more understood,[2] it is still not understood where in the reduced phases the additional lithium ions are located, nor how this process proceeds.

Electrochemical charging presents challenges to the study of organic anode materials as the additives required for successful cycling can complicate sample recovery, dilute sample volume and mask signal in NMR experiments. A chemical lithiation method circumvents these challenges and allows the charged phases to be studied using powder X-ray diffraction (PXRD) and solid-state NMR spectroscopy.[3] Together, and in conjunction with DFT calculations on candidate structures for the lithiated phases, we are able to gain valuable insights into the structures of the charged phases of organic anode materials.

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P-224 - Iron oxide-based MNPs: shape and size effect on their ^1H -NMR relaxation properties

Miss Margherita Porru

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: Iron oxide-based magnetic nanoparticles (MNPs) are exploited as contrast agents for MRI: their magnetic nature shortens the nuclear relaxation times ($T_{1,2}$) of the hydrogen nuclei, enhancing the image contrast according to their bio-distribution. The nuclear relaxation mechanisms are correlated to the particles' shape and size, on which this study is based.

Aims: The purpose of this work is to understand how fundamental microscopic characteristics, such as shape and size, influence MNPs' magnetic and relaxometric properties, to tailor them to the selected field of application.

Methods: This study is focused on three differently shaped sets of MNPs: nanospheres, nanoflowers, and nanocubes, with different sizes (from 6 to 35 nm), and coated with different biocompatible molecules (DMSA, PAA, and CM-dextran).

The samples are morpho-structurally and magnetically characterized by TEM, XRD, IR, TG and VSM analysis.

The relaxation properties of the colloidal MNPs solution are investigated by ^1H -NMR measurements of the longitudinal (T_1) and transverse (T_2) nuclear relaxation times as a function of frequency. The relaxivity ($r_{1,2}$) vs frequency (i.e., static magnetic field), determines the MNPs' efficiency as MRI contrast agents. The frequency behaviour below 7.2 MHz is investigated utilizing the Fast-Field-Cycling (FFC) technique, which resorts to Pre-Polarized (PP) acquisition sequences (Saturation Recovery SR and Spin-Echo SE), while higher frequencies are explored with an electromagnet, through SR-SE and Carr-Purcell-Meiboom-Gill (CPMG) sequences.

Results: The morpho-structural and magnetic characterization confirmed the superparamagnetic nature of the cores and the presence of the coatings surrounding them. The shapes of the ^1H -NMRD profiles are explained in terms of the different relaxation mechanisms and associated spectral densities and are influenced sizeably by MNPs' shapes and sizes.

Conclusions: Different sizes and shapes lead to different spin dynamics depending on the frequency regime. Through the present study, we suggest some possible ways to tailor the investigated MNPs' microscopic characteristics for MRI uses.

P-254 - Local dynamics in triptycene-based porous polymers by multi-nuclear and multi-technique solid-state NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Polymers with intrinsic microporosity (PIMs) [1-3] have been assessed as promising materials for the development of energy efficient gas-separation membranes with high CO₂ permeability and selectivity [4], even if critical limitations, such as the trade-off between permeability and selectivity, plasticization, and physical aging, still need to be overcome [5]. The comprehension of the gas adsorption and transport mechanisms at the molecular level and of the complex structure-properties relationship is at the base of the development of materials with improved and optimized performances. To this end, solid-state NMR spectroscopy (ssNMR) can play an important role, enabling the study of structure and dynamics on wide ranges of spatial lengths and motion time scales [6].

Aims and Methods

In this work, ssNMR has been used to characterize the structure and dynamics of PIMs constituted by benzo-triptycene structural units and perfluorinated biphenyl linkers (Figure 1a). Selective information on the dynamics of t-butyl, triptycene and biphenyl moieties have been obtained by the combined analysis of ¹H, ¹⁹F and ¹³C T₁ relaxation times recorded at different Larmor frequencies and at variable temperature (Figure 1b). The analysis of ²H static spectra of a sample deuterated on the t-Butyl groups has also been performed.

Results and Conclusions

The presence of fast motions with characteristic frequencies in the MHz regime involving the perfluorinated biphenyl linkers and the t-Butyl groups has been detected, while the triptycene unit seems to be frozen in the investigated time scale.

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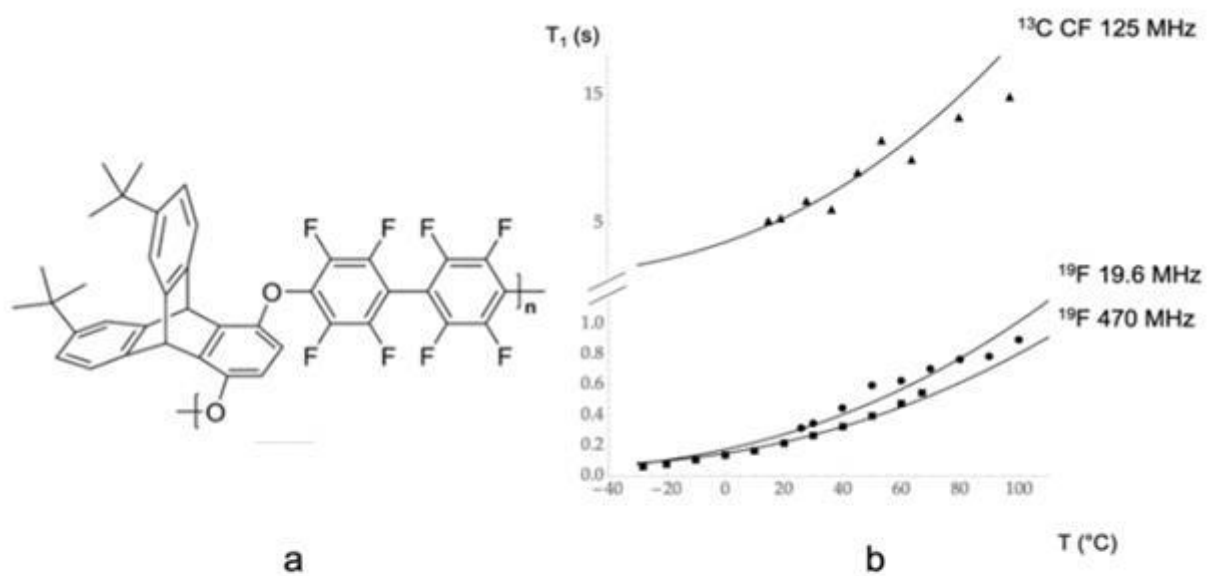


Figure 1 (a) Structure of the investigated PIM. (b) Analysis of the variable temperature ^{19}F and ^{13}C T_1 data.

P-312 - From weak to strong: an interplay between NMR, in-silico approaches and 3D structures in drug discovery

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The immense power of NMR is illustrated with two recent successful applications to our drug discovery portfolio. In the first example [1], we describe the rapid identification of potent binders for the WD40 repeat domain (WDR) of DCAF1. This was achieved by two rounds of iterative focused NMR screening of a small set of compounds selected based on in-house WDR domain knowledge followed by hit expansion. Subsequent structure-based design led to nM binders with a clear exit vector enabling DCAF1-based PROTAC exploration. In the second example [2,3,4], we describe the discovery of the first class of small molecules potentially inhibiting the YAP-TEAD interaction by binding at one of the main interaction sites of YAP at the surface of TEAD, providing a path forward to pharmacological intervention in the Hippo pathway. In both examples, hit to lead optimisation was carried out by an integrated approach, where in-silico methods, NMR, X-ray crystallography, computer-aided drug design and biochemical assays played a crucial role. As a result, potent, highly selective ligands for these two protein targets were developed that exert their desired mode of action at the cellular level.

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P-264 - Investigation of exhaled breath condensate by NMR based metabolomics

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Asthma is an umbrella term that encompasses various presentations and clinical characteristics, the complexity of asthma is manifested in its varied pheno- and endo-types¹, overlaid by circadian rhythms², which makes accurate diagnosis challenging, and a range of diagnostic methods need to be employed in the clinic. Exhaled breath condensate (EBC) is a biospecimen relevant to airways diseases³. Asthma is a complex disease well-suited to metabolomic profiling, both for the development of novel biomarkers and for the improved understanding of pathophysiology⁴. Here we investigate the use of NMR to identify the presence of possible metabolic biomarkers in EBC samples, for the potential diagnosis of asthma. The preliminary study involves collecting data on the variation between days for circadian rhythm. The longer term goal is to be able to diagnose and subclassify asthma patients from the NMR spectral profile of EBC samples, and secondary to identify the critical metabolites. This information can then be used to develop fast and inexpensive diagnosis tools. EBC sample are collected using RTubes, and subjected to NMR analysis. Experiments also include identification of pollutants from collection systems and air. ¹H-NMR spectra were obtained on 800MHz NMR spectrometer with cryoprobe using the 1D NOESY presaturation pulse sequence. Metabolites identification are based on 2D NMR experiments, databases, and published literature.

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P-256 - Deciphering the composite GlycA/B inflammation markers in NMR spectra of human blood

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

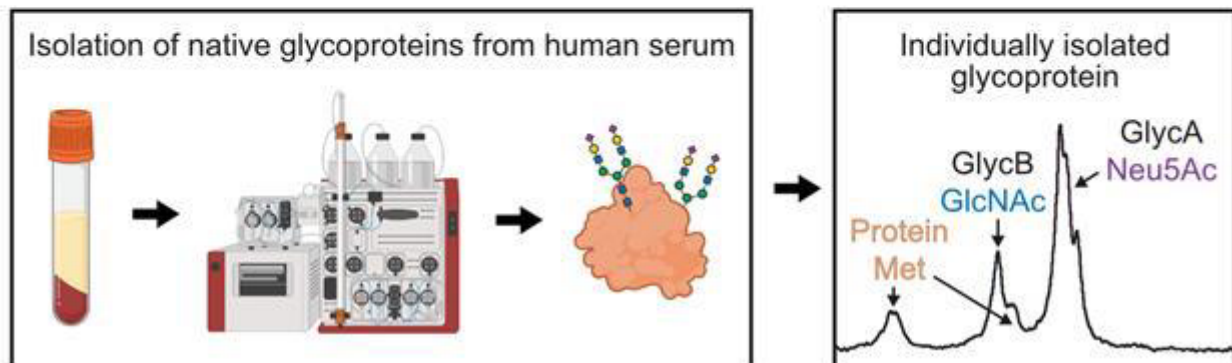
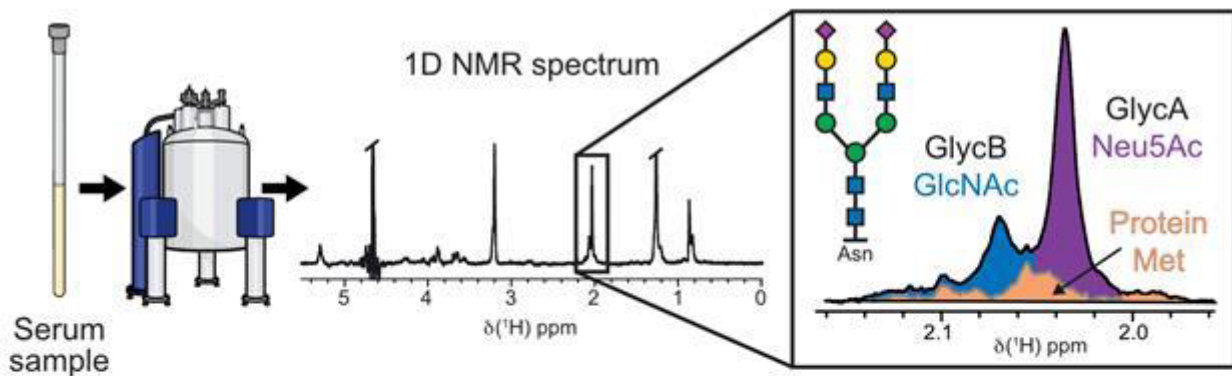
Introduction: Nuclear Magnetic Resonance (NMR) spectra of human serum and plasma reveal two intense glycoprotein glycan signals, known as GlycA and GlycB, originating from N-acetyl methyl groups of neuraminic acid and N-acetylglucosamine, respectively. The GlycA/B signals are considered biomarkers for various inflammatory conditions, including cardiovascular disease, rheumatoid arthritis, and COVID-19. α 1-acid glycoprotein, haptoglobin, α 1-antitrypsin, α 1-antichymotrypsin, and transferrin have been suggested as major contributors to these signals. Yet, the NMR characteristics of a larger number of individually isolated glycoproteins, including disease-related changes in glycosylation profiles, have not been studied in-depth.

Aims: Our goal is to decipher the GlycA/B NMR signals by identifying the contributing glycoproteins and their respective glycosylation profiles. Therefore, we aim to establish an efficient method for the isolation and further NMR characterisation of individual glycoproteins from human serum.

Methods: Unlike previously reported isolation methods, our approach allows for the isolation of up to ten serum glycoproteins in their native form from a single serum aliquot. Purification follows a two- to four-step strategy involving gradient ultracentrifugation and chromatographic techniques, e.g., size-exclusion, anion-exchange, and pseudo-affinity chromatography. Isolated proteins were characterised using polyacrylamide gel electrophoresis and Western blotting. NMR spectroscopic techniques and enzymatic digestions were utilised to analyse glycosylation profiles of individual glycoproteins and determine their contribution to the GlycA/B resonances.

Results: Several human serum proteins, including those described as major contributors, and additionally IgGs, α 2-macroglobulin, hemopexin, α 2-HS-glycoprotein, and ceruloplasmin have been successfully isolated and afford NMR characterisation of each isolated glycoprotein.

Conclusions: New methods for efficient chromatographic isolation of individual glycoproteins were optimised for the characterisation of glycoproteins by NMR. NMR analysis of glycoproteins isolated from different patient cohorts will aid in evaluating the potential of GlycA/B as biomarkers, particularly in inflammatory conditions. This work paves the way for future studies utilising NMR proteo-metabolomics in clinical research on inflammatory diseases.



P-234 - Molecular-mechanical link in shear-induced self-assembly of a functionalized biopolymeric fluid

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction. Mechanical properties of many non-Newtonian fluids are determined by the existence of specific short range intra- and intermolecular arrangements making fluids nano- or micro-structured. Molecular conformation greatly affects the rheological properties of the fluids.

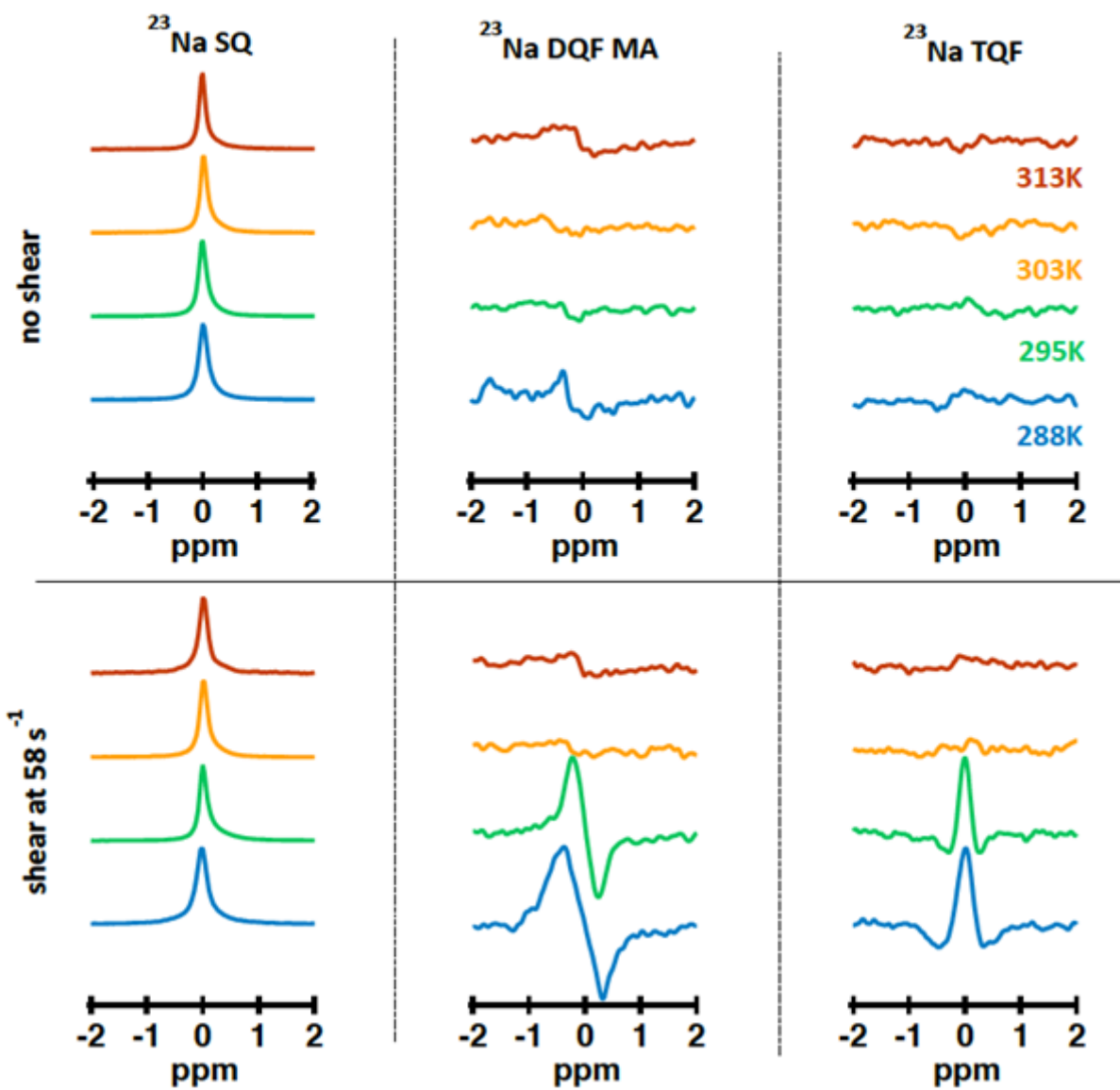
Aims: We seek to establish the molecular – mechanical link in shear thinning biopolymeric fluids to explore the temperature dependence of shear induced molecular alignment.

Methods: We applied multiple quantum filtered (MQF) spectroscopy of ²³Na (nuclear spin $I = 3/2$) in a rheo-NMR system with a rotating Couette cell during the temperature ramp to monitor shear-induced molecular order in 0.5% κ -carrageenan fluid, a linear sulphated anionic polysaccharide, that is used in food sciences, pharmaceutical industry, biotechnology, tissue engineering and medical applications

Results: Using a temperature ramp from 288K to 313K, the molecular phase transitions from helices to random coils of the biomacromolecules was captured in 0.5% κ -carrageenan solution. It was found that ²³Na MQF signals were observed only under shear and when fluid demonstrated yielding or shear-thinning behaviour in separate measurements using bulk rheometric methods. No ²³Na MQF signals were observed with or without the shear at temperatures of 303K when molecular phase transition to random coils occurs and the fluid becomes Newtonian.

Conclusions: The observation of a shear induced alignment in a fluid without an explicit liquid-crystalline phase, demonstrates the potentially higher impact of ²³Na rheo-NMR for many biologically relevant fluids, such as synovial fluid, blood and various polysaccharide solutions used in drug delivery. The method is sensitive enough to utilize the endogenous sodium ion concentration of approximately 0.02% that is about 45 times lower the physiological concentration.

Figure 1. ²³Na single quantum (SQ), double quantum filtered magic angle (DQF MA), and Triple quantum filtered (TQF) spectra of 0.5% aqueous κ -carrageenan solution at different temperatures in Couette cell with and without shear.



P-252 - Probing Multidimensional Metal Halides at the X-site using $^{79/81}\text{Br}$ NMR, NQR, and DFT

Mr Riley Hooper

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: Inorganic metal halide semiconductors are under intense research for myriad applications. These materials offer extraordinary optoelectronic tunability through compositional and elemental variation but often feature complicated phase behaviour (from the presence of multiple phase transitions or phase segregation) and dynamic properties as well as multiple degradation pathways and disorder in their structure. These phenomena are integral in the resulting bulk-level chemical and physical properties of the semiconductors, with the halide site being implicated in many of the above issues. Understanding the halogen chemical environment in these materials is essential to addressing many of the challenges precluding their implementation into real-world applications.

Objectives and Methods: Our goal is to use magnetic resonance techniques aided by DFT computations to probe the halogen local chemical environments in a series of energetically relevant inorganic lead bromide semiconducting materials. Doing so will provide a benchmark for studying more complex state-of-the-art mixed compositions found in the devices of tomorrow. This presentation will feature a combined $^{79/81}\text{Br}$ ultra-wideline NMR, NQR, and GIPAW DFT quantum chemical approach for interrogating the X-site in the perovskite and related compositions CsPbBr_3 , CsPb_2Br_5 , and Cs_4PbBr_6 . It will also discuss some additional considerations when expanding this approach to $^{35/37}\text{Cl}$ and ^{127}I .

Conclusions: Quadrupole coupling constants (CQ) were extracted via wideline $^{79/81}\text{Br}$ NMR and NQR spectroscopy for each material. The three-dimensional perovskite phase (CsPbBr_3) exhibits the largest measured CQ and the zero-dimensional Cs_4PbBr_6 phase the smallest, providing insight on the EFG at each distinct axial and equatorial bromine site in these materials. GIPAW DFT computations are shown to be an excellent in silico pre-screening tool for estimating the EFG at the X-site for Br materials.

P-302 - DITOPIC MACROCYCLIC LANTHANIDE(III) COMPLEXES WITH A METHYLENE-BIS(PHOSPHINATE) BRIDGE

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

The GdIII complexes of H4dota (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) derivatives are used as MRI contrast agents. However, these complexes have suboptimal properties as MRI contrast agents in the context of technological advancement of MRI scanners (mainly, the higher magnetic fields). For such magnetic fields, GdIII complexes of multitopic ligands with a medium size/molecular weight (up to cca 5–10 kDa) are more suitable. Furthermore, GdIII complexes of phosphinate derivatives of H4dota have properties desirable for MRI e.g., a high hydrophilicity, a fast coordinated water exchange, and a high extent of second-sphere hydration. However, the studies of phosphinate-containing multitopic contrast agents are limited.

Aims

We studied LnIII complexes of a ditopic ligand (DO3AP)2CH2 (Figure) by multinuclear NMR to determine its properties important for MRI. This ligand was designed as a model for phosphinate-containing multitopic contrast agent.

Methods and Results

We used ¹H NMR, ³¹P NMR and 2D EXSY to study solution isomerism and dynamics of the model LnIII complexes. Moreover, the same data were measured for LnIII complexes of an analogous monotopic model ligand DO3APPIN (Figure) which was used as a reference. The T1 relaxation times of bulk water in presence of GdIII monocomplex and (GdIII)₂ homodicomplex of (DO3AP)2CH2 as well as of GdIII–DO3APPIN complex were measured to determine their relaxivity.

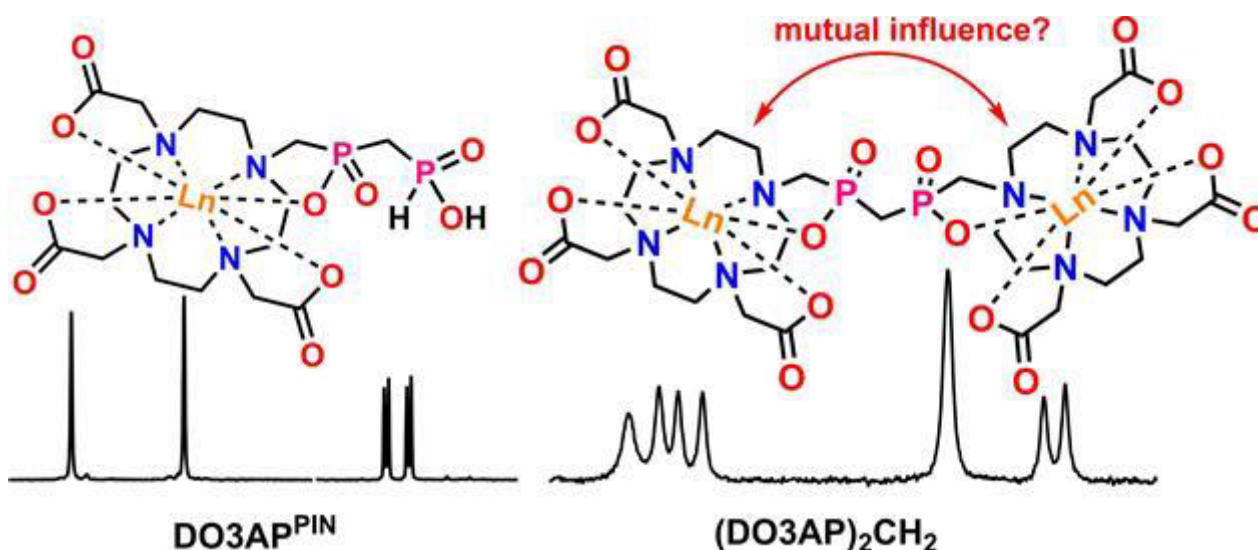
Conclusions

Based on our results, relaxivity of the GdIII homodicomplex is one of the highest ever measured for dinuclear complexes. Furthermore, behavior/isomerism of the macrocyclic subunits of the LnIII complexes is mutually independent. These findings deepen understandings of such systems and may be used to design more effective MRI contrast agents.

Acknowledgments

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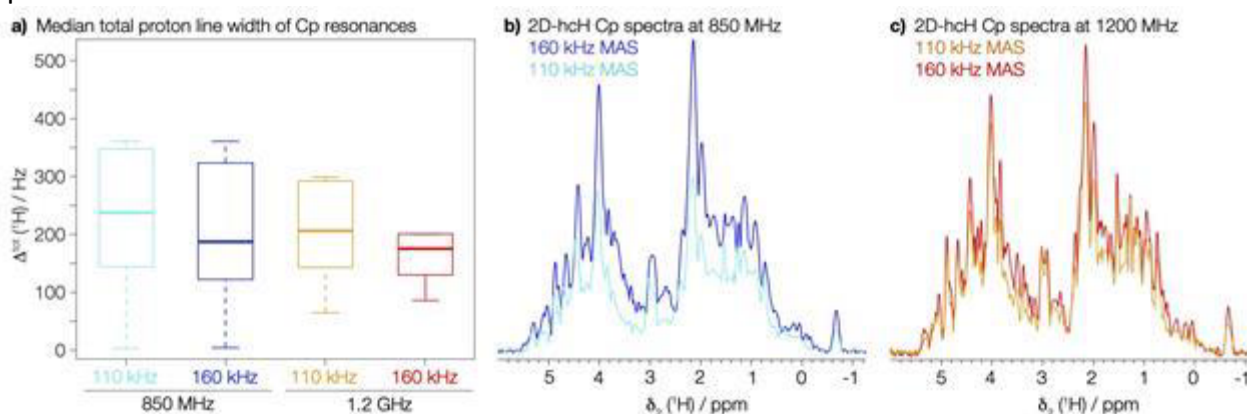
P-380 - Side-chain proton detection and assignment at 160 kHz MAS and 1200 MHz magnetic field

Mrs Morgane Callon¹, Dominique Luder², Dr Alexander Malär², Prof. Dr. Thomas Wiegand³, Dr Vaclav Rimal², Dr Lauriane Lecoq¹, Mrs Anja Böckmann¹, Prof. Ago Samoson⁴, Prof Beat Meier²

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

We investigated the benefits of fast magic-angle spinning (MAS) at 160 kHz, using a 0.5 mm probe (Prof. Ago Samoson, Darklands OÜ, Estonia), to enhance the resolution of side-chain resonances in proton-detected spectra of protonated proteins. These resonances are often part of a strongly coupled network of protons since many of them have similar chemical shifts. This leads to strong-coupling effects and additional line broadening when compared to amide or Ha protons. This induces severe overlap of many of these resonances, preventing their assignment, even at 110 kHz MAS. However, these resonances form most of the essential contacts that define the protein fold and are therefore crucial for determining protein structure and characterizing biomolecular interactions by their localization at the interface. We first show on a model system (ortho-phospho- L-serine) that a reduction of the homogeneous linewidth by a factor two can be achieved when comparing data recorded at 110 and 160 kHz MAS. A combination with high magnetic field (1.2 GHz) allows to further reduce line widths by increasing the chemical shift dispersion on a Hz-scale. In an application to the core protein (Cp), forming the 4 MDa Hepatitis B virus capsid, we could assign 60% of the aliphatic protons using a combination of hCCH Total through Bond correlation Spectroscopy (TOBSY) and hNCH experiments. These experiments also benefit from the lengthening of the transverse relaxation time T_2' with faster spinning, which could as well allow observing flexible residues in the solid-state using INEPT-based pulse sequences often inefficient at slower MAS due to too-fast relaxation. The availability of side-chain protons for structure determination and protein-protein interaction studies will open new possibilities for the studies of complex proteins that are often produced in small quantities.



P-322 - Variable Repetition Time for accelerated 1D NMR experiments

Ms Margot Sanchez

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: NMR is a powerful tool for structural elucidation. Therefore, 1D techniques are currently used for routine experiments on medium-field spectrometers but their signal-to-noise ratio (SNR) is often low for nuclei such as ^{13}C .

Aim: VRT¹, which is based on the shortening of the pulse sequence repetition time together with the evolution period incrementation, has been previously proposed to reduce the analysis time of 2D experiments. We show here that this method is also well suited to improve the SNR per time unit of 1D spectra.

Methods: 1D sequences were modified by introducing progressive reduction of the repetition time. After 2D Fourier transform, central lines were extracted and summed to obtain the 1D spectrum.

Results: We investigated the use of 1D-VRT with: different sequences (single pulse, DEPT or APT), different compounds (ethanol, ibuprofen and cholesterol) and different nuclei (^{13}C , ^2H and ^1H), in order to demonstrate its versatility. Preliminary results, obtained with routine setups, showed that we could easily divide the experimental time by 3 to 4, depending on the equation used for the evolution curve of the repetition time. Variation of relative areas of around 15% were observed in these non-quantitative conditions, with almost no SNR loss for a given number of scans.

Conclusion: Further work is needed on the quantitative aspect of 1D-VRT, but previous studies have already demonstrated the compatibility of such a method with a precision of a few permils in 2D experiments². In the future, VRT could be applied to any 1D experiment, to gain either time or sensitivity, which could be an important asset for both high-throughput routine NMR and the study low concentration samples.

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P-140 - On the multiple roles of Copper Ions into the DNA-cleaving-DNA process: an EPR/ESR study

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Cleavage and ligation of DNAs and/or RNAs can be classified as a vital process in all living systems. For example, topoisomerase enzymes resolve topological problems of DNA in replication, transcription and other cellular transactions by cleaving one or both strands of the DNA; restriction enzymes protect the cell against virus infection by cleavage of the foreign DNA or by degrading cellular DNA during apoptosis of the affected cell. DNAzymes (known also as deoxyribozymes) have been isolated by in vitro selection; they can be classified as “artificial enzymes”. DNAzymes can form duplex and triplex substructures that flank a highly conserved catalytic core. Insights on the structural and mechanistic features of DNAzymes are severely lacking. The role of metal cofactor(s), often paramagnetic species, in such systems has not yet been elucidated. Electron Spin Resonance is the method of choice for deciphering structural and mechanistic features of the paramagnetic species involved into the chemical reactions. Hyperfine and dipolar spectroscopy have been used for detecting short- and long-range interactions, respectively. In particular, the role of copper ions both for stabilization of the 3D architectures and for the oxidative reaction has been described. The preliminary results obtained on selected cleaving/cleaved architectures pave the way for analysis on mixtures where also metals/lanthanides are used as cofactors, having reached resolution of single nucleotide and beyond. Selective mutations of the DNAzyme sequence could validate the proposed model.

P-094 - The response of Mincle, a C-type lectin receptor, to infection by *Mycobacterium tuberculosis*

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

C-type lectin receptors (CLRs) are host proteins that act as sensors of pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs). As such, CLRs play a key role in the innate immune system. Transmembrane CLRs consist of a short cytosolic N-terminal sequence and an extracellular domain including a C-terminal carbohydrate recognition domain (CRD). The extracellular and cytosolic domains are connected by a single-pass transmembrane domain. Certain CLRs, expressed in macrophages and dendritic cells, play key roles in infection by *Mycobacterium tuberculosis*. Binding of trehalose dimycolate (cord factor), a glycolipid from mycobacteria, to human Mincle leads to activation of NF- κ B via Syk-Card9-Bcl10-Malt1. Signal transduction requires FcR γ , a small, single-pass transmembrane protein bearing cytosolic ITAM motifs which are phosphorylated by Src family kinases in the first intracellular step of this signalling pathway.

Methods

Mincle has proven recalcitrant to large-scale production in *E. coli*, hindering detailed study of ligand binding and the mechanism of signal transduction. In a first step to breaking this deadlock, we have undertaken expression of the CRD of human Mincle in the Origami 2(DE3) pLysS strain of *E. coli* to circumvent the need for refolding from inclusion bodies. This has enabled the production of isotopically labelled protein for structural studies by solution-state NMR.

Results

We present here our progress to date on resonance assignment, ligand interaction studies and determination of the dynamic properties of Mincle. The latter are assessed through ¹⁵N relaxation measurements, coupled to molecular dynamics simulations, aimed at capturing conformations related to the formation of a hydrophobic groove which seems essential for proper ligand binding. By characterising the effects of extracellular interactions on human Mincle, we begin to define the molecular response to binding events that leads to activation of the signalling pathway.

P-250 - Exploring Spin-Orbit Coupling and Octahedral Tilting in A_2SnX_6 (A = K, Rb; X = Cl, Br, or I) Materials

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: A_2BX_6 compounds, which typically adopt a K_2PtCl_6 type structure, have gained traction as possible non-toxic alternatives to lead halide perovskites with improved ambient stability. From materials of this class, A_2SnX_6 (A= K, Rb; X= Cl, Br or I) compounds have been known for decades, but their suite of characterizations has lacked a detailed experimental and theoretical examination.

Methods: Solid-state nuclear magnetic resonance (NMR) spectroscopy and density functional theory (DFT) calculations were used to explore the microscopic structure of A_2SnX_6 materials.

Results and Conclusions: ^{39}K and ^{87}Rb NMR spectroscopies of the A-sites show that static octahedral tilting gives rise to chemical shift anisotropy (CSA) and sizeable quadrupole coupling constants (CQs) for A-site nuclei in compounds that deviate from cubic symmetry. High field (21.1 T) NMR analysis combined with periodic DFT calculations enables successful resolution of the relative orientation between the electric field gradient (EFG) and CSA tensors for ^{39}K in the monoclinic K_2SnBr_6 . ^{119}Sn NMR spectroscopy of the B-site polyhedra shows that the ^{119}Sn chemical shift follows a normal halogen dependence (NHD). Scalar relativistic and spin-orbit (SO) DFT calculations on cluster models demonstrate that the NHD is driven almost entirely by SO effects, which is explained in terms of the frontier molecular orbitals and the involvement of the Sn and X atomic orbitals in the Sn–X bonds. Proper relativistic treatment of heavy atoms is also underscored by a comparison of the calculated ^{119}Sn chemical shifts at different levels of theory.

P-120 - NMRTTools.jl: a library for NMR analysis in Julia

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Julia is an open source, high-level, high-performance dynamic programming language designed specifically for numerical and scientific computing [1]. Its speed, simplicity, and expressiveness make it an ideal choice for scientific research and data analysis, while its integrated package and environment manager ensures reproducibility. Here, we introduce NMRTTools.jl, a simple, open source library being developed to facilitate reading, writing and analysing NMR data within Julia [2].

Aims

We aim to develop a light-weight and easy-to-use interface to a collection of composable data types appropriate for handling NMR data and their associated metadata (including both processing and acquisition parameters), together with recipes for plotting and visualisation.

Results

An initial version of NMRTTools.jl has been released [2] allowing the import of spectra from NMRPipe, the automated parsing of Bruker acquisition parameters, and simple plotting of one- and two-dimensional data. Leveraging Julia's multiple dispatch paradigm, data can be accessed directly as arrays, or through chemical shift-based selectors that provide easy access with no detriment to performance. At this stage, the library design continues to evolve, and input from the community in this process is welcomed.

Conclusions

NMRTTools.jl provides a simple, easy-to-use interface for handling NMR data within Julia. While still at a relatively early stage of development, we have already found it useful for a variety of applications, including ¹⁹F lineshape analysis [3], two-dimensional lineshape fitting of ¹H-coupled multiplets for the analysis of cross-correlated relaxation in methyl groups [4], and the analysis of real-time protein phosphorylation kinetics [5]. Interested users are invited to contribute to the development of this ongoing project.

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P-340 - Future Proofing Scotch Whisky

Mr Kacper Krakowiak¹, Dr David Ellis¹, Dr Ruairaidh McIntosh¹

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Scotch Whisky is one of Scotland's most important and famous exports. Many Scotch Whiskies have characteristic, smoky flavours imparted into the spirit through burning of peat.¹ Peat is a limited resource, due to many years of excavation for fuel and compost. Destroying natural peat deposits through exploitation releases large amounts of carbon dioxide into the atmosphere and causes various undesirable effects on the ecosystem.²

Methods

In order to identify the compounds connected to flavours mentioned above, several analytical techniques were employed, principally high resolution nuclear magnetic resonance with multiple solvent suppression pulse sequences, complemented by gas chromatography coupled with mass spectrometry and pyrolysis. These methods have been proven to produce high quality data when used for analysis of complex mixtures.^{3 4}

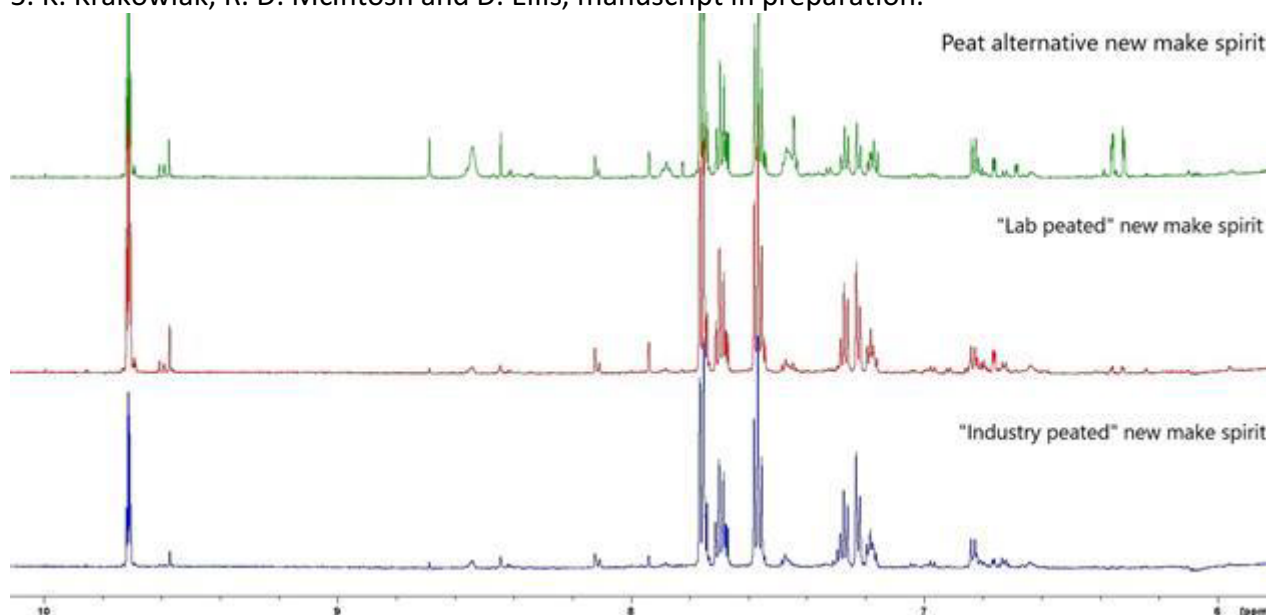
Results

Some potential replacement candidates for peat have been selected due to the high content of biopolymers similar to those found in peat. Initial results show that despite the differences between the materials, smoke produced by them contains similar compounds, including congeners of interest, such as cresols, phenol, guaiacol.⁵

Conclusions

Methods outlined above were used effectively in this project to investigate the composition of peat and possible alternatives, through study of extracts, smoke and spirit produced using them. This methodology can be further extended to analysis of other complex mixtures and study of thermal decomposition of biopolymers.

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P-394 - STRUCTURE AND DYNAMICS OF AMORPHOUS CALCIUM CARBONATE BY MAS NMR

Mr Sanjay Vinod Kumar

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Amorphous calcium carbonate (ACC) is an essential precursor phase in the controlled biomineralization required for the formation of skeletal parts of marine organisms.[1] ACC contains structural water in an approximate ratio of 1:1 with CaCO_3 . Knowledge of the structure of ACC is key to understanding biomineralization pathways and how organisms determine the properties of resulting crystalline materials. Molecular dynamics (MD) simulations have produced a handful of ACC structures, which more or less reproduce the pair distribution functions,[2] but a comprehensive structural model is lack

Magic angle spinning (MAS) NMR spectroscopy can provide unique information on the structure and dynamics of amorphous materials like ACC. Comparison of ^1H - ^{13}C cross-polarization (CP) and ^{13}C direct excitation (DE) spectra (figure 1a) reveals that ^1H to ^{13}C polarization transfer is inefficient for the carbonates of ACC.[3] In addition, comparing the carbonate slice from a 2D ^1H - ^{13}C wideline separation (WISE) experiment to the ^1H direct detection (DE) spectrum (figure 1b) suggests the presence of a large fraction of mobile protons, which lack effective dipolar coupling to the carbon nuclei due to restricted motion. Observation of a nuclear Overhauser effect (NOE) confirms the presence of mobility in ACC at a fast timescale. Analysis of NOE data is in progress and should provide the cross-relaxation rate (σ) and C-H distance. The observation of ^1H mobility by NMR is in agreement with AFM studies, which show ionic conductivity in ACC nanoparticles.[4]

Figure 1: MAS NMR spectra of ACC stabilized by polyaspartic acid (PAsp) at 400 MHz and 10 kHz spinning.

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P-080 - Structural analysis and binding studies of lanthanide-peptide complexes derived from calmodulin's EF-Hand

Ms Yulia Perevedentseva¹, Mr Nikolai Klishin¹, Dr Ruslan Nediellkov¹, Dr Heiko Möller¹

¹Potsdam University, Potsdam, Germany

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The problem of recycling and reusing lanthanides now attracts special attention. As the opposite to the chemical separation of lanthanides, biological separation using modified peptides is now being developed. A combination of theoretical and practical approaches is needed to select the most suitable system. The study of such peptides using NMR is of particular interest because this method allows to reveal the structural features of the peptide-Ln. It should also be noted that lanthanides play a special role in NMR due to the presence of additional effects related to paramagnetism. The quantitative analysis of these paramagnetic effects provides plenty of structural information. The combination of these effects with normal structure determination methods will give us more detailed information about the nature of the interaction between the peptide and the lanthanides. As is known, lanthanides effectively replace calcium in calmodulin because of their very similar ionic radii. Calmodulin contains four calcium binding sites per mole. By a different approach, it was possible to obtain derivatives of EF-hand peptide that bind preferentially to Ln instead of Ca. However, the biophysical reasons for this preference are still not fully understood. In this report, we present the first results of combining the study of the complex system by different approaches on a model system EF-hand 4, which has been chosen for its initial good affinity to lanthanides. We report the last results of structure calculations based on different NMR 2D and 3D techniques such as NOESY, RDC, PSC and PRE. These structure calculations will help us to go into the mechanism of lanthanide peptide binding.

P-136 - Magnetstein: tool for complex mixture analysis in NMR spectroscopy

Miss Barbara Domżał

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

NMR spectroscopy is a potent tool for quantitative and qualitative analysis of mixtures. It can be used particularly effectively when supported by libraries of spectra of pure components.

Aims

Unfortunately, the analysis (especially quantitative) is impaired for spectra with high peak overlap. This problem is common, particularly for low-field spectra, e.g., from benchtop spectrometers. Another difficulty stems from differences between the measurement conditions of individual components from the library and the mixture, leading to peak shifts. Existing methods of analyzing mixture spectra only partially handle both problems.

Methods

Here we present an excellent remedy – a new approach based on the Wasserstein metric, known from the fields such as probability theory and machine learning. To deconvolve a spectrum of a mixture, we formulate the problem as linear regression. The Wasserstein distance is used as a measure of dissimilarity between spectra.

Results

We show the potential of our method under challenging spectra from a benchtop spectrometer and spectra of mixtures of metabolites that differ in measurement temperature from the base spectra. In all the cases magnetstein either outperforms or gives comparable results to the algorithm provided by ACD/Spectrus 2020.1.1 Targeted Profiling tool (ACD/Labs, Canada).

Conclusions

We conclude that the Wasserstein distance is an adequate and powerful tool for complex mixture analysis. Our algorithm is available in open-source Python package on <https://github.com/mciach/masserstein>.

P-314 - Title: Rethinking the screening strategy. Two new NMR experiments for fragment based drug discovery.

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

¹H NMR Fragment Based Drug Discovery (FBDD) is a highly efficient screening method. It allows for the identification of hits from a small library of fragments in a relatively short time. Various NMR methodologies, such as ¹H STD NMR, T2-Filter, Waterlogsy, DOSY, and ¹H CSP, are routinely used at different stages of a screening campaign. However, one limitation of NMR is its relatively low sensitivity leading to longer experimental times. To address this issue, screening using a mixture of fragments is commonly employed. While this is a powerful approach, it has certain drawbacks. For instance, the use of mixtures increases the overall molar concentration of the ligands in solution, which may be incompatible or compete for the same protein binding site.

Aims

To overcome these challenges, we propose to redesign the FBDD process by using a single ligand in combination with sensitivity enhanced NMR experiments.

Methods

Our approach revolves around modified ¹H STD and ¹H CPMG NMR experiments. We have thoroughly tested and validated these methods, comparing them with their standard variants to ensure their robustness and reliability.

Results

By multi-fold signal amplification relative to the standard pulse sequences, the two new NMR techniques designed reduce the acquisition time to just a few seconds per ligand .

Conclusions

The enhanced sensitivity of the ¹H STD NMR and ¹H CPMG techniques allows for a re-evaluation of the screening strategy and the hits discovery process.

P-202 - Hyperpolarization effects with ansa-aminoboranes and parahydrogen in different solvents

Miss Karolna Konsewicz¹, Dr Vladimir Zhivonitko¹, Dr Kristina Sorochkina², Docent Konstantin Chernichenko², Docent Timo Repo²

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Hyperpolarization in NMR is a sensitive tool for analysing chemical compounds.[1] In parahydrogen-induced polarization (PHIP), the hyperpolarization effects can be dependent on physical and chemical character of the used solvent. Properties of solvents such as polarity might affect this phenomenon. Understanding correlations between the solvent properties and NMR enhancement is important to optimize polarization in NMR experiments with parahydrogen. In this study we investigate PHIP effects using metal-free ansa-aminoboranes (AAB) as catalysts in different solvents. AABs contain nitrogen-borane frustrated Lewis pair, which plays a role of moiety reacting with parahydrogen, leading to the hyperpolarization effects.[2]

Aim

The aim of this work is to understand mechanisms behind activation of parahydrogen with AABs in different solvents.

Methods

We measure ¹H NMR spectra of AABs with various groups at the boron site (mesityl, i-propylphenyl, phenyl) in a range of temperatures in toluene-d₈, dichloromethane-d₂ and acetonitrile-d₃ with and without parahydrogen added. We determine equilibrium parameters for the reaction between the AABs and H₂.

Conclusions

We observe a strong influence of solvent on hyperpolarization effects in the reaction of AABs and parahydrogen. It is noticeable that polarity has an impact on both the concentration of AAB-H₂ adducts and the enhancement of NMR signals. The higher polarity of the solvent, the lower concentration of AAB we obtain. These findings of solvent-dependent behaviour of AABs give us the important information for further hyperpolarization studies.

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P-292 - Unmasking of SOCS2-targeting phosphotyrosine prodrugs observed by in cell NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Suppressor of cytokine signalling 2 (SOCS2) is a substrate receptor of Cullin5, that provides negative feedback in the JAK/STAT pathway by binding and degrading the phosphorylated cytokine receptor. Its diverse functions in cell cycle regulation and cancer as well as the possibility to use an SOCS2 inhibitor as a synthetic growth hormone make SOCS2 an attractive drug target.

Phosphotyrosine is a worthwhile starting point for ligand design despite the complications in terms of synthesis, cell permeability and stability arising from the presence of a phosphate group in the ligand, as SOCS2 binds it with very high ligand efficiency. Our group has recently developed MN551, a phosphotyrosine-derived covalent chemical probe for SOCS2. To overcome the permeability issues arising from the charge and polarity of the phosphate group, a prodrug approach was necessary. We used in cell 19F NMR to characterize cellular uptake of these prodrugs and observe unmasking. We could show that POM-protected MN551 efficiently enters the cell, is deprotected with less than an hour half-life time of the prodrug and leads to high enrichment of the active species in the cell. Once unmasked, MN551 covalently targets a cysteine near the phosphotyrosine binding site of SOCS2.

I will also present ongoing work to exploit the reactivity of this cysteine to find non-phosphotyrosine based ligands for this site by using spinlabels and a paramagnetic relaxation enhancement (PRE) based fragment binding screen.

P-034 - Intrinsically disordered regions modulate the DNA binding behavior of the oncogenic transcription factor MYC:MAX via intramolecular interactions

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

The intrinsically disordered protein MYC belongs to the family of basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factors (TFs). In complex with its partner MAX, MYC preferentially binds to E-Box promotor sequences where it controls fundamental cellular processes such as cell cycle progression, metabolism, and apoptosis. Dysregulation of MYC and its tight network of interaction partners is associated with tumorigenesis and cancer proliferation.

Aims:

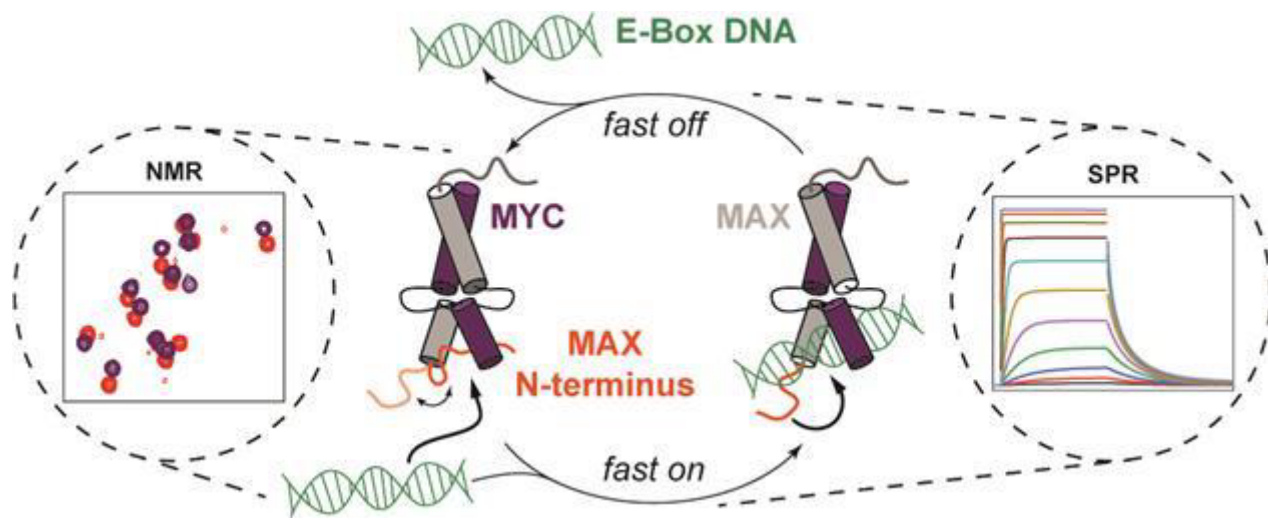
In this study, we set out to elucidate intramolecular interactions in MYC and MAX, as this aspect of MYC:MAX regulation has not yet been investigated in detail. Ultimately, we aim to modulate MYC IDRs to enable new modalities for drug discovery.

Methods and Results:

To this end, we use Nuclear Magnetic Resonance (NMR) spectroscopy to identify and map intramolecular interactions between disordered MYC and MAX regions and the folded MYC:MAX DNA binding domain (DBD). We find that these binding events are mainly driven by electrostatic interactions and that they are competitive with DNA binding. Using Surface Plasmon Resonance (SPR), we demonstrate that the identified IDR:DBD interactions provide specificity for E-Box DNA by lowering the affinity of the DBD to non-cognate DNA by competition. Furthermore, our data show that binding kinetics are accelerated in the presence of the MAX N-terminus. We also establish that these effects are further enhanced by phosphorylation of the MAX N-terminus, allowing for faster screening of the DNA for the relevant E-box sequences.

Conclusions:

We speculate that the negatively charged MAX N-terminus – especially when phosphorylated – serves as DNA-mimicking folding template for the dimeric DBD, allowing efficient pre-formation of this crucial binding motif, prior to DNA engagement and thereby increasing the association rate. Our work provides new insights how bHLH-LZ TFs are regulated by intramolecular interactions between disordered regions and folded DNA binding domains, which opens up new paths to drug discovery.



P-270 - HighER-resolution relaxometry with pure-shift NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Intro

High-resolution NMR relaxometry is the approach that combines the possibility of fast field cycling with the advantages of using high-resolution detection by mechanically shuttling the sample away from the magnetic center of a high-field magnet.

Recently, we developed a systematic method to directly identify and characterize weak metabolite-macromolecule interactions in complex media.[1] The method detects interactions from the presence of a dispersion at low fields in the relaxometry profile of a metabolite transiently bound to a macromolecule.

A classic problem to overcome when dealing with complex mixtures is the poor resolution of ¹H-spectra which can hinder the possibility to extract data from all the components in solution. Pure shift NMR techniques can greatly alleviate this problem.

Aim

In this work we want to implement pure-shift techniques for routine use in high-resolution relaxometry experiments to facilitate the analysis of complex mixtures.

Methods

A 14 T Bruker spectrometer equipped with the new development state-of-the-art fast-shuttle system was employed.[2] The sequence includes a relaxation delay combined with PSYCHE[3] elements working with Saltire pulses and WET blocks for water suppression.[4] Additional SAPPHIRE cycles were employed to reduce harmonic artifacts from evolution under residual coupling.[5] Dispersion profiles were measured on a model sample.

Results

The sequence gives comparable results in terms of quality and sensitivity to non-shuttled version in a mixture of glucosamine and lysozyme.

Conclusion

Pure-shift sequences for longitudinal relaxation measurements were adapted to enhance resolution for the analysis of metabolites-macromolecules interactions by relaxometry.

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P-354 - Spherical Tensor Analysis for Studying Relaxation in Liquids

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Spherical tensor analysis (STA) enables complete decomposition of the NMR signal into components, which pass through different irreducible spherical tensor operators (ISTO) at any point during an experiment.¹ Such a decomposition is achieved by performing multiple experiments different only in a single pulse sequence element accomplishing a rotation of the spin polarisation through a predefined set of Euler angles.

Despite its great potential in separating spin operators not only by coherence order but also based on their spherical rank, only a few STA studies have been reported in solids^{1,2} and in anisotropic liquid phase.³ The main limitation of STA is the large number of experiments required to separate all spherical signal components. However, often one is only interested in the evolution of a smaller set of operators, allowing the number of experiments to be significantly reduced. Notably, the coherence order of the spin operators remains unchanged under free evolution, within the secular approximation. Filtration of the operators based on their coherence order is easily achieved by phase cycling and/or pulsed field gradients in the solution state, which can be further separated by their spherical rank.

It is demonstrated that the number of experiments required for STA can be reduced to the number of ISTOs to be separated, permitting multiple decomposition steps within a sequence. This allows determination of not only the auto-relaxation rate of all ISTOs but also all cross-relaxation rates between them. Application of these new types of STA experiments to study the relaxation properties of strongly coupled spin-1/2 systems at high field and in field cycling experiments will be presented.

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P-054 - Functional cycle of the human Hsp70 chaperone BiP at atomic resolution

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Hsp70 chaperones are molecular machines essential to all kingdoms of life. Their functional cycle is crucial to their role as central client processing hubs interacting with multiple co-factors. Segments of the functional cycle have been studied by structural and biophysics methods, but the connections between these segments and the interplay with co-chaperones are only poorly understood. Here we show how the superior power of methyl NMR spectroscopy combined with an ATP regeneration system can be harnessed to probe the functional cycle of the human Hsp70 BiP at unprecedented spatial and temporal resolution. Determination of the kinetic reaction rate constants under turnover conditions results in a quantitative description of the underlying non-equilibrium thermodynamic energy landscape. The data resolve that BiP undergoes a unique five-state functional cycle that includes two previously unrecognized high-energy conformations. Analysis of NMR parameters on the background of published structural data resolves for the first time the clockwork mechanism underlying Hsp70 function. One of the novel high-energy conformations retains ADP deep-locked inside the protein, serving as a timer of the functional cycle. We also illustrate how the ATP regeneration setup allows studies of the interplay between diverse co-chaperones at the atomic level by reaction-step-specific enhancement factors and determining interaction interfaces. The technology sets a paradigm for studies of dynamic networks of biomolecules in general and a template for Hsp70 functional cycles in all kingdoms of life.

P-222 - Ex situ and operando NMR studies of the redox-two-dimensional covalent organic framework (2D-COFs) cathodes for durable aluminum batteries

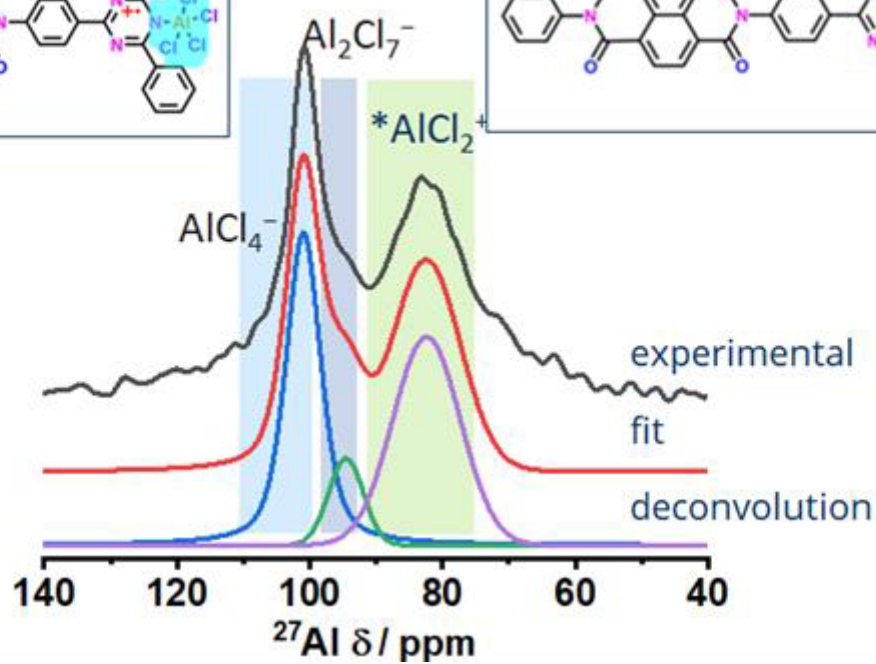
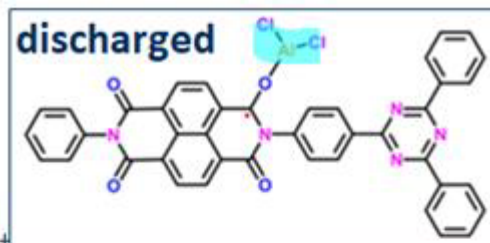
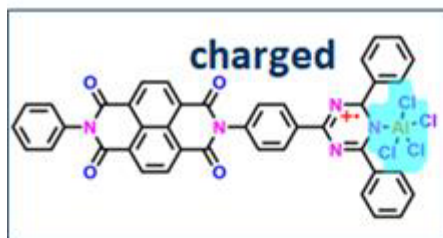
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Rechargeable aluminum batteries (RABs) offer a promising option for next-generation energy storage technologies due to the high abundance of Al, low cost, and safety. However, the development of RABs requires appropriate cathode materials for hosting Al-based ions (e.g., Al^{3+} , AlCl_2^+ , AlCl_2^+ , AlCl_4^- , and Al_2Cl_7^-)¹. Organic COFs^{2,3} with redox moieties are potential candidates enabling the coordination with charge-compensating ions unlike inorganic electrode materials.² Herein, we report studies on new 2D-COFs with unique bipolar redox-capability over ca. 4,000 cycles. NMR techniques were used to understand the structure and fundamental charge-storage mechanism.

Ex-situ and operando NMR are powerful tools to understand the charge-storage mechanism of RAB cathode.⁴ Ex-situ MAS NMR experiments were applied to study the structure of the 2D-COF-based electrodes and their interaction with Al ions at different charge states. The ^{27}Al signals of AlCl_4^- (103 ppm) and Al_2Cl_7^- (97 ppm) were detected in both the ionic liquid (IL) -electrolyte and 2D-COF electrode at open circuit voltage. Interestingly, only the signal at 103 ppm is detected in the fully charged 2D-COF electrode due to inserted anionic Al species, mainly AlCl_4^- . In the fully discharged 2D-COF electrode, an additional broad signal at ca. 82 ppm appears pointing towards the presence of cationic³ AlCl_2^+ as charge carrier. The analysis of ^{27}Al -T₂ of fully discharged 2D-COF electrode by echo experiments indicates strong binding interaction between the 2D-COF and AlCl_2^+ due to the short T₂ time of AlCl_2^+ . In addition, ^{27}Al {1H} HETCOR shows the correlation of aromatic 1H spins of the 2D-COF with ^{27}Al signals of AlCl_4^- and AlCl_2^+ . Furthermore, the decreasing intensity of the ^{13}C CP MAS signal of imide C=O indicates the reduction of C=O in the discharged 2D-COF electrode because AlCl_2^+ binds to the C=O. Moreover, operando ^{27}Al NMR studies of the monomer cathode reveal reversibility of intensity and shift of IL.



P-272 - ¹H NMR metabolomics in honeybee research: individual analysis of honeybee body parts and castes comparison (worker and queen bees)

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Agriculture and human food security sectors heavily rely on honeybees. It is estimated that honeybees pollinate around one-third of the world's crop production. Metabolomics using NMR spectroscopy provides valuable insight into biological functions that are applied in various scientific disciplines. In honeybee research, NMR metabolomics is a promising diagnostic tool that has yet to be fully explored.

The purpose of this study was to employ ¹H NMR to understand the distribution of metabolites in the body parts of honeybee workers and queens (head, thorax, abdomen, and gut), as well as the metabolic characteristics between the castes.

The samples consisted of pooled bees, nurses, foragers, and queens. Bees were anesthetized with CO₂ before processing. Each body part was dissected and collected separately. Homogenized samples were extracted with methanol, reconstituted in D₂O, and analyzed using a Bruker Avance III spectrometer with a BBFO probe operating at 500.18 MHz for ¹H NMR. The spectra were pre-processed, and the selected spectral regions were integrated. This resulted in 156 buckets, of which 73 were identified using Chenomx and an in-house library. Multivariate analysis of PCA, OPLS-DA, and hierarchical clustering were applied.

Each body part of the workers and queens contained metabolites corresponding to their physiological functions. The heads of queens and workers have markedly high concentrations of 10-hydroxy-2-decenoic acid, a royal jelly acid. Notable concentrations of (2E)-9-oxodecenoic acid, a queen pheromone, were found in the queens' heads. In contrast, workers did not possess any (2E)-9-oxodecenoic acid, whereas 3-hydroxykynurenine levels were significantly high. Among worker bees, nurses' heads had significantly higher concentrations of glucose and fructose than foragers' heads. The results of this study suggest the characteristic biomarkers of bee body parts and support the expansion of NMR-based metabolomics studies in honeybee research.

This research is supported by the Czech Republic's Ministry of Agriculture (QK21010088).

P-102 - The bacterial actin homolog ParM as a model to study actin dynamics at the atomic level.

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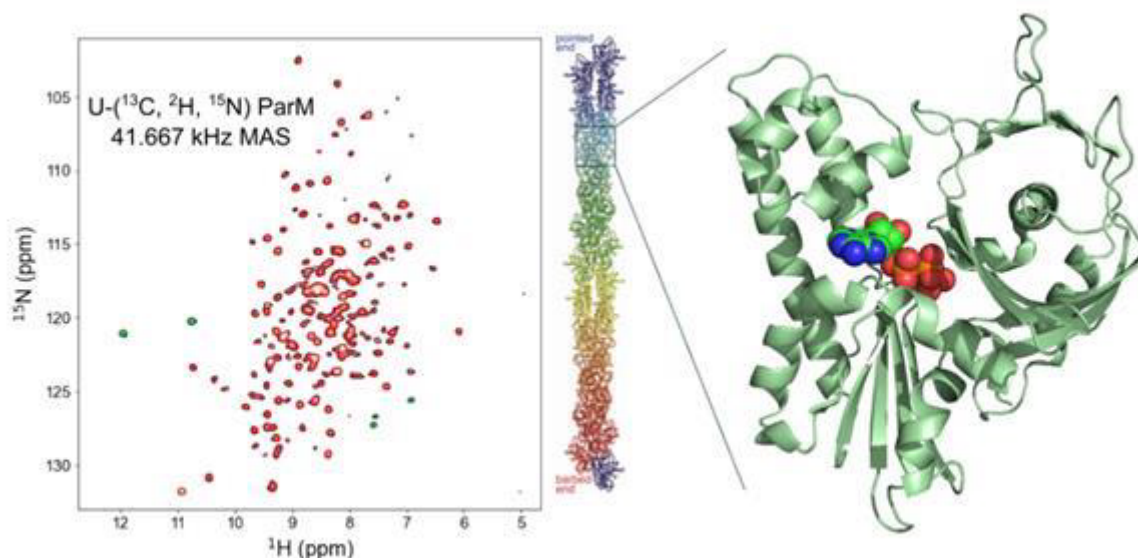
Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Actins are ubiquitous proteins that control several cellular processes by responding to the presence/absence of different nucleotides and binding partners which modulate their polymerization, depolymerization, and branching. In spite of a large body of work on actin biophysics, atomic level details of how actins dynamically respond to nucleotides, cofactors, and membranes to achieve their function is lacking. The discovery of actin-like cytomotive proteins in prokaryotes has also opened up questions about the evolutionary and regulatory aspects these proteins. The bacterial actin homolog ParM is an ideal candidate where we can begin to answer some of these questions.

ParM is a key component of plasmid-segregating machinery for bacteria containing low copy-number plasmid. Despite having only 10% of sequence similarity to actin, its nucleotide-binding pocket is conserved indicating that the mechanism of filament formation might be same. ParM exhibits ATPase activity but unlike most actin-like proteins, it shows dynamic instability. It can be stabilized in the F-state if the monomers at the ends of the polymer are ATP-bound.

Our first aim is to understand atomic-level details of how nucleotide binding initiates polymerization, and how nucleotide hydrolysis is primed to trigger disassembly. This will be uncovered by studying site-specific dynamics of ParM in the ATP and ADP-bound F-state using solid-state NMR.

ParM is a 37 kDa protein, which makes it difficult to study by traditional ¹³C-detected NMR. Instead, fast magic-angle-spinning (40-100 kHz) ¹H-detected ss-NMR will allow us to study this protein at the atomic level. I will be presenting our work that involves preparation of uniformly labelled, either U-(¹³C, ²H, ¹⁵N) or U-(¹³C, ¹⁵N) ParM in the F-state using non-hydrolysable nucleotide. This enabled us to obtain well-resolved spectra's for nearly complete assignment, using multidimensional experiments. I will also present initial Rotational-echo double-resonance (REDOR) based experiments that give us insights into site-specific dynamics in this protein.



P-300 - Unraveling Structure and Interactions of Azithromycin Conjugates: An Integrated NMR, Fluorometric and Molecular Modelling Approach

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Azithromycin belongs to a 15-membered macrolide class of antibiotics possessing broad spectrum of antibacterial potency and favourable pharmacokinetics [1]. It exerts its activity by binding to the bacterial ribosomal 23S rRNA in domain V at the peptidyl transferase region and blocks the exit tunnel thus inhibiting the bacterial protein synthesis. The overuse and misuse of antibiotics worldwide have led to increasing bacterial resistance which present serious threat to human health worldwide. Hence, there is a high need for discovery of new antibiotics to combat resistance. Recently discovered conjugates of azithromycin and thiosemicarbazones, the macrozones, represent new class of macrolide derivatives that exhibit promising activities against resistant pathogens [2,3]. NMR experiments including transferred nuclear Overhauser effect spectroscopy (trNOESY), saturation transfer difference (STD), WaterLogsy, diffusion and solvent paramagnetic relaxation experiments (PRE) coupled with molecular modelling have been employed to characterize binding epitopes and bound conformations of macrolides and macrozones [4, 5]. Subsequently, fluorimetry has provided binding constants of macrozones in interactions with E. Coli ribosome and bovine serum albumin (BSA).

The presented results can further be exploited in the process of design and discovery of novel macrolide compounds with activity against resistant bacteria.

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P-266 - SCORE-metabolite-ID – Identification of metabolites from complex mixtures by correlation of 1D- ^1H or 2D-HSQC NMR, MS and LC data

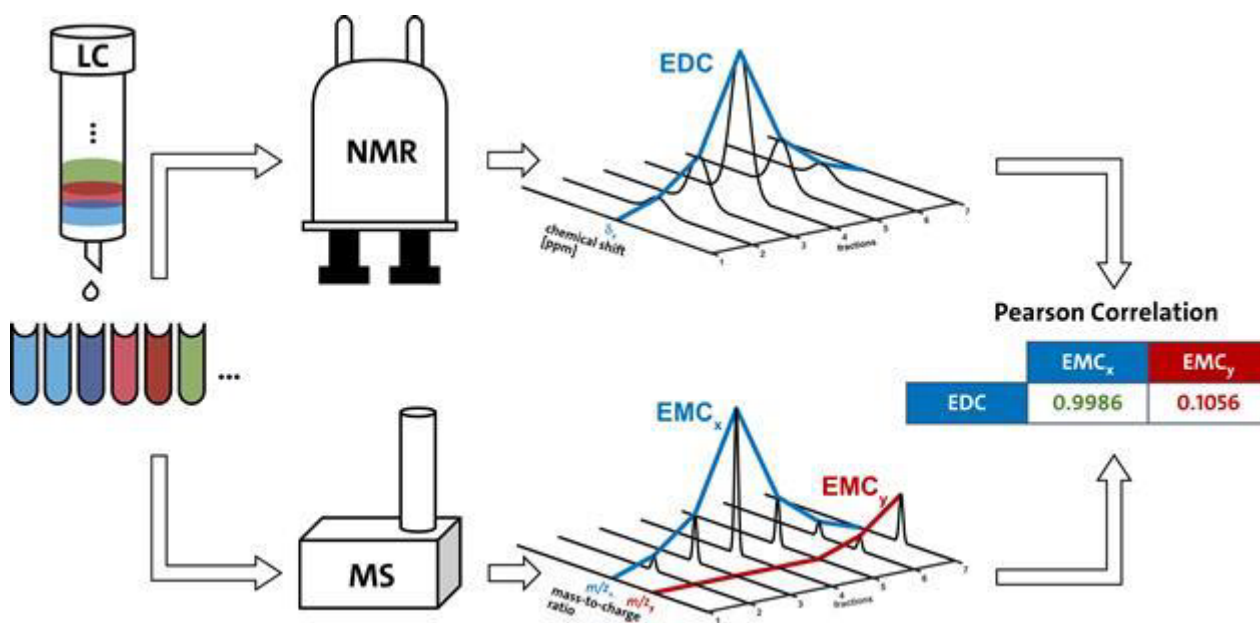
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The identification of metabolites is an extremely relevant factor in metabolomics studies. Due to the large number of metabolites, which also occur in strong concentration differences, resulting in strong signal overlaps, the identification of individual metabolites from either NMR or MS spectra of natural products is hardly possible.

Since NMR and MS provide complementary information, the combination of both analytical methods is a promising approach. In our SCORE-metabolite-ID approach, this combination is achieved by correlation of NMR and MS data of the time course of a chromatographic fractionation (cf. figure 1). This correlation strategy leads to the detection of related signals in the NMR spectra themselves as well as associated mass-to-charge ratios from the ESI mass spectra. This correlation of NMR and MS data can be performed semi-automatically. As a result, the MATLAB based app enables simple, efficient and reliable identification of metabolites without the need for individual isolation. The tool can be used either with 1D ^1H NMR spectra or 2D HSQC spectra.



P-364 - Ultrahigh Resolution Fluorine and Phosphorus NMR Spectroscopy

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

NMR measurements in systems containing fluorine or phosphorus are becoming increasingly common due to their use in areas such as pharmaceuticals and environmental science. ¹⁹F and ³¹P NMR provide important information on structure and conformation which can easily be extracted when these nuclei are sparse and peak overlap is rare. However, in systems highly abundant in such nuclei, such as perfluoroalkyl compounds, spectral information can be nearly impossible to extract due to the presence of multiple homonuclear scalar couplings. Homonuclear decoupled, or 'pure shift', NMR experiments are highly effective at suppressing the effects of homonuclear scalar couplings in proton NMR,[1-3] but existing methods have insufficient bandwidth to cover the large chemical shift ranges commonly observed in fluorine and phosphorus NMR and therefore require modification.

Here, we present a new family of ultra-high resolution NMR experiments for the simplification of wide NMR spectra to give one singlet per chemical shift environment. The benefits of this new pure shift NMR approach will be demonstrated in the ¹⁹F and ³¹P analysis of two highly challenging mixtures, suppressing the effects of homonuclear scalar couplings that are as large as 330 Hz and cover a chemical shift range of up to 350 ppm. The substantial improvement in resolution shown in the NMR spectra of these two mixtures highlights how pure shift methods can be used to aid in the analysis of even the most complex systems.

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P-346 - Spectral Decomposition of Complex Mixtures with HPLC/DOSY and PARAFAC

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Abstract

Mixture analysis is one of the most active areas of research in NMR. The development of powerful methods as diffusion-ordered spectroscopy (DOSY) can allow the recovery of individual component spectra. However, when the diffusion coefficients of mixture components are similar and/or their signals overlap, DOSY is insufficient to achieve complete separation of component spectra.

Enhancements to DOSY can be introduced, such as addition of an extra spectral dimension to offer more resolution e.g., HSQC-DOSY 1. Another way is to combine diffusion encoding and spectral data with a third dimension encoding changes in the concentrations of mixture constituents, e.g., by acquiring diffusion NMR data during a chemical reaction 2,3. This generates a 3D dataset that can be analysed with powerful multivariate statistical methods such as Parallel Factor Analysis (PARAFAC). PARAFAC exploits independent variation in three or more dimensions to extract the NMR spectrum of each component.

In this work, changes in component concentrations during a liquid chromatography run were used as the third dimension, for a mixture that was only partially resolved by HPLC. Fractions were collected as the mixture eluted, and for each fraction a diffusion NMR data set was acquired using the Oneshot DOSY sequence 4. Data were processed and PARAFAC analysis performed using the General NMR Analysis Toolbox (GNAT) 5.

This research complies with the conference theme involving solution NMR methods for small molecules/ drug discovery, as it can be applied for the analysis of mixtures of small molecules including pharmaceutical compounds.

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P-040 - Interaction of SARS-CoV-2 unique domain with 3'-untranslated region of cellular messenger RNAs

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: Nsp3 is the largest non-structural protein of SARS-CoV-2. It functions as an essential component of the replication-transcription complex, cleaves non-structural proteins, promotes cytokine expression and blocks the host's innate immune response. Several putative domains of Nsp3 are conserved among different coronaviruses. Macrodomain Nsp3c, however, is missing in all coronaviruses except SARS-CoV and SARS-CoV-2, suggesting it is responsible for increased pathogenicity of the later. The influence of Nsp3c on apoptosis and the immune response is hypothesized to be achieved through binding to cellular nucleic acid targets.

Aims: We have aimed to characterize the interaction of positively charged stretch of lysine residues found in macrodomain Nsp3c with its nucleic acid binding partners to answer the question of how Nsp3c achieves modulation of protein expression.

Methods: Potential Nsp3c target sequences of 3' untranslated regions of genes involved in the apoptotic and inflammatory response were analyzed with the G4 hunter algorithm. The interaction between Nsp3c peptides and several target sequences of human mRNAs was characterized with liquid-state NMR spectroscopy and complementary biophysical methods.

Results: Titration with different oligonucleotides showed that the Nsp3c peptides bind nucleic acids in general and specific modes via different amino acid residues. Tyrosine residues were mainly involved in the general binding mode of a ribosomal RNA hairpin, while specific binding to guanine-rich oligonucleotides was achieved through arginine and lysine residues. The identification of the most stable RAB6B mRNA structures allowed precise analysis of the RNA binding site at the 3'-end of the oligonucleotide and the two terminal G-quartets. Complex formation resulted in a significant broadening of a subset of peptide and oligonucleotide signals, suggesting that the complex is dynamic and alternates on a millisecond timescale. However, since the binding of the entire Nsp3c domain is in the nanomolar range, additional binding surfaces must be involved in the interaction.

P-176 - A method to follow real-time hyperpolarized [1-13C]-pyruvic acid metabolism in living bacteria

Mrs Arianna Ferrari

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Hyperpolarized MR has enabled real-time metabolic imaging in humans and animals. However, it is yet unclear if hyperpolarized [1-13C]pyruvic acid MR is suited to follow the metabolism in microbes.

Our aim was to assess hyperpolarized [1-13C]pyruvic acid utility to reveal aerobic glycolysis in *E. coli* bacteria. Hyperpolarization was prepared with dissolution dynamic nuclear polarization (dDNP).(1)

E. coli (K12, and TOP10(2)) were grown in LB medium overnight (250 rpm, 37°C). The bacteria were collected, washed with HEPES (40 mM, pH 7.3), resuspended to HEPES and filled into an NMR tube. The tube was placed in a 400 MHz NMR at 37°C, while hyperpolarizing a standard dDNP sample (~ 50 mM of pyruvic acid and 30 mM of trityl radical) with a cryogen-free dDNP polarizer.(3) About 26 s after the dissolution, the hyperpolarized sample (~40° C), was injected into the NMR tube containing *E. coli* bacteria for signal acquisition.

With this method, we consistently observed three signals at 124.60 ppm, 160.27 ppm, and 182.5 ppm, which were assigned to CO₂, bicarbonate, and lactate. An average liquid state ¹³C polarization was 26.4±4.75 %. The peaks were slightly shifting to the right during the experiment, because of temperature changes. We noticed an improvement in aerobic glycolysis when the bacteria were “starved” in the poor-nutrient medium.

With this, we present the protocol for the observation of the pyruvate metabolism of microbes using dDNP hyperpolarization. Next, we will study the metabolism of other metabolites in *E. coli* LF82 isolated from Inflammatory Bowel Disease (IBD) patients.

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P-072 - Interaction of N-and C-terminal SH3 domains of Drosophila adapter protein Drk with Sos and Dos

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Drk, a Drosophila homologue of human GRB2, binds towards the phosphotyrosine residue of Sev via its SH2 domain, while the N- and C-terminal SH3 domains (Drk-NSH3 and Drk-CSH3, respectively) are responsible for the interaction with proline-rich motifs of Son of sevenless (Sos) or Daughter of Sevenless (Dos). Isolated Drk-NSH3 is in a conformational equilibrium between the fold and unfolded states, and it interacts with the PxxPxR motifs in Sos. Drk-CSH3 is supposed to bind the PxxxRxxKP motifs in Dos. To identify the functional differences between the two SH3 domains, structure determination of Drk-CSH3 as well as the NMR titration experiments with Sos- and Dos-derived peptides were performed. The Drk-SH2 domain's solution structure is also determined to investigate the interdomain interaction.

Methods

The titration experiments with SH3 domains were conducted with peptides from Sos, YRAVPPPLPPRR (1339–1350, Sos-S1), and GELSPPIPPRL (1377–1389, Sos-S2), or Dos, DCPVNRKLKP (638–648, Dos-S1) and GPPSVDRKCKP (690–700, as Dos-S2). The dissociation constants were calculated from the chemical shift perturbation (CSP) and the docking simulations of the abovementioned peptides were performed using the program AutoDock CrankPep (ADCP) 0.1.

Results and Conclusion

The solution structure and the ¹⁵N-relaxation data showed that Drk-SH2 and Drk-CSH3 consist of stable domains. Unlike Drk-NSH3, Drk-CSH3 exhibits completely folded. Titration data reveals that a large CSP was commonly found around the RT loop and the hydrophobic patch of both SH3 domains during the Sos and Dos-peptide interactions. Sos-derived peptide interactions shifted the equilibrium of Drk-NSH3 to folded state whereas Dos-derived peptides did not. Sos-derived two peptides with PxxPxR motif showed stronger affinity towards both the SH3 domains. Aiming at investigating the cooperativity of the two SH3 domains in Sos- and Dos-binding, further NMR experiments using full-length Drk are in progress.

P-280 - Flow encoding established by optimal control RF pulse

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Flow encoding MRI is traditionally achieved by applying bipolar gradients which cancel the even terms of Taylor expansion of the magnetization phase with respect to time, and the odd terms lead to a predominantly velocity-dependence of the phase. In this study, we show that velocity can be encoded into phase during the excitation time. First, a mathematical model is designed. Then, optimal control (OC) theory is used to design the required flow encoding RF pulse. Also, GRAPE algorithm is employed to minimize the cost function in the OC problem, and a constraint is added to make the RF pulse slice selective.

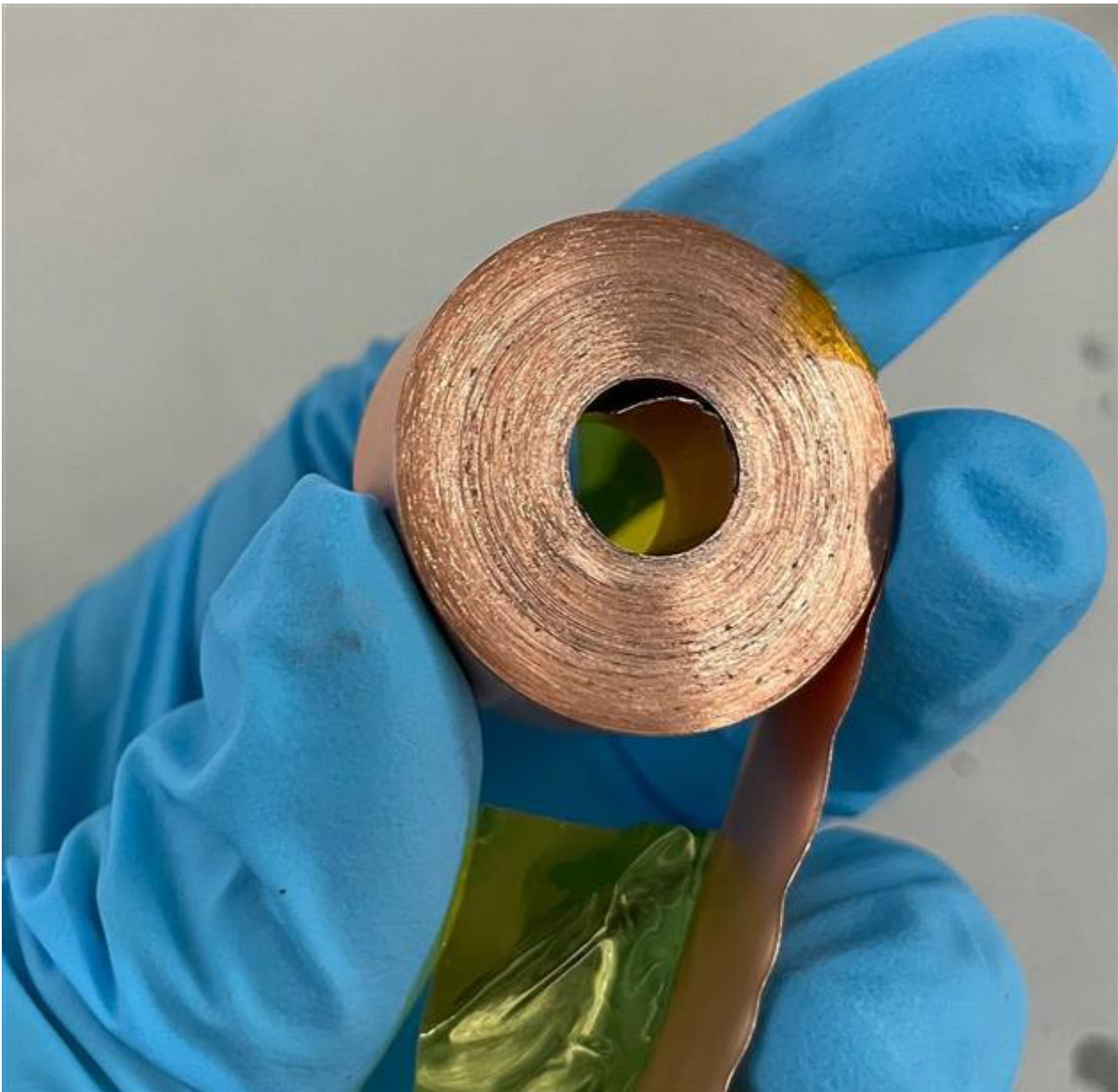
Since in this method encoding is achieved based on a mathematical model, it opens new doors to many beneficial abilities and flexibilities which are not accessible by traditional methods. First, during this process, encoding is established without the reliance on switching the gradients. This avoids the adverse effects of gradient induced eddy currents. Second, it enables one to achieve shorter echo times hence enhance signal-to-noise ratio. Furthermore, bipolar gradients lead to a linear relationship between phase and velocity; but in this method, a non-linear relation can be made which improves phase-SNR of regions with lower flow rates, for example close to vessel walls. Regarding further studies, many other parameters can be taken into account in the mathematical model. For example, OC problem can be asked to improve contrast in multiphase flows or address adverse effects of acceleration in turbulent flows.

P-160 - Watch-sized 7 Tesla Magnet

Dr Pin-Hui Chen¹, Dr. Chukun Gao¹, Dr Nicholas Alaniva¹, Dr Snædis Björgvinsdóttir¹, Mr Alexander Däpp¹, Ronny Gunzenhauser¹, Michael Urban¹, Dr. Ioannis Pagonakis¹, Prof. Dr. Alexander Barnes¹
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Dynamic nuclear polarization (DNP) can boost NMR sensitivity by orders of magnitude, allowing applications in in-cell NMR, structural biology and functional materials. Up to now the highest field demonstrated with DNP is 21.1 T. We aim to build an all-high-temperature-superconducting (HTS) gyrotron magnet for DNP at 28 T. Gyrotron magnets require less field homogeneity and stability than NMR magnets. For instance, gyrotron magnets only need a homogeneity of 1% over a few millimeters, while NMR magnets typically require a homogeneity of 0.01 ppm. This, combined with the current-carrying capabilities of high temperature superconductors (HTS) above 4.2 K and feasibility of non-persistent mode operation, frees magnet development paths from conventional restrictions. Later, such compact ultra-field magnets could be designed and shimmed for NMR use. Here, we present our progress towards building a 28 T gyrotron magnet using only high temperature superconductor (HTS). HTS, made of rare earth barium copper oxide (REBCO) ceramics, can sustain higher current density than low-temperature superconductors and therefore generate very high magnetic fields. Here, HTS tape is wound in a pancake coil geometry using a customized winding machine. The magnet has an inner diameter of 8 mm, an outer diameter of 25 mm and a height of 10 mm. Note, the total tape length is only 9.4 m. With a current of 1100 A driven in non-persistent operation, this miniature magnet achieved a stable field of 7 Tesla in a liquid helium bath (4.2 K). The small bore size greatly reduces the amount of HTS tapes required for high-field magnets. The miniature magnets could enable better magnets at higher fields and operation with cryogen-free systems, or liquid nitrogen. This miniature magnet is an important step towards realization of 28 T magnets for gyrotrons and pulsed DNP at 792 GHz.



P-324 - Effect of surfactant aggregation on cloud formation and aerosol cooling potential

Professor Ville-veikko Telkki¹, Dr Anne Selent¹, Dr. Pär Håkansson¹, Dr. Sampo Vepsäläinen¹, Ilari Ainasoja¹, Dr Sanna Komulainen¹, Pau Mayorga Delgado¹, Dr Otto Mankinen¹, Jiří Mareš¹, Dr Ritu Ghanghas¹, Silvia Calderón¹, Dr Vladimir Zhivonitko¹, Jack Lin, Jussi Malila¹, Nønne Prisle¹

¹University of Oulu, Oulu, Finland

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Organic surfactants, which are common constituents of atmospheric aerosols, change both surface tension and water activity of cloud droplets. The aggregation of surfactants significantly affect the cloud droplet growth, inducing yet not well-known potential effects on global climate.

Aims

This work aims at improved understanding of aggregation phenomena of surfactants in aqueous solutions as well as the effect of aggregation on cloud droplet growth.

Methods

We combine advanced relaxation and diffusion NMR techniques with state-of-the-art relaxation modelling, in which dynamic and structural parameters are extracted from MD simulations, to elucidate the aggregation of surfactants. Aqueous sodium decanoate was used as a representative model system. Furthermore, we use thermodynamic models and Köhler theory to estimate the effect of aggregation on growth of cloud droplets.

Results

Contrary to the common understanding, our NMR analysis implies that surfactants form small clusters below the critical micelle concentration (CMC). Furthermore, it shows that the micelle size above the CMC is significantly smaller than the value provided by a conventional empirical model. Water diffusion experiments revealed water-encapsulating aggregates at higher concentrations and exchange rates between the encapsulated and free water pools were determined by ultrafast diffusion exchange spectroscopy. Thermodynamic modelling of aerosol hygroscopic growth and cloud droplet formation showed that the aggregation phenomena may significantly affect the hygroscopic growth factors at lower humidity conditions, leading to misrepresenting the aerosol cooling potential.

Conclusions

Combined experimental relaxation and diffusion NMR and relaxation modelling provided groundbreaking information about clustering, micelle sizes and water encapsulation in aqueous surfactant solutions. Thermodynamic modelling showed that the aggregation needs to be taken into account to avoid biased predictions in climate models. [1,2]

[1] O. Mankinen et al. Ultrafast diffusion exchange NMR. Nat. Commun. 11, 3251 (2020).

[2] A. Selent et al. Surfactant aggregation may bias aerosol cooling potential. To be submitted.



P-078 - NMR studies of Artificial Metalloenzymes in vitro and in bacterial cells

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Effective protein design for new-to-nature reactions requires a detailed understanding of the relationship between reactivity and the enzyme conformational landscape, including its dynamics. Previously, we showed that employing magic-angle spinning (MAS) in-cell solid-state (ss) NMR spectroscopy[1] is a powerful spectroscopic tool that can be used to probe an artificial metalloenzyme (ArM) at atomic level inside *Escherichia coli* cells[2]. Our research focused on an ArM composed of the paramagnetic cofactor copper(II)-phenanthroline bound to the homodimer of the lactococcal multidrug resistance regulator (LmrR). This complex is capable of catalyzing Friedel-Crafts alkylation of indoles with high yields and exceptional enantioselectivity[2]. Recently, we used solution-state NMR spectroscopy to study the binding events and protein dynamics of a variant of the ArM in vitro. This variant has been optimized through directed evolution and can bind the diamagnetic zinc(II)-phenanthroline cofactor. Our results reveal that the successive directed mutations that improve reactivity cause multiple backbone conformations to appear of the LmrR homodimer in its apo state that are modulated when a cofactor binds. Additionally, titrating Friedel-Crafts reaction substrates to the complex has revealed additional protein sites that could be targeted and may further improve ArM activity in vivo. To further elucidate the conformational space of the LmrR complex in cells we study whole cell lysates and employ dynamic nuclear polarization (DNP) MAS-ssNMR at high magnetic field (800 MHz/527 GHz) conditions, allowing us to make use of recent advancements in DNP radical design[3,4].

[1] S. Narasimhan et al. *Nat Protoc.* 2021, 16, 893-18. [2] S. Chordia et al. *Angew Chem Int Ed.* 2021, 60, 5913-20. [3] X. Cai et al. *Chem Eur J.* 2021, 27, 12758-62. [4] R. Yao et al. *Chem Sci.* 2022, 13, 14157-64.

P-274 - MRI and CT of lung cancer patients: a comparison between radiomic features from two different imaging modalities

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¹University of Pavia, Pavia, Italy, ²INFN, Pavia, Italy, ³I.R.C.C.S. San Matteo Hospital, Pavia, Italy

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction_Magnetic Resonance Imaging (MRI) of lung is far from reaching Computed Tomography (CT) performances, due mainly to low signal and to lack of standard acquisition protocols. Despite such obstacles, as MRI provides anatomical and functional information in a non-invasive way, great efforts are directed to improve the lung MRI images quality. Additionally, to increase the quality and quantity of information extracted from medical images, radiomics methods are nowadays used.

Aims_Within the above framework, our goal was to investigate the statistical reliability of CT and MRI lung cancer radiomic features and the agreement between two radiomics platforms, Pyradiomics and LIFEx.

Methods_We analyzed both CT and MRI segmented images from 26 patients. Three different preprocessing methods were applied and 66 IBSI-compliant features common to the two radiomics software were selected. The correlation between Pyradiomics and LIFEx features for each imaging technique, and the correlation between CT and MRI features for each radiomics platform was investigated both visually, on graphs, and quantitatively, using the Spearman coefficient and the Intraclass correlation coefficient (ICC).

Results_For all voxel resampling methods considered, MRI images have shown good/excellent ICC reliability for nearly 90% of features, while the same level has been reached by 85% of CT features. As regards the comparison between the two different imaging modalities, for both radiomics software less than 15% of features has shown excellent and good ICC reliability.

Conclusions_Radiomics is a powerful tool through which is possible to analyze the abilities of different imaging modalities to extract novel clinical biomarkers. ICC reliability between the two software was excellent for at least 45% of the features in both datasets (CT and MRI). The analysis of the volume and sphericity tumor area can be evaluated by means of both imaging modalities, and MRI may be chosen to reduce patients' exposure to radiation and radiotracers.

P-048 - Structural basis of metabolite transport by the chloroplast outer envelope protein OEP21 by solution NMR

Dr Umut Günsel^{1,2}, Kai Klöpfer¹, Dr. Elisabeth Häusler², Dr Manuel Hitzenberger³, PD. Dr Bettina Bölder⁴, Dr Laura Sperl¹, Prof. Dr. Martin Zacharias³, Prof. Dr. Jürgen Soll⁴, Mr Franz Hagn^{1,2}

¹Bavarian NMR Center (BNMRZ), Department of Bioscience, School of Natural Sciences, Technical University of Munich, Garching, Germany, ²Institute of Structural Biology, Helmholtz Munich, Neuherberg, Germany, ³Lehrstuhl für Theoretische Biophysik (T38), Department of Bioscience, School of Natural Sciences, Technical University of Munich, Garching, Germany, ⁴Biozentrum, LMU München, Department of Biology, Planegg-Martinsried, Germany

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Chloroplasts, as the primary site of photosynthesis, are vital organelles in plant cells, by fixing CO₂ into triose phosphates (TPs) through the Calvin-Benson-Bassham cycle. In addition, they are involved in the synthesis of various metabolites, such as amino acids, fatty acids, nucleotides, and vitamins. Chloroplasts are encircled by the inner and outer envelopes (IE, OE). Thus, the transport of metabolites is assumed to be tightly regulated by specific transporters and channels. Although the transport across the IE is relatively well understood, our knowledge of the transport proteins in the OE remains sparse. In this study, we used nuclear magnetic resonance (NMR) to determine the high-resolution structure of an OE channel, OEP21, the main exit pore for TPs in C₃ plants. OEP21 adopts a cone-shaped β -barrel pore structure with 12 anti-parallel β -strands and a wider opening toward the intermembrane space. The inner pore surface is predominantly decorated with positively charged amino acids, suggesting specificity for negatively charged metabolites. Indeed, we observed a charge-dependent interaction mechanism, where the positively-charged residues bind to various negatively charged phosphorylated metabolites in a competitive manner. Interestingly, our biochemical assays with OEP21 reconstituted in liposomes revealed that metabolite transport across OEP21 is relatively fast with a size cutoff of 1 kDa. Furthermore, we found that ATP has a significant impact on the stability and monomer-to-oligomer equilibrium of OEP21, favoring the monomer and keeping the channel open. Taken together, our results suggest that OEP21 is a promiscuous membrane pore for phosphorylated metabolites whose activity is modulated by ATP. Together with other OEPs, this promiscuous mechanism enables the translocation of a broad range of essential metabolites by a limited number of transport proteins.

Günsel, U., et.al. & Hagn, F., (2023), Structural basis of metabolite transport by the chloroplast outer envelope channel OEP21, Nat. Struc. Mol. Biol., principally accepted

P-164 - Development of a Broadband On-Chip EPR Spectrometer.

Ms Selina Eckel

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Conventional Electron Paramagnetic Resonance (EPR) spectrometers are limited by their narrow bandwidth and large physical dimensions. To overcome these limitations, we present a path towards a broadband on-chip EPR spectrometer. The broadband approach allows applications such as the distinction between field-dependent and field-independent processes like the Electron-Zeeman Interaction and Zero-Field Splitting. Moreover, an on-chip spectrometer can reduce size, cost, and complexity of the overall system. Such a device can be realized by using a broadband transmission line instead of a narrowband resonator-based coupling structure.

As a first step, we compare three different system architectures to realize the envisioned EPR spectrometer. All measurements are done at a frequency range from 0 to 28 GHz with a coupling structure based on a meandered co-planar transmission line. The first architecture is a superheterodyne receiver where the EPR signal is measured on a fixed intermediate frequency. The second architecture extends this with an interferometer to cancel the unwanted large signal so that only the small EPR signal is present on the receiver. The third architecture is a millimeter-wave detector with lock-in detection.

For a first implementation of an on-chip EPR spectrometer, the third architecture is chosen because it has the lowest requirements for the millimeter-wave components and has a high signal-to-noise ratio due to the inherently good filter capabilities of the lock-in principle. Two components central to the prototype, a single-ended broadband amplifier and a corresponding detector, were designed and manufactured. The characterization of the two integrated circuit components is presented.

P-016 - Putting the cat among the pigeons: a study on the large-scale impacts of mutagenesis in a dynamic NRPS protein

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Objectives

Nonribosomal peptide synthetases (NRPSs) biosynthesize several antibiotics and therapeutics and are targets for production of novel pharmaceuticals. Within their flexible, multidomain architecture, cyclization (Cy) domains catalyze peptide bond formation and cyclodehydration between two amino acid substrates. These substrates are funneled into Cy domains by two interacting thiolation (T) domains. Engineering NRPSs could produce novel therapeutics but mutagenesis results are often confounding. We developed an NMR approach to determine how 52 kDa Cy domains recognize their T domain partners and their attached substrates and how mutagenesis affects this communication.

Methods

We determined the solution structure of the Cy domain, Cy1 (52 kDa), from yersiniabactin synthetase. We used relaxation dispersion to determine the dynamics of Cy1 and developed an NMR readout to monitor its allosteric response with one of its substrate-loaded thiolation domain partners, T1. With this approach, we could determine how mutagenesis affects both dynamics and function.

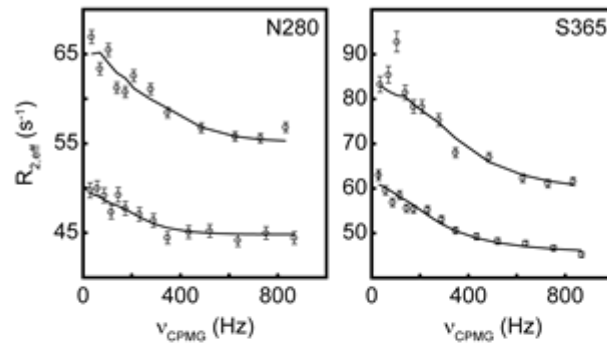
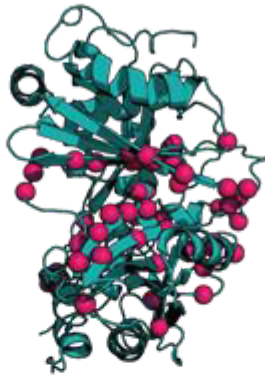
Results

The structure of Cy1 displayed substantial heterogeneity, and relaxation dispersion revealed a network of dynamic residues linking the two T domain binding sites pointing at allostery. We then uncovered a substrate-specific allosteric response of Cy1 to its T domain partner. We could then demonstrate that introducing a mutation shown to reduce activity produced a global response that both significantly damaged Cy1's dynamics and knocked out its allosteric response.

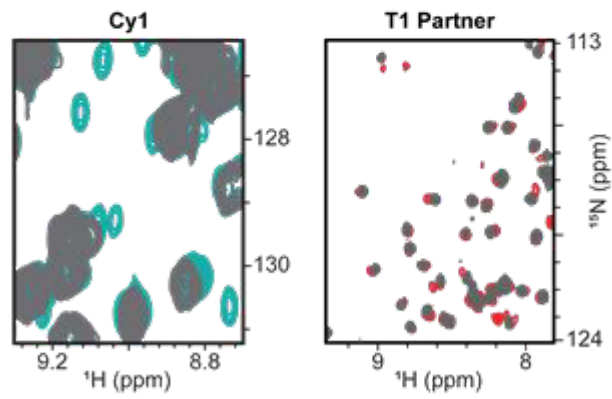
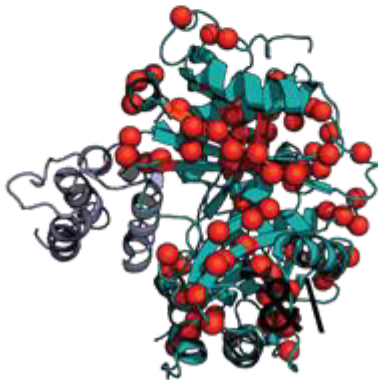
Conclusions

We tied dynamics to Cy1's allosteric response using mutagenesis. This allostery may be required to ensure that Cy domains only engage their T domain partners when both hold substrates, a critical consideration for bioengineering strategies. That mutagenesis globally impacted Cy1 highlights the need to integrate dynamics into new NRPS mechanisms.

Cy1 Dynamics



Cy1 Allostery



P-044 - Combination of solution and solid-state NMR at fast MAS for the structural characterization of SARS-COV-2 ORF6 membrane protein

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

By using fast MAS solid-state NMR we propose a structural model for ORF6 that differs from the proposed AlphaFold2 models.

While X-ray diffraction and cryo-EM are effective techniques for determining the structures of large molecular systems, smaller systems can only be accurately determined using NMR. However, small membrane proteins present a challenge for solution state NMR due to the size of the detergent micelles required for their solubilization. In addition, fewer structures of this protein group have been experimentally solved, resulting in less accurate predictions by AlphaFold2. ORF6 is a 61-amino acid, membrane-associated accessory protein of SARS-COV-2, which is found to be the most cytotoxic protein of the virus by interfering with nuclear pore transport and causing interferon inhibition. However, the relationship between its membrane localization and the nuclear pore remains unclear. We aim to decipher the structure and oligomeric state of ORF6 protein using detergent micelles or lipid membranes. To better understand the oligomerization behavior of ORF6, we employed a range of biophysical techniques, including SEC-MALS, analytical ultracentrifugation and NMR. A combination of solution- and solid-state NMR was used to gain insight into its structure and dynamics.

Our data show that the micelle-associated ORF6 have a not defined oligomeric state, which depends on the protein to micelle ratio. This suggests that detergent micelles cannot mimic the native membrane. The combination of solution and solid-state NMR data allowed us to get a complete picture of the protein in different environments. In detergent micelles allowing to specifically assign the C-terminal flexible amino-acids, and in lipid membranes allowing the detection of more rigid residues. Specific amino-acid labelling and paramagnetic relaxation enhancement allowed us to assign the structured region and to identify significant structural differences from the models obtained by AlphaFold2.

With this comprehensive approach, we propose a new structural model of ORF6.

P-336 - Computationally-Assisted Multifrequency STD NMR Toolkit for the Fast Characterisation of Low Affinity Protein-Ligand Interactions

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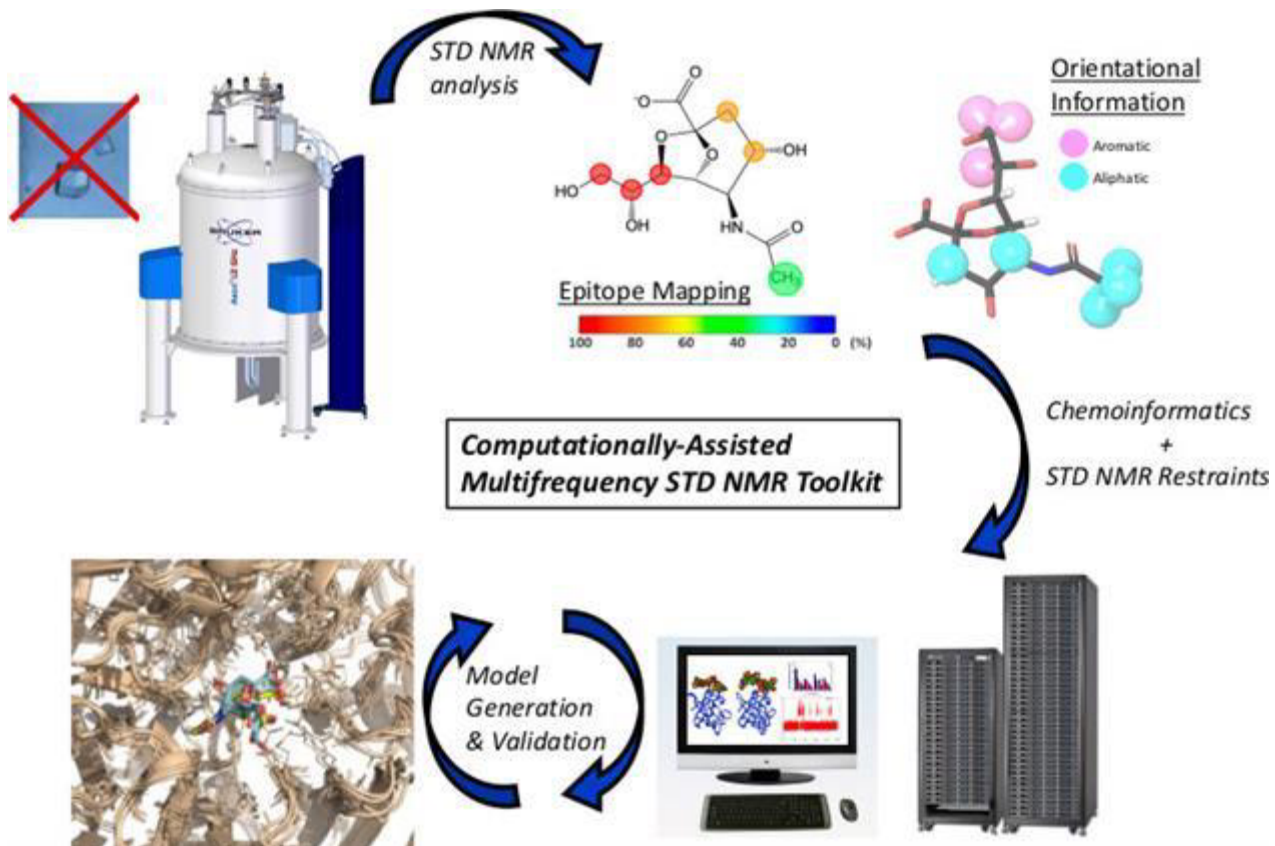
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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Saturation transfer difference (STD) NMR spectroscopy has revolutionized the study of low affinity receptor-ligand interactions due to its versatility and popularity, as evidenced by the myriad of approaches and applications developed. Methodologies such as (i) inter-ligand STD NMR to characterise fragments' interactions in adjacent protein subsites, DEEP-STD NMR to gain ligand orientation information, KD determination, SSTD to study mutual site chemical exchange processes, and ligand binding epitope mapping are some elegant examples.

Low affinity protein ligand/fragment complexes are particularly challenging to characterise by standard methods such as X-ray crystallography due to unsuccessful crystal formation or to low ligand occupancy in the crystal. Hence, our aim is to provide the NMR and drug discovery communities with a computationally-assisted NMR toolkit that allows for the rapid characterisation and validation of 3D models of protein-ligand complexes where crystallisation fails. Towards pursuing this goal, we have recently developed the RedMat software, which relies on full relaxation matrix calculations simplified by the concept of Reduced Matrix, for the structural evaluation of static and dynamic protein-ligand 3D models using in-solution STD NMR data. We have also developed analytical and machine learning (ML)-based approaches for fast ligand binding epitope mapping analysis, significantly reducing the experimental and data analysis time required.

Herein, we provide an overview of the strengths and limitations of STD NMR spectroscopy and present a toolkit that combines STD NMR approaches assisted by computational and ML-based methods for the fast experimental acquisition and characterisation analysis of low affinity protein-ligand interactions. Specific examples will be presented to illustrate how our toolkit can provide very important insights into challenging protein-ligand systems that would otherwise be discarded, which is particularly relevant for Fragment Based Drug Discovery (FBDD) projects.



P-232 - Structure of Titanium Surface Sites in Ziegler-Natta Pre-Catalysts from 47/49Ti MAS NMR Signatures

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¹ETH Zurich, Zürich, Switzerland, ²CP2M Lyon, Lyon, France, ³EPFL, Lausanne, Switzerland

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Ziegler-Natta catalysts (ZN) are of the highest importance for worldwide production of polyethylene (PE) and polypropylene (PP). Yet, their development was mainly empiric in the view of high complexity of the heterogeneous catalytic system and various factors influencing activity and selectivity of ZN, including Ti sites, support, various additives etc. For rational development, structure-activity relationships are necessary, which require molecular-level understanding of Ti sites, known to form active sites in polymerization.

While 47/49Ti NMR is highly sensitive to Ti local surrounding and electronic structure, it is extremely complicated because two Ti isotopes are i) quadrupolar nuclei, ii) low abundant, iii) have rather large quadrupole moment, and vi) are in close proximity in NMR spectra (within 267 ppm), often yielding overlapping broad lines. For ZN, situation is worsened by low Ti content and heterogeneity of Ti sites. In this work, we tackle these challenges by i) using the high magnetic field (900 MHz) and low temperature (100 K) to significantly narrow down the NMR lines and gain sensitivity due to temperature, ii) extensive DFT modelling of NMR parameters including a libraries of molecular complexes (benchmarking) and surface models of Ti sites in ZN, and iii) using BCl₃-treated ZN-PE, to obtain Ti sites, expected to have rather symmetric electric charge distribution around Ti, hence lower CQ values critical for enabling experimental observation of Ti surface species in ZN.

As a result, we obtained NMR spectroscopic signatures of Ti sites in ZN that enabled to resolve the structure of Ti surface sites adsorbed on MgCl₂ (i.e. representative industrial precatalyst) surface using DFT modelling. Analysis of chemical shift and CQ helped to highlight the influence of the local environment and MgCl₂ morphology on the electronic structure of the Ti sites. This work constitutes a key step towards establishing structure-activity relationships in ZN catalysis.

P-328 - The Aharonov-Anandan phase and geometric double-quantum excitation in strongly-coupled nuclear spin pairs

Dr Mohamed Sabha¹, Dr. Christian Bengs¹, Professor Malcolm Levitt¹

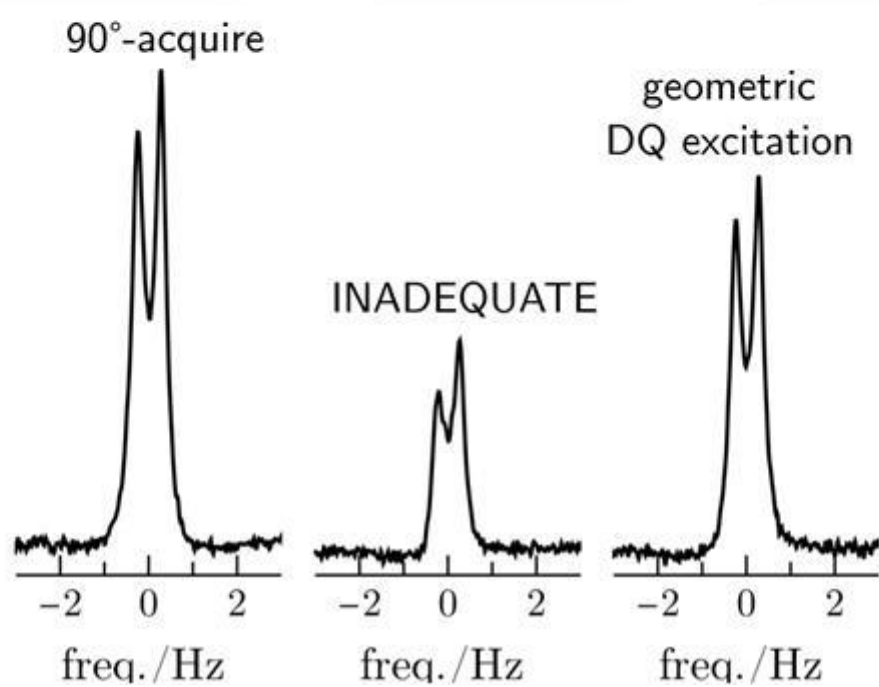
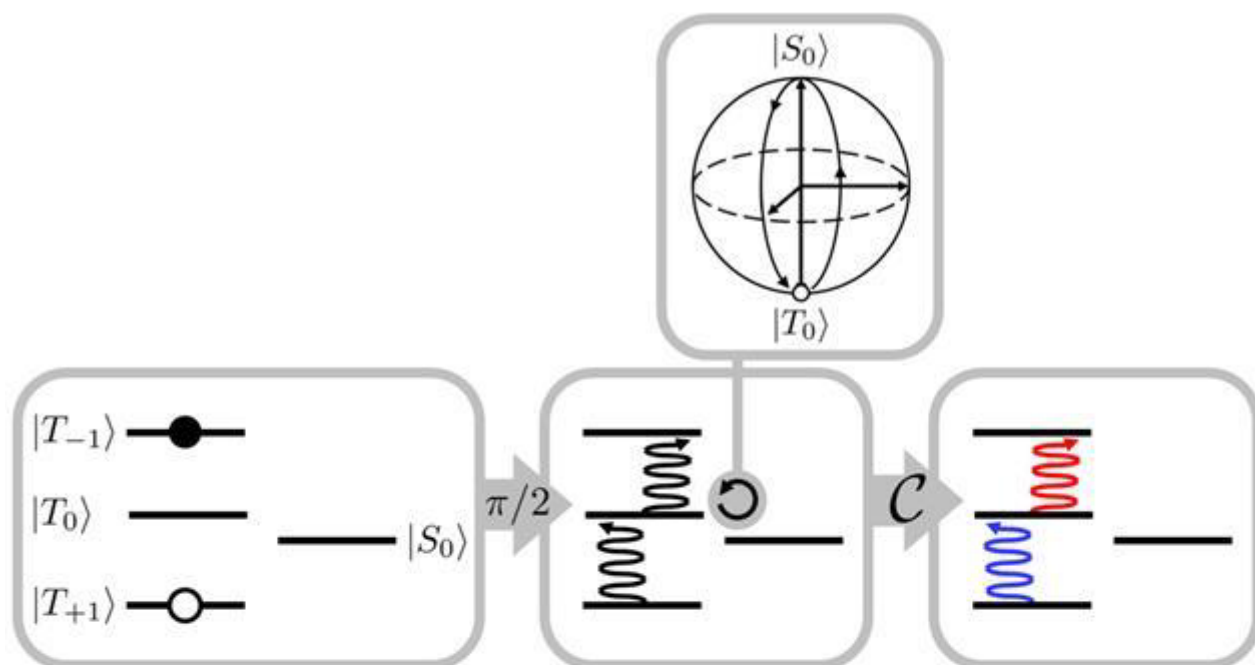
¹University of Southampton, Southampton, United Kingdom

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The Aharonov-Anandan phase is a contribution to the phase acquired by cyclic evolution of a quantum state which depends only on the geometric properties of its trajectory.

We report the study and the exploitation of the Aharonov-Anandan phase by NMR interferometry techniques in homonuclear spin-1/2 pairs in the near-equivalence limit. We introduce a new method for engineering effective zero-quantum Hamiltonians with arbitrary phase in the transverse plane. We use this method to generate a variety of cyclic zero-quantum paths enabling direct study of the geometric Aharonov-Anandan phase to probe the rotational characteristics of the zero-quantum subspace.

Double-quantum excitation is routine in solution NMR, with several applications to spectral filtration, peak assignment, and the study of cross-correlated relaxation processes. However, double-quantum excitation is challenging outside of the weak-coupling limit. We show that the geometric Aharonov-Anandan phase may be used for efficient double-quantum excitation in strongly coupled spin pairs. Geometric double-quantum excitation comfortably outperforms the standard method (INADEQUATE) in the near-equivalence limit.



P-008 - Benchtop NMR relaxometry to monitor Ni(II) removal by resin and activated charcoal.

Miss Marie Bernardi

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Water pollution by heavy metals is a major environmental concern. In this context, removal of heavy metal from wastewater by ion exchange resins or adsorbents is often necessary. However, current techniques to study adsorption efficiency are indirect and sometimes destructive. Some heavy metal ions like Ni(II) have paramagnetic properties that can affect the NMR relaxation times T1 and T2 of water protons, which can be measured by benchtop NMR relaxometry. Therefore, T1 and T2 can be used to monitor the removal of paramagnetic heavy metals by a sorbent. In this work, the removal of Ni(II) by Dowex Marathon MSC resin and commercial activated charcoal was studied.

Batch experiments were carried out to study the ion exchange/adsorption isotherms: a sample containing 10 mg of resin or 50 mg of activated charcoal was put in contact with aqueous solutions containing different concentrations of Ni(II), before being shaken by a vortex mixer. Once the equilibrium reached, T1 of the solution was measured which allowed the determination of the amount of adsorbed metal. The study of the loaded sorbent was also carried out.

The equilibrium isotherms of Ni(II) were satisfactorily described by the Langmuir model for both studied sorbent. The longitudinal and transverse relaxation of the wet resin is shown to be biexponential. The relaxation rates of the fast relaxing water fraction of the Ni(II) loaded wet resin can be correlated with the metal content obtained independently by AES after acidic digestion of the samples. Loaded activated charcoal, due to its important microporosity but also because of a partial desorption of Ni(II) ions, exhibits a more complex relaxation behavior.

Based on these results, a column experiment will be set up inside the NMR coil in order to follow the loading of the sorbent in real-time through the evolution of the NMR signal.

P-216 - Large Volume Purification of Hyperpolarised Noble Gases via Multi-stage Combustion after Spin Exchange Optical Pumping

Mr Arthur Harrison

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: Hyperpolarised (HP) noble gas MRI enables probing of lung function and microstructure. The HP state is achieved via Spin Exchange Optical Pumping (SEOP); however the process necessitates temporary dilution of the pumped species with a buffer gas and a radiation quenching agent that must be removed before MRI application. In previous work, catalytic combustion demonstrated the removal of molecular hydrogen after usage as SEOP buffer gas and radiation quenching agent. This novel process makes the purification of HP^{83}Kr despite its nuclear electric quadrupole moment possible. The protocol may streamline HP^{129}Xe production.

Aims: Prototype demonstration of efficient HP^{83}Kr and HP^{129}Xe purification via combustion to allow for preclinical MRI applications.

Methods: We have built a prototype device for the automated purification and recompression of large volumes of HP^{83}Kr and HP^{129}Xe . The system utilises multi-stage oxidation with catalytic and spark initiated combustions. A fully computer-controlled combustion reactor and SEOP process can operate via either 'batch' or 'semi-continuous' mode.

Results: Combining catalytic combustion with spark ignition capabilities, multiple combustion phases can purify the HP noble gas deliveries to over 95%. The combustion process can be tailored to either HP^{83}Kr or HP^{129}Xe to maintain the respective HP state. 'Batch' mode concentrates a single delivery of HP gas very quickly and is scalable to accommodate any quantity of gas from the SEOP polariser. 'Semi-continuous' mode utilises both a catalytically induced oxygen flame and spark triggered ignitions to purify multiple batches of HP gas. This permits scaling of the accumulated HP gas to meet gas quantity requirements, irrespective of the polariser capabilities.

Conclusions: Combustion purification methodology for HP noble gases has matured to attain sufficient noble gas quantities and purities for HP MRI applications.

Figure 1: 'Batch mode' combustion purification of HP noble gas. Including pressure profile within the chamber (A) and associated images of the oxidation process (1,2,3,4).

P-060 - The backbone dynamics of Interleukin-17 gives insights to function

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Knowledge of protein dynamics is fundamental to the understanding of biological processes. We have combined NMR and 2D-IR spectroscopy to gain a new understanding of the dynamic nature of the cytokine IL-17. Interleukin-17's (IL-17) are proinflammatory cytokines which are associated with several serious autoimmune diseases (e.g., psoriasis). Previous crystallographic studies have been unable to explain the dramatic differences in affinity observed between IL-17 dimers and their receptors, suggesting there are factors that cannot be fully explained by the analysis of static structures alone. Here, we show that the IL-17 family of cytokines have varying degrees of flexibility which directly correlates to receptor affinity. In addition, a small molecule inhibitor which was thought to function by either causing steric clashes or structural changes appears to function via an alternate mechanism of inhibition. The small molecule rigidifies the protein so causing a reduction in affinity. These results indicate an induced fit model of cytokine:receptor binding, with the more flexible cytokines having a higher affinity. The approach presented here could be applied to other systems where the inhibition of a protein-protein interaction has proved intractable. The targeting of allosteric sites which modulate protein dynamics could open new avenues for novel therapeutic development.

P-196 - Using SABRE to facilitate the detection of a range of non ^{15}N labelled substrates with long lifetimes.

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

One inherent issue with NMR is low sensitivity. Consequently, at a field of 9.4 T and a temperature of 298 K only around 1 in every 31,000 ^1H nuclei act positively to create the observed NMR signal. Hyperpolarisation techniques address this problem by artificially increasing the imbalance between nuclear spin orientations, dramatically improving our ability to detect such nuclei.¹ More recently, the latent spin order of parahydrogen has been used to sensitize the detection of a growing range of materials through both chemically changing hydrogenation and Signal Amplification By Reversible Exchange (SABRE).² SABRE is a form of molecular catalysis that uses a metal complex to transfer magnetism from parahydrogen without a change in chemical identity.

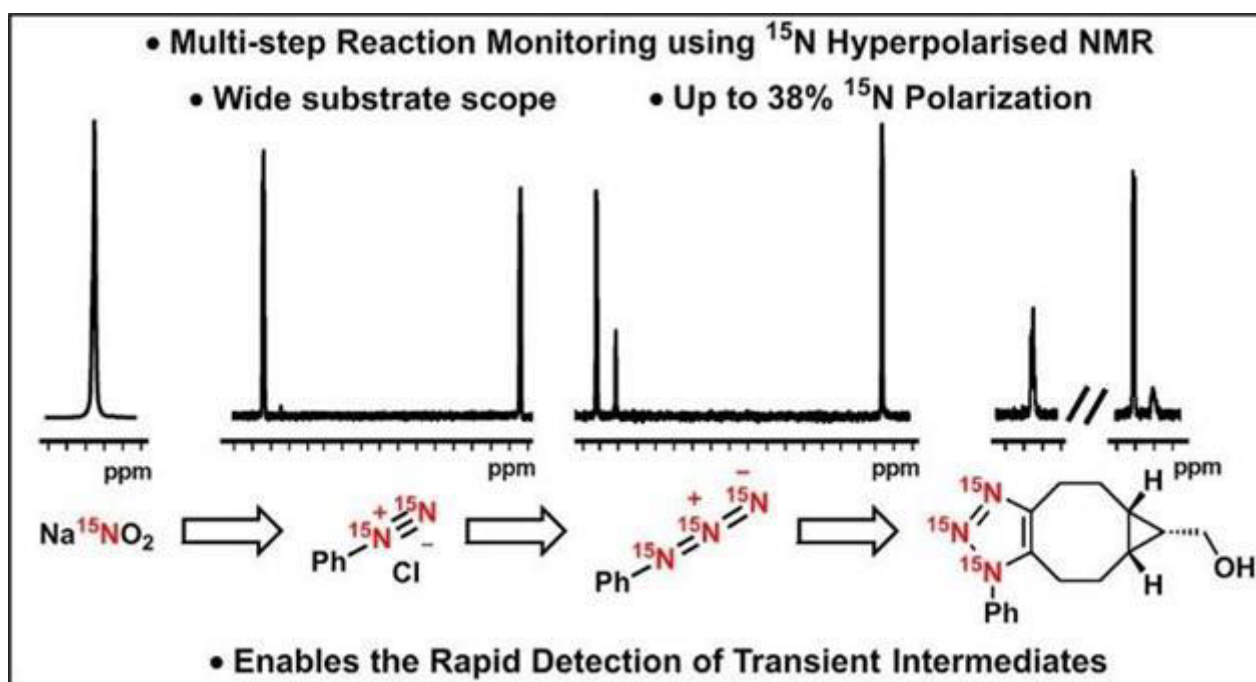
This study involves the examination of pharmaceutically relevant functionality such as nitrite, triazines, nitriles and pyridines. Strategies to overcome their weak interactions with a metal centre are developed to improve complex stability and hence facilitate SABRE. The chemical evolution of the catalyst system as it attains this point has been studied and a number of reaction intermediates identified by multi-nuclear NMR. Through optimisation, we then use the SABRE process to hyperpolarise a range of their ^{15}N nuclei, which are classically $\sim 260,000$ times harder to detect than ^1H signals at 9.4 T. ^{15}N polarisation levels of $>10\%$ are achieved for all substrates, with the highest being 38%. This makes the molecules readily detected in a single scan without the need for labelling.

This increase in sensitivity has been used to facilitate ^{15}N -DOSY measurements, which have been validated by comparison to an internal ^1H standard. Furthermore, the long T_1 of ^{15}N has been harnessed to facilitate reaction monitoring through the evolution of the SABRE enhanced response.³

¹J. H. Ardenkjaer-Larsen, PNAS, 2003, 100, 10158-10163.

²R. E. Mewis, Elsevier, 2018, pp. 145-212.

³P. J. Rayner, JACS, 2022, 144, 8756-8769.



P-070 - Beyond short linear binding motifs: chemical-exchange NMR reveals the dynamics and kinetics of interactions in intrinsically disordered proteins

Mr Duc Duy Vu

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction:

Interactions between intrinsically disordered proteins (IDPs) and their partners are vital in various biological functions. These interactions often occur through short linear binding motifs and are typically studied using short peptides in X-ray crystallography, as flexible parts would hinder crystallization. However, truncating IDPs to short motifs may suppress some essential features of the interactions. In contrast, chemical-exchange NMR techniques can provide information on both the rigid and flexible parts of IDPs, whether free or bound.

Aims:

Here, we used chemical-exchange NMR to investigate the interaction between the Ku-binding motif (KBM) in full-length MRI2 and the vWA domain of Ku80 (Ku80_vWA), which is involved in regulating the activity of the non-homologous end-joining pathway for DNA double-strand break repair.

Methods:

We used an integrative approach combining ¹⁵N Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion with ¹⁵N, ¹³CO, ¹³CA, and ¹³CB chemical-exchange saturation transfer (CEST) to investigate the kinetics of the interaction and to characterize both the structure and the dynamics of the bound form of KBM. The methodology only requires the observation of the signals from free MRI2 in the presence of sub-stoichiometric amounts of Ku80_vWA.

Results:

We found that the interaction between Ku80_vWA and MRI2 can be described using a two-state binding mechanism with well-defined kinetic parameters. Importantly, the residues adjacent to the KBM residues formed an alpha-helix upon binding, which was not previously detected in the crystal structure obtained with a too-short peptide of MRI2. Introducing a mutation in MRI2 that increased the helical propensity of this region in its unbound state resulted in faster binding kinetics.

Conclusions:

Our study revealed the important role of residues near the core binding motif of KBM_MRI2 in interacting with Ku80_vWA while illustrating the power of chemical-exchange NMR techniques for studying interactions of IDPs beyond short linear motifs.

P-022 - Intrinsically Disordered Tardigrade Proteins Self-Assemble into Fibrous Gels in Response to Environmental Stress

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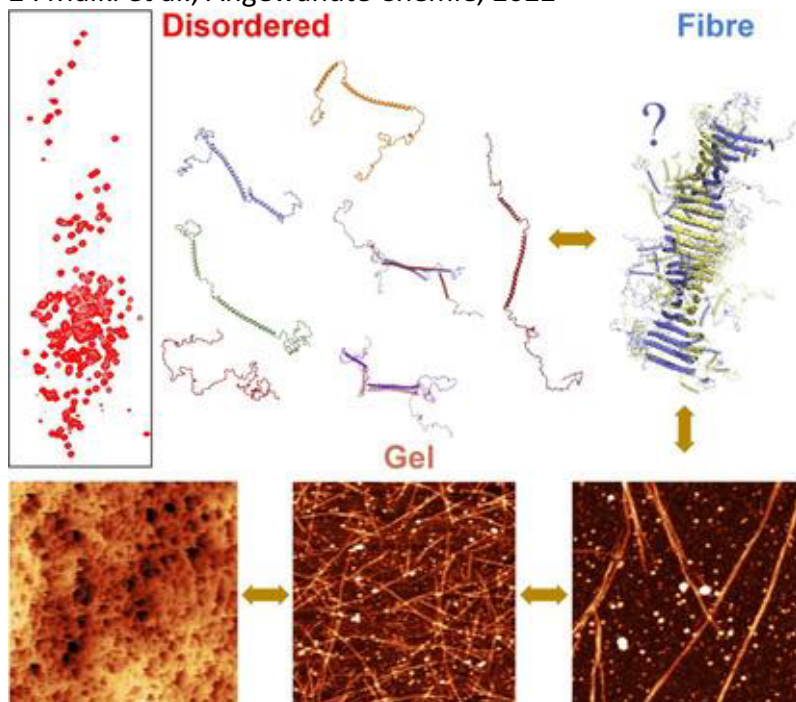
¹Institut De Biologie Structurale, CNRS, CEA, UGA, Grenoble, France, ²niChe Lab for Stem Cell and Regenerative Medicine, Department of Biochemical Science and Technology, National (Taiwan) University, Taipei, Taiwan

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Tardigrades are microscopic animals famous for their ability to survive harsh stress conditions as diverse as extreme temperature or pressure and desiccation. However, despite centuries of interest, the molecular mechanisms responsible for this unusual resistance to environmental stress remain unknown. Recently, tardigrade-unique intrinsically disordered proteins have been shown to play an essential role in tardigrade survival to extreme dehydration¹. We've characterized the conformational and physical behaviour of one of those unique tardigrade proteins, CAHS-8, from the *Hypsibius exemplaris* species. Using NMR spectroscopy we determined that the protein comprises an extended central helical domain flanked by disordered termini and calculated its conformational ensemble. Through the measurements of Paramagnetic Relaxation Enhancement experiments and Residual Dipolar Couplings, we characterized the tertiary structure of the protein. Using a combination of NMR, AFM, DLS and SAXS, we have also demonstrated that upon concentration, the protein successively forms oligomers, long fibres, and finally gels constituted of fibres in a strongly temperature-dependent manner. The helical domain forms the core of the fibrillar structure, while the disordered termini remain highly dynamic within the gel and therefore observable by solution-state NMR. Finally, we showed that soluble proteins can be encapsulated within cavities in the gel, that their functional form is maintained but that their translational diffusion is impaired². This ability to reversibly form fibrous gels and the possibility of trapping other proteins within it may be associated with the enhanced protective properties of these unique tardigrade proteins.

1 : Boothby et al., Molecular Cell, 2017

2 : Malki et al., Angewandte Chemie, 2022



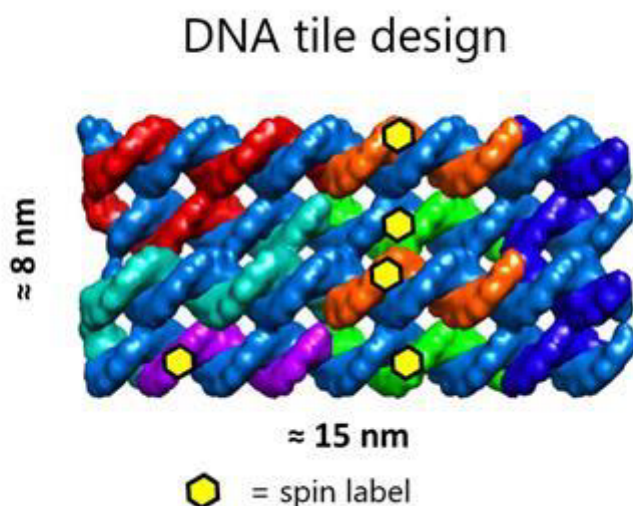
P-142 - A DNA Tile Electron Spin Breadboard

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Adding probes detected via magnetic, electrochemical, or other non-optical modalities to DNA nanostructures has the potential to create materials with emergent properties and, more generally, broaden the DNA nanotechnology toolbox. DNA origami, with typical dimensions of approximately 100 nm, is the most common DNA nanostructure used to organize objects at the nanoscale. Although more cost effective than DNA origami, DNA tiles are not as commonly used for such applications. We have designed a DNA tile, with dimensions of 10 nm x 8 nm, on which up to five molecular probes can be placed at defined positions, resulting in five unique inter-probe distances. This DNA tile provides an affordable, versatile biomolecule-based “ruler” for a variety of measurement modalities. Such rulers containing more than two labels are typically fabricated either via complex synthetic chemistry procedures or molecular biology methods requiring specific expertise. Here, we report our progress on the assembly and characterization of our DNA tile, labeled with a spin label for magnetic resonance measurements in two different locations as well as a doubly-spin labeled construct.



P-358 - Dissecting complex mixtures by heteronuclear SCALPEL

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

NMR analysis of mixtures can be a challenge due to the overlapping signals with complex multiplicities observed in ¹H spectra and the range of different concentrations of multiple species often present. As the small chemical shift dispersion of ¹H resonances (typically ~10 ppm) often means severe signal overlap for mixtures, the use of more advanced NMR experiments is useful to increase resolution. Different methods for dealing with this complexity have been previously reported, including band-selective methods (e.g. REST, selective relaxation-encoded TOCSY)¹ and pure shift methods (e.g. PSYCHE, Pure Shift Yielded by CHirp Excitation)². Even though these methods are very helpful, highly complex mixtures still represent a very significant challenge. The SCALPEL (Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components)³ technique allows mixtures of complex spectra to be separated into their component subspectra by encoding multiple independent properties (e.g. diffusion coefficient, chemical shift evolution and T2) of the species in a mixture and using tensor decomposition methods (e.g. PARAFAC) to extract the spectra of spin systems that shared the same encoded properties. Here, a new approach for heteronuclear SCALPEL, using ¹³C, is demonstrated for a mixture of sugars. Separating the spectra of coupled spin systems by SCALPEL is much less invasive than traditional methods of separation and purification, such as chromatography, and allows the investigation of intact mixtures. This new class of experiments is faster than traditional multidimensional NMR methods because it requires only a small number of measurements for each encoded property.

References

1. Chem. Comm., 2017, 53, 7461-7464.
2. Angew. Chem. Int. Ed. 2014, 53, 6990–6992.
3. J. Am. Chem. Soc., 2019, 141, 5766-5771.

P-128 - FAST DEEP LEARNING RECONSTRUCTION TECHNIQUES FOR MAGNETIC RESONANCE FINGERPRINTING

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Magnetic Resonance Fingerprinting (MRF) simultaneously measures multiple tissue properties through a time-efficient acquisition routine. The traditional post-processing procedure, however, is relatively slow and requires significant storage capacity. Deep Learning (DL) methods are an interesting strategy to overcome these limitations.

Aims

We propose a DL method and a hyperparameters optimization strategy to reconstruct T_1 and T_2 maps acquired with two MRF sequences.

Methods

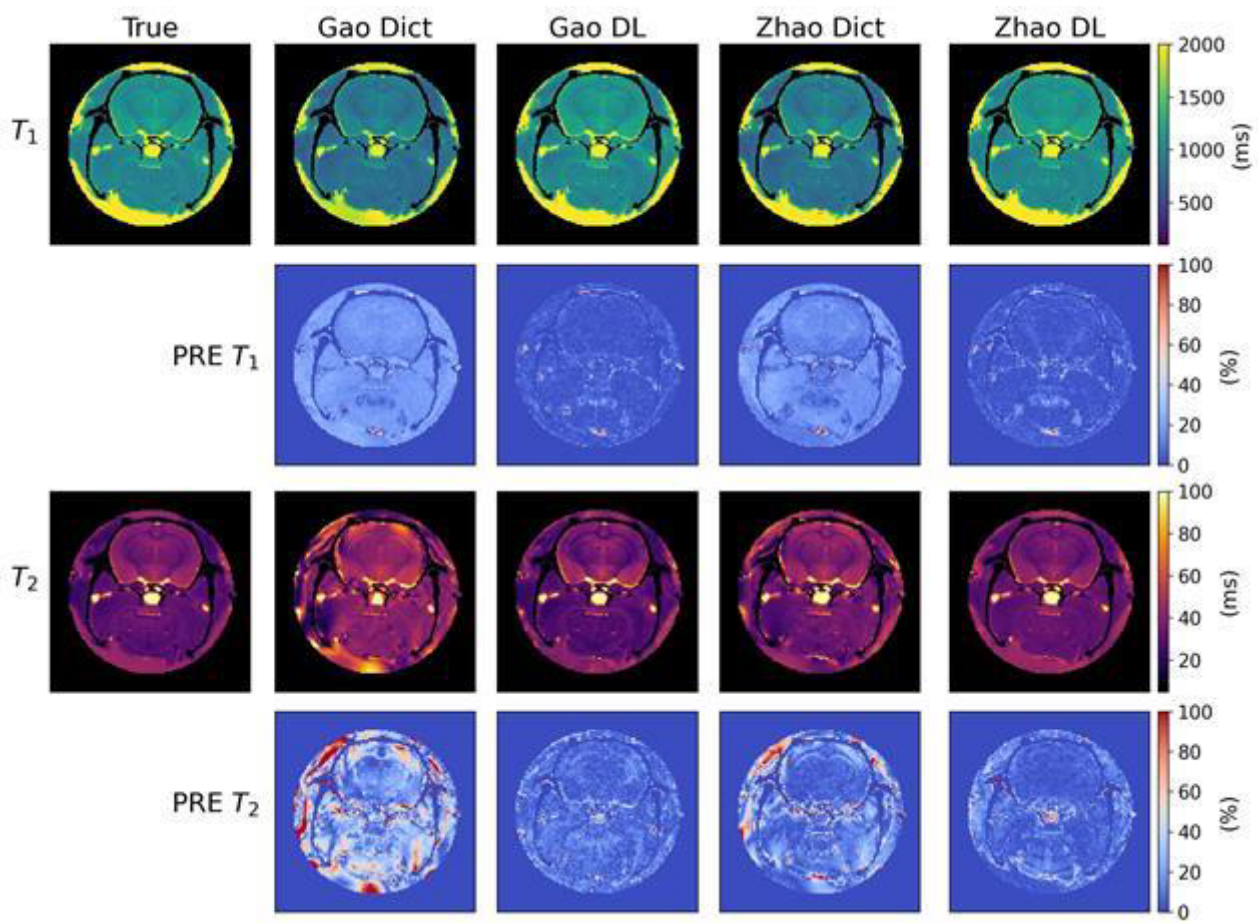
The dataset consisted of 6 slices of two ex-vivo brain rat phantoms: 5 slices from the first phantom and one slice from the second one. We implemented the two MRF sequences (Gao et al. 2015, Zhao et al. 2018) and the reference T_1 , T_2 mapping sequences on a 7-Tesla Scanner. Gao and Zhao MRF dictionaries were simulated by Extended-Phase-Graph formalism. We defined a multilayer perceptron and a recurrent neural network as DL models to perform a voxel-wise regression of the T_1 , T_2 parameters, given the voxel-wise MRF signal timecourses as input. 5 slices of the first phantom were used to train and validate (80/20%) both the models using the Mean Squared Error as loss function. The slice of the second phantom was used as an independent test set. The set of optimal hyperparameters of the model and the training algorithm were established through an automatic optimization procedure.

Results

The DL reconstruction method resulted in a mean percentage relative error (PRE) for Zhao dataset of $5\% \pm 8\%$ for T_1 ($5\% \pm 8\%$ for the Gao dataset) and $10\% \pm 14\%$ for T_2 ($11\% \pm 12\%$ for the Gao dataset). For the dictionary-based method, the PRE for the Zhao dataset was $16\% \pm 8\%$ for T_1 ($19\% \pm 7\%$ for the Gao dataset) and $20\% \pm 24\%$ for T_2 ($35\% \pm 29\%$ for the Gao dataset), Figure 1.

Conclusion

The similar performances between DL model and the traditional reconstruction method suggested that DL techniques may be a time-saving approach for T_1 and T_2 maps reconstruction.



P-360 - ^1H NMR as a tool for investigating the chemical origins of life

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction: Understanding how life first began on Earth is one of science's greatest questions. In order to form biomolecules such as RNA and amino acids, organic precursor molecules are required. The conditions under which these precursor molecules form are still under debate. This study investigates the hypothesis that these precursor molecules form at sub-zero temperatures in seawater. It has been shown that CO_2 and CH_4 can undergo electrochemical conversion in brines at low temperatures to form small molecules such as formic acid and ethanol.¹ This project is exploring the role of minerals believed to have been present at the time, such as siderite (FeCO_3). NMR can detect¹ and quantify organic molecules from these reactions non-invasively and in situ. Using chemical shift imaging (CSI), it becomes possible to screen multiple reaction mixtures simultaneously. By studying the electrochemical reaction in the spectrometer, the reaction can be studied in real time and in operando.²

Aims: ^1H NMR spectroscopy was used to characterise and quantify reaction products under conditions which mimic prebiotic Earth.

Methods: The limit of detection for organic products was determined using sodium formate solutions over a concentration range 1 μM to 1 mM. ^1H NMR spectra were acquired for reaction products with siderite over a range of experimental conditions.

Results: The limit of detection was established within an experiment time of 3 hours. A range of organic molecules have been observed in solutions extracted from siderite reactions.

Conclusions: ^1H NMR is able to monitor reaction products at the concentrations produced by these reactions. High throughput screening using CSI of multiple reaction tubes will enable a range of reaction conditions to be explored. In operando observation of the electrochemical reactions will be developed.

1 Sargeant et al., ACS Catal, 2020, 10, 7464

2 Britton, ChemPhysChem, 2014, 15, 1731

P-150 - Strategies for Frequency and Time Domain Simulations of Pulsed ^{19}F -ENDOR Experiments

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

ENDOR spectroscopy is a magnetic resonance technique with many different applications such as the recently developed intermediate range distance measurements using ^{19}F -nuclear spin labels.[1] However, to derive detailed structural information from experimental data careful analysis and simulation of the experimental data are crucial.

The most widespread approach is the use of frequency domain simulations, where ENDOR resonance frequencies are calculated from the spin Hamiltonian and simulated as a 'stick'-spectrum. It is then convoluted with a line-shape function containing line broadening and effects of the pulse sequence. We expanded this approach by first implementing a statistical analysis of the experimental spectrum through application of a 'drift-model'[2], and subsequently introducing Bayesian optimization for the simulation parameters. Together with bootstrapping this allows to determine parameter uncertainties.[3] The full 'analysis-pipeline' from the experimental data to the derived structural parameters will be presented here.

A more precise tool to directly simulate the effect of pulse sequences are time domain simulations, which calculate the influence of the pulses on the spin density matrix over the whole time evolution of the experiment.[4] It thereby provides a theoretical tool to identify the potential strengths and weaknesses of pulse sequences without the necessity of intensive optimization and comparisons in practice.

Here, we present the use of time domain simulations to evaluate and understand the spin-dynamics in pulsed ^{19}F -ENDOR experiments such as the length and strength of the radio frequency pulse.

Literature:

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[4] I. Bejenke, R. Zeier, R. Rizzato, S. Glaser, M. Bennati, *Mol.Phys.*, 2020

P-384 - Unravelling the Reactivity of Framework Lewis Acid Sites towards Methanol Activation on H-ZSM-5 Zeolite with Solid-State NMR Spectroscopy

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Zeolite catalysts are important catalysts in chemical and petrochemical industrial processes. The acidity of zeolites is generated by Brønsted acid sites (BAS) and Lewis acid sites (LAS), which are governed by the structure, coordination state and distribution of Al species in either framework or extra-framework positions. In contrast to the well-defined Brønsted acidity, the complexity of the Lewis acidic Al sites hampers the understanding of their nature and catalytic role despite their high potentials in many important reactions. The origins and structures of framework Lewis acidic Al sites (FLAs) remain a subject of debate.

Aims

The present work aims to detect and reveal the structure of framework Lewis acidic sites in ZSM-5 zeolite and understand their activity in catalytic reactions.

One and two-dimensional (2D) solid-state NMR spectroscopy in combination with density functional theory (DFT) calculations.

Methods

On the calcined H-ZSM-5 samples, ³¹P NMR using trimethylphosphine oxide (TMPO) as a probe molecule shows the presence of different FLAs. ³¹P-{²⁷Al} PT-D-HMQC NMR experiments confirmed these Al species in tetrahedral states, providing evidence about the existence of three types of tri-coordinated FLAs. ¹H NMR and FTIR spectra suggest the association of hydroxyl groups with these tri-coordinated Al species. Detailed structures of the FLAs were refined with assistant of DFT calculations, namely, one type of framework tri-coordinated Al and two types of framework-associated tri-coordinated Al with attached hydroxyl groups. In the methanol activation, the tri-coordinated Al with two hydroxyl groups enables formation of surface methoxy group even at room temperature, showing higher activity than the BAS.

Conclusions

The structures of the framework-associated tri-coordinated Al bound with one or two hydroxyl groups are ascertained on ZSM-5 zeolites by using 1D and 2D NMR experiments coupled with DFT calculations. We provide direct evidence for the high reactivity of these framework-associated tri-coordinated Al in methanol activation.

P-154 - Cryogen-free 400 MHz (9.4 T) Solid state MAS NMR system with liquid state NMR potential

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¹Cryogenic Ltd, London, United Kingdom, ²Warwick University, Coventry, United Kingdom

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Cryogen-free systems are widely used for Electron Paramagnetic Resonance and Dynamic Nuclear Polarisation experiments. Besides the low maintenance costs (especially nowadays with skyrocketing prices for liquid helium due to the ongoing helium supply crisis), the cryogen-free technology presents two other major innovations for NMR. First, the magnet is much more compact. The probe can be inserted from the top of the magnet, that eliminates need to bring the magnet up onto the long legs. Second, the magnetic field in cryogen-free magnets is always ready to be changed. That is opening the possibility to use the same magnet at different fields.

Method

We made a 400 MHz MAS NMR system and measured numbers of liquid and solid state samples to demonstrate the performance of the system. The temporal magnetic field distortion generated by the mechanical nature of the cryogen-free cold head is the reason why this technology is still rarely used for high resolution NMR.

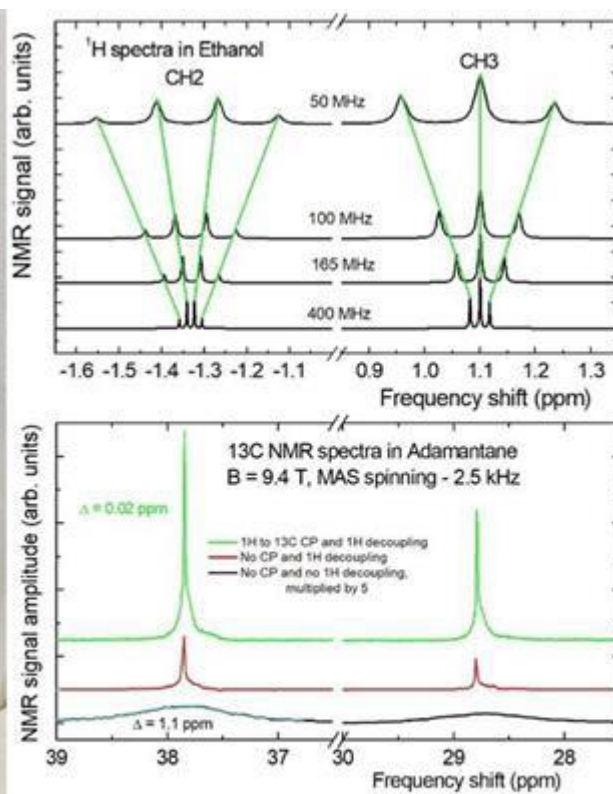
Results

We showed that these distortions can be made smaller than one part per billion with respect to the main magnetic field. That makes the cryogen-free magnets acceptable for solid state MAS NMR and possibly for liquid state NMR as well.

We also showed that the field settling process can be completed within an hour after the field change. That makes possible to change the field in the magnet in a day to day basis without compromising the measurements resolution.

Conclusion

We showed that the cryogen-free magnets have a good potential to replace the conventional magnets with the field up to 750 MHz in near future.



P-192 - Targeted sample formulation developments for the study of biological samples using dissolution-dynamic nuclear polarization

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The aim of our studies is to analyse complex samples, such as live cell cultures to model diseases and response to treatments, or natural abundance biological sample such as blood, urine or cellular media.

Our two research groups at Université Paris Cité and École Normale Supérieure operate together a 3-polariser platform for dissolution dynamic nuclear polarisation (D-DNP). These three setups operate at fields ranging from 3.35 T to 9.4 T and enable us to dissolve samples into spectrometers operating at 400, 500, 600 and 800 MHz. This has allowed us to start tackling a variety of applications, from the in-vitro monitoring of enzymatic reactions to fast MRI in a micro-imaging probe at 800 MHz.

However, while D-DNP experiments can theoretically reach signal enhancements higher than four orders of magnitude, these are much more complex to achieve when the samples differ from standard formulations, and tailor-made developments are necessary.

We will present recent hardware and methods developments that enabled us to work more and more efficiently using nitroxide radicals coupled to cross-polarisation. By optimising specific formulations for our metabolites of interest, we have reached solid-state polarisations higher than 30% for isotopically enriched glutamine and asparagine and higher than 40% for uniformly labelled glucose. We also report our latest results on mixtures of metabolites developed without usual DNP glassing agents with MRI intent. We have successfully used these formulations to follow several enzymatic reactions within the time-frame of a D-DNP experiment, to obtain measurements using cellular extracts, and performed some ¹³C micro-imaging on a basic sample using glutamine as the hyperpolarised target.

These results pave the way to more advanced and challenging studies, from natural abundance metabolite mixtures to biological samples, and to more sophisticated detection schemes taking advantage of the signal enhancements brought by D-DNP to analyse complex mixtures of metabolites.

P-018 - A protonated cytidine stabilizes the ligand-binding pocket in the preQ1 riboswitch in thermophilic bacteria

Mrs Stefanie Rückriegel

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Riboswitches are bacterial mRNA structure elements regulating either transcription or translation of downstream genes in response to high-affinity binding of a low molecular weight ligand. Among this diverse group of RNA structures, the class-I preQ1 sensing riboswitches (QSW) stand out since they are the smallest known natural riboswitches. QSW combine ligand sensing and functional control within a single structural domain that adopts a pseudoknot conformation encapsulating both the cognate ligand and the ribosome binding site. QSW also occur in thermophilic bacteria. In these cases, their tertiary structures have to be stable even at temperatures above 60 °C to be functional at the organism's optimal growth temperatures. Despite the available high-resolution structures of these riboswitches, it is not yet understood which tertiary interactions are primarily responsible for their exceptional temperature stability. Here, we show that an intricate network of non-canonical interactions involving various non-neighboring nucleobases is the origin of the riboswitch's thermostability. An essential part of this network is a so far undetected stably protonated cytidine. It is characterized by an exceptional high pK_a value of >9.7 and could be unambiguously identified through the application of modern heteronuclear detected NMR experiments. Thus, the presence or absence of a single proton can modulate the formation of an RNA tertiary structure and ligand binding capacity under extreme environmental conditions.

Figure 1: (A) Amino selective ¹³C direct detected CN-HSQC for the cytidine resonances. (B) Region of the HNN-COSY for amino protons reporting on the H-bond between the amino proton of C7+ and G11. (C) Chemical structures and H-bond interactions between the nucleobases forming the ceiling of the binding pocket. The H-bond shown in (B) is marked in pink. (D) Scheme of the binding pocket of QSWTt with its three layers floor, binding core and ceiling.

P-204 - Novel paramagnetic metal polarizing agents for site-specific DNP

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Dynamic nuclear polarization (DNP) has shown tremendous potential to increase sensitivity in numerous MAS NMR applications [1]. Even though in conventional DNP experiments uniform signal enhancements are typically obtained, DNP itself can act as a source of specificity as well [2]. The hyperfine interaction is mediating the initial step of the complex mechanism of the overall DNP transfer. Therefore, the effects of the distance dependence of the dipolar hyperfine interaction between the electron spin (source) and nuclear spin (target) are of exceptional importance. By microwave irradiation, electron-nuclear coherences are generated which finally result in nuclear hyperpolarization. If subsequent spin diffusion is restricted, this transfer can act as a measure for hyperfine interaction and thus electron-nuclear distance. However, the competing paramagnetically enhanced spin-lattice relaxation counteracts the creation of a polarization gradient, theoretically leading to uniform, distance-independent DNP enhancement [3]. Nevertheless, the direct-DNP build-up rate can act as a direct measure of this interaction and can thus yield distance information in biomolecules [4].

Currently, systematic investigations of electron-nuclear distances and their effect on paramagnetic relaxation and DNP rates are lacking. Here, we present an approach to quantitatively investigate the distance dependence of DNP transfer based on paramagnetic metal polarizing agents such as Gd(III) complexes which are decorated with specifically isotope-labeled functional groups as molecular rulers. We show details about the chemical synthesis as well as EPR and NMR spectroscopic characterization. Computational methods for validation of the expected distance distributions between source and target spin for correlation with DNP-derived rates are also presented.

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P-316 - Mutual Diffusion coefficients by NMR spectroscopy

Ms Sarah Mross

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Information on mutual diffusion coefficients is particularly needed for modeling mass transfer processes and is important for process design. However, due to a lack of data in literature empirical estimates are often used, which can lead to large errors. Nuclear magnetic resonance (NMR) spectroscopy is an excellent method for the measurement of self-diffusion coefficients [1-2]. By extrapolating the results of concentration dependent measurements of self-diffusion coefficients to infinite dilution, the important mutual diffusion coefficient at infinite dilution can be determined. NMR spectroscopy was used in the present work for studying self-diffusion coefficients of binary mixtures of poly(oxymethylene)dimethyl ethers (OME) (1) with alkanes (2). OME are a new class of synthetic fuels [3-6], that may also be used in mixtures with hydrogenated vegetable oils (HVO), which are represented here by alkanes. The measurements were carried out at high dilution of either (1) or (2), so that an extrapolation of the results to infinite dilution is facilitated. OME with chain lengths $n = 1...4$ were studied, the alkanes were dodecane and hexadecane. The temperatures were between 25 and 80 °C. The new results can be used for developing models of transport properties of OME and OME-containing mixtures, model-based design of OME production processes, simulation of combustion processes, and for testing molecular models of OME [7].

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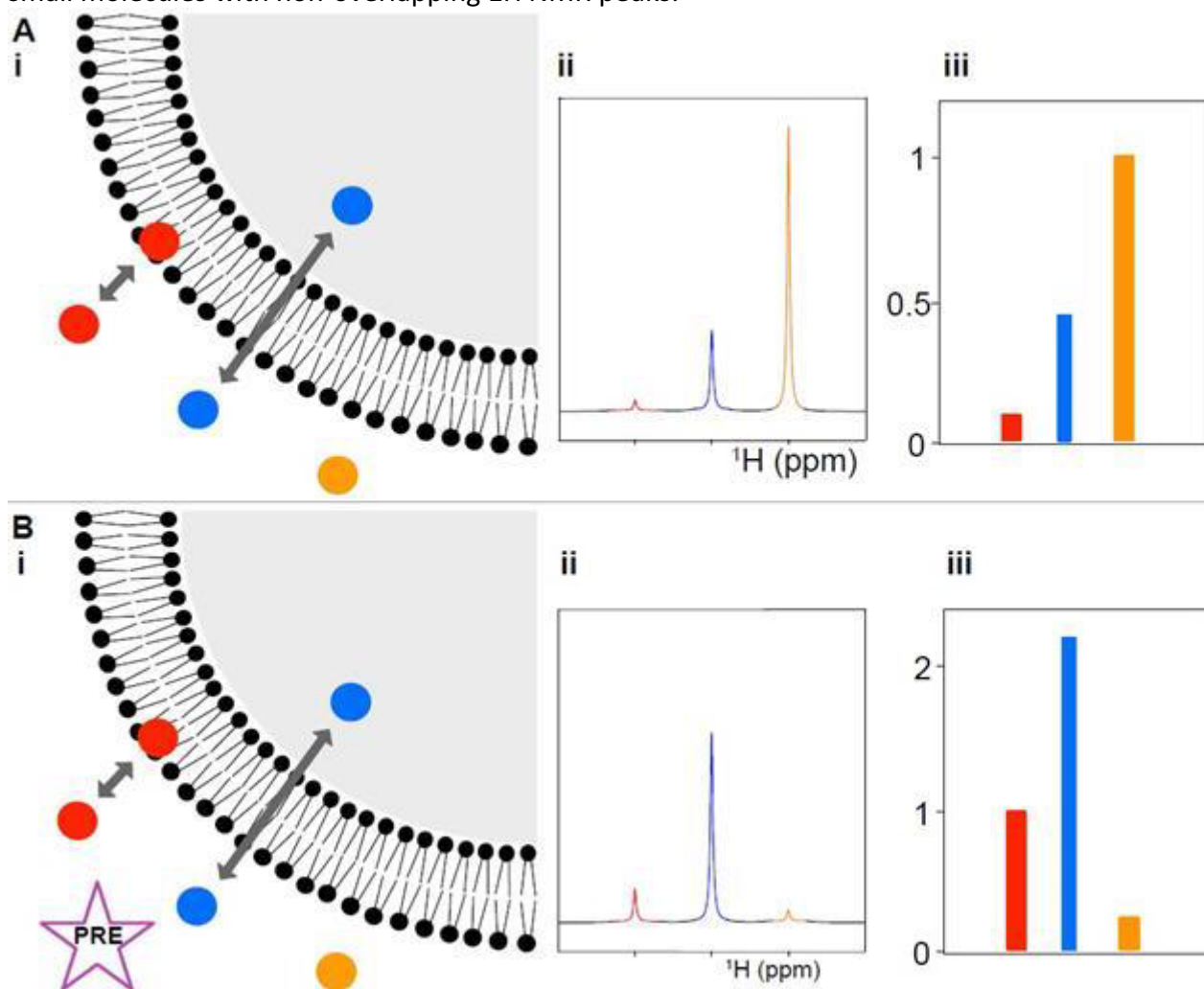
P-350 - Elucidation of small molecule passive permeation across lipid membranes using conventional solution state NMR methods

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¹University Of Kent, Canterbury, United Kingdom

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Quantifying small molecule uptake across a biological membrane in any cell system is crucial for the development of efficacious and selective drugs. However, obtaining such data is not trivial in any system. Herein, we present an accessible, high-throughput (20 mins), solution state ¹H NMR assay which enables the quantification of passive permeation and/or comparative degree of membrane interaction for a target compound into a phospholipid membrane of a desired composition. The method exploits the NMR solvent PRE effect using ¹H 1D CPMG NMR experiments, and the ability of membrane permeable molecules to travel to the interior of lipid vesicles, shielding themselves from the solvent PRE reagent. As proof-of-principle we apply this methodology to candidates from a class of supramolecular self-associating amphiphiles, members of which have been shown to interact with biological phospholipid membranes and elicit an antimicrobial effect, allowing the determination and comparison of their membrane permeability and interaction properties. In conclusion, the assay can be used with vesicles of any lipid composition, including lipids extracted from natural sources, allowing permeation studies of antimicrobials, and can assess the permeability of any mixture of small molecules with non-overlapping ¹H NMR peaks.



P-230 - NMR of C₆₀ endofullerenes

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

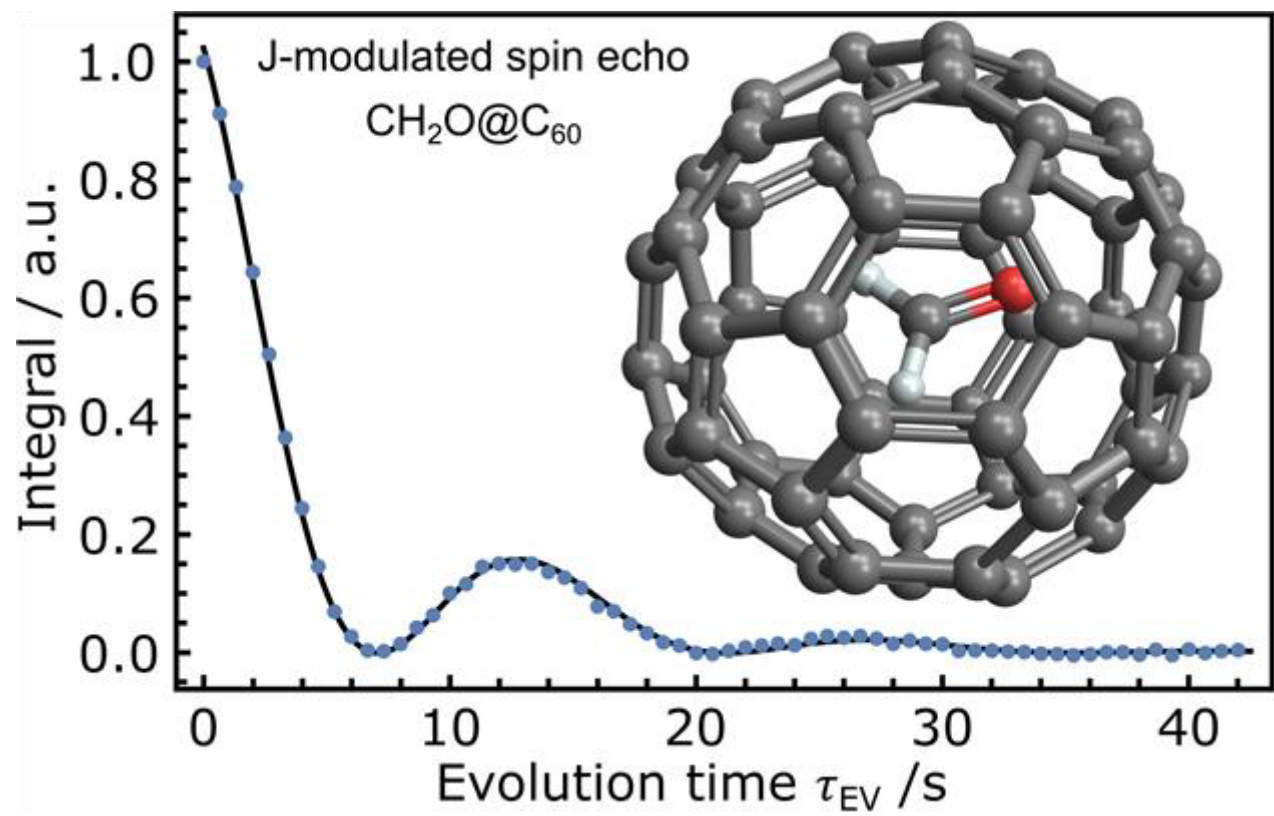
The C₆₀ fullerene molecule has raised great interest of scientists and mathematicians due to its fascinating truncated icosahedron shape, with a very high degree of symmetry. Endofullerenes are supramolecular complexes where one small (endohedral) atom/molecule is completely confined within a bigger, fullerene, molecule which acts as an enclosing cage.¹ Endofullerenes offer an ideal “particle in a box” nano-laboratory to observe quantum mechanical phenomena.

A small J-coupling (~75 mHz) between ³He and ¹³C in the C₆₀ cage was observed due to confinement, in the ³He@C₆₀ endofullerene.² Confinement keeps the nuclei in close proximity for an indefinite amount of time and if suitably long relaxation times are present a “non-bonded” J-coupling can be observed between the endohedral species and the enclosing carbon cage. This is attributed to a ⁰JHeC-coupling, where the “zero” represents the number of chemical bonds between Helium and Carbon.² The ⁰JHeC-coupling displays a peculiar temperature dependence which can be explained by “particle in a box” type models. The same type of ⁰J-coupling is observed between ¹H and ¹³C in the CH₂O@C₆₀ endofullerene, with similar magnitude to ⁰JHeC. See figure for a J-modulated spin echo measurement, used to estimate the ⁰JHC-coupling in CH₂O@C₆₀. Signs of another ⁰JHC-coupling are observed in CH₄@C₆₀. Furthermore, ³He NMR measurements of ³He@C₆₀ in solution and solid state were performed from room temperature down to cryogenic temperatures.

When the C₆₀ molecule is filled with an endohedral species, the C₆₀ ¹³C resonance is shifted downfield depending on the species. The shift appears to reflect the “pressure” a single atom/molecule exerts on its container, in this case the C₆₀ molecule. A theoretical model which quantitatively explains the experimental observations in terms of the pressure of a single atom will be presented.

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P-388 - Ex-situ solid state NMR investigation of NASICON-based electrode materials

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The current shift towards sustainable energy requires development of energy storage technologies based on non-critical materials. Aqueous Na-ion batteries due to enhanced safety, reduced cost and sustainability could solve many issues of Li-based technology. Though, this is at the expense of reduced energy density which is less important for stationary power grid stabilization applications. Here we present the recent results achieved by developing NASICON-structured (Na Super Ionic CONductor) materials for the electrochemical applications such as battery electrode materials. NaTi₂(PO₄)₃ (NTP) was chosen as starting material which was covered by carbon nano-layer to increase the electronic conductivity. The formed composite with binder and carbon filler was coated on current collectors by doctor blading method. These electrodes were used to assemble electrochemical cell which was cycled. Upon cycling the electrochemical cells were disassembled and the NASICON-based electrode materials were prepared for solid state NMR measurements. ³¹P and ²³Na MAS measurements have revealed the formation of the amorphous phases upon cycling the electrochemical device. The amount of amorphous phase increases up the degradation of the electrochemical device.

Further measurements employing ^{47/49}Ti and ⁵¹V MAS and other NASICON-based materials such as Na_{3-x}V_{2-x}Ti_x(PO₄)₃ (NVTP) are planned.

Funding by the Research Council of Lithuania under grant P-MIP-23-146 is gratefully acknowledged.

P-124 - Integrating new functionality into CcpNmr Analysis: Macros, Plug-ins and the NMR Exchange Format

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction and Aims

The Collaborative Computational Project for NMR (CCPN) had developed one of the most popular spectral analysis programs for biomolecular NMR data: CcpNmr Analysis [1]. A core aim has always been allowing others to contribute code and features to the program and to enable easy integration with other major NMR software packages and databases.

Methods and Results

The latest version 3 provides users with a simple data structure and API to interact with, accessible even to novice programmers.

Small, self-contained elements of code for customisation or stand-alone features can be written and contributed as macros.

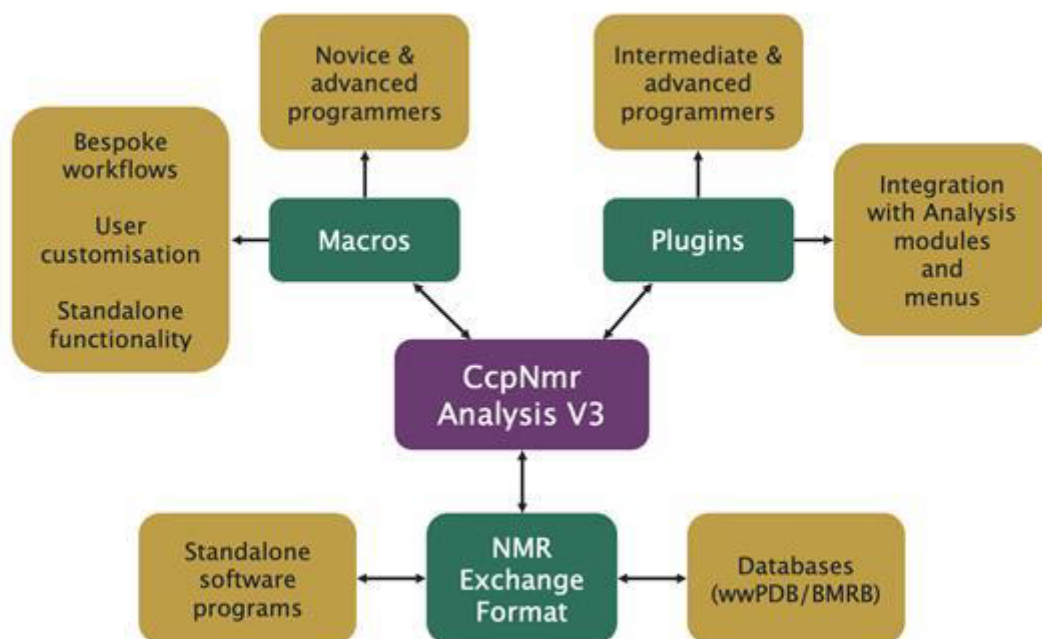
Integration with other software packages and databases such as XPLOR-NIH, ARIA, the PDB and BMRB now successfully proceeds via the NMR Exchange Format (NEF). NEF is easy to implement and extend. Crucially, it makes data traceable (e.g. peak IDs are maintained) and includes accurate documentation of stereo-specific and non-stereospecific assignments – enabling consistent, accurate data validation by external programs for the first time.

On an intermediate level of integration, we introduce an early structure for plugins, a popular way of allowing users to add their own features and customisation used across many modern software packages. Within CcpNmr Analysis, plugins will enable users to write code which interacts directly with the program's modules and menus. We present some early examples of plugins being developed in collaboration with the groups of Hari Arthanari (Harvard Medical School) and Ilya Kuprov (University of Southampton) which integrate new assignment methods, including AI methods, into the CcpNmr Analysis backbone assignment workflow.

Conclusions

By integrating their data analysis methods with CcpNmr Analysis in one of the ways outlined, NMR spectroscopists involved in method development can facilitate rapid global uptake of their latest advancements.

[1] Skinner et al. (2016) J. Biomol. NMR <https://doi.org/10.1007/s10858-016-0060-y>



P-162 - Automated Bullet-Dynamic Nuclear Polarization

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Bullet-DNP [1] is a form of dissolution-dynamic nuclear polarization. In bullet-DNP the hyperpolarized target material and a suitable radical are dissolved in a glass-forming solvent, and the solvent is placed into a bullet. The sample is then hyperpolarized under standard D-DNP conditions and then ejected from the polarizer into an injection device for detection in solution NMR magnet. For many years, DNP experiments have been performed without sufficient attention to automating the whole process. Automating this not only eliminates possible human errors during sample preparation and placing it inside the DNP, but also increases the accuracy, efficiency and throughput of the experiments and enables remote experiments over nights or over weekends. In addition, due to the automation of the process, it is possible to perform the experiments with higher reproducibility. We recently presented a semi-automated bullet system, in which such experiments can be carried out repeatedly without the need to remove the injection device from the NMR magnet [2]. The instrument presented in Ref [2] still needed manual intervention between experiments. Here we provide automation solutions for the bullet-DNP. The new fully automated system includes sample selection through a 96 wells microtiter plate, sample injection into the bullet, freezing at -180 °C, automatic loading of the bullet into the DNP insert, shooting from the DNP insert into the injector device for liquid state NMR, and at the end ejection of the used bullet and preparation of the system for the next experiment. The system is still under final development, but will facilitate unsupervised DNP experiments in the near future.

P-332 - Adventures with Ultra-High-Field ^{15}N detection of proteins

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

With the introduction of high field heteronuclear direct detection cryoprobes first at 700 and 800 MHz and now also at 950 MHz and above, ^{15}N detection has become sensitive enough to be a prospect for proteins at realistically achievable protein concentrations. We have introduced a ^{15}N detected BEST-TROSY variant a few years ago and are now exploring situations where despite the much lower inherent sensitivity there may be advantages to switching from ^1H to ^{15}N detection. We are looking into divided evolution techniques and the measurement of Residual Dipolar Couplings as well as intrinsically disordered systems and will report on our progress with using ^{15}N detection at 950 MHz

P-046 - Structure-function relationship in processing of pre-miRNA-31

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

MicroRNAs(miRNAs) play an important role in post-transcriptional regulation of gene expression [1,2]. Therefore, the production of individual miRNA must be strictly controlled to maintain cellular homeostasis. The biogenesis of many, but not all, miRNAs is mediated by trans-acting protein partners through a variety of mechanisms, including remodeling of the RNA structure [3,4]. miRNA-31 functions as an oncogene in numerous cancers and interestingly, its biogenesis is not known to be regulated by protein binding partners. Therefore, the intrinsic structural properties of miRNA-31 can provide a mechanism by which its biogenesis is regulated.

Using NMR spectroscopy, we investigated structural features of precursor element of miRNA-31 (pre-miRNA-31) with the aim to understand structure-based regulation mechanisms of its biogenesis [5]. The high-resolution model of 71-nt pre-miRNA-31 revealed the key structural elements for Dicer processing. We found that mismatches within the helical stem do not strongly influence Dicer processing of pre-miRNA-31. In contrast, both the apical loop size and structure at the Dicing site are important for discrimination by Dicer. Interestingly, we identified a presence of a triplet of base pairs that link the Dicer cleavage site and the apical loop. Our results enrich our understanding of the active role that RNA structure plays in regulating Dicer processing which has direct implications for control of gene expression.

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P-318 - More than ADEQUATE: doubling the sensitivity of ^{13}CH – ^{13}CH correlations in double-quantum NMR experiments

Mr Justinas Sakas

Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

The INADEQUATE (Incredible Natural Abundance Double QUAntum Transfer Experiment) NMR experiment [1,2] is of substantial interest to chemists as it allows tracing out the carbon skeleton of a molecule. However, it suffers from inadequate sensitivity as it relies on the presence of two coupled ^{13}C spins, which only exists in approximately 1-in-8,300 molecules. Gains in sensitivity have been achieved through the use of ^1H detection in experiments such as INEPT-INADEQUATE or ADEQUATE. [3, 4]

It has been demonstrated that removing proton terms from heteronuclear coherences increases their relaxation times [5] and this can be used to increase sensitivity, especially in experiments optimised for long-range carbon-carbon correlations containing longer delays. Additionally, sensitivity is lost when the multiple-quantum coherences present during the t_1 period contain proton terms causing a leakage of the signal into zero-order coherences. [6]

Both these issues can be tackled by refocussing $^1\text{J}(\text{CH})$ couplings and decoupling protons during $\text{J}(\text{CC})$ evolution intervals. In this work we apply these modifications to the ADEQUATE experiment which more than double the sensitivity of carbon-carbon correlations of ^{13}CH – ^{13}CH moieties and strongly attenuate HSQC-like artefacts. [7] Doubling the sensitivity results in an impressive 4-fold reduction of experimental time, allowing ADEQUATE spectra to be recorded overnight instead of over multiple days. These sensitivity enhancements increase the potential of using ^{13}C – ^{13}C correlations for structure elucidation and will benefit areas such as carbohydrates, natural products or mixture analysis, as well as general NMR applications when sample quantities are limited.

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P-042 - DEVELOPING TOOLS TO MONITOR THE IMPLICATION OF CROSS-TALKS BETWEEN tRNA CHEMICAL MODIFICATIONS IN BACILLUS SUBTILIS WITH NMR

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Intro.

Transfer RNAs (tRNAs) are by far the most post-transcriptionally modified RNAs in all domains of life. These modifications play a key role in their function, affecting either their folding and stability or the fidelity and efficiency of protein synthesis. The insertion of certain modifications may depend on the presence of prior ones, creating cross-talks between modification events that affect their kinetics of introduction. In cell, tRNA modification levels changes depending on the cellular conditions, in particular in reaction to cellular and environmental stresses.

Aims.

We previously introduced NMR spectroscopy as a tool to monitor the maturation of tRNA in yeast in a time-resolved fashion (Barraud et al. Nat Commun 2019). We now aim at exploring changes in tRNA modification pathways in response to stress, in the Gram-positive model bacterium *Bacillus subtilis* (Bs).

Method.

We use NMR of ¹H-¹⁵N imino signals, carried by uridines and guanosines, to follow tRNA modification in a time-resolved fashion in cell extracts. In addition, we use Bs strains deleted each of one tRNA modification enzyme to monitor tRNA maturation in the corresponding cell extracts, thereby enabling the observation of the modification cross-talks with NMR spectroscopy.

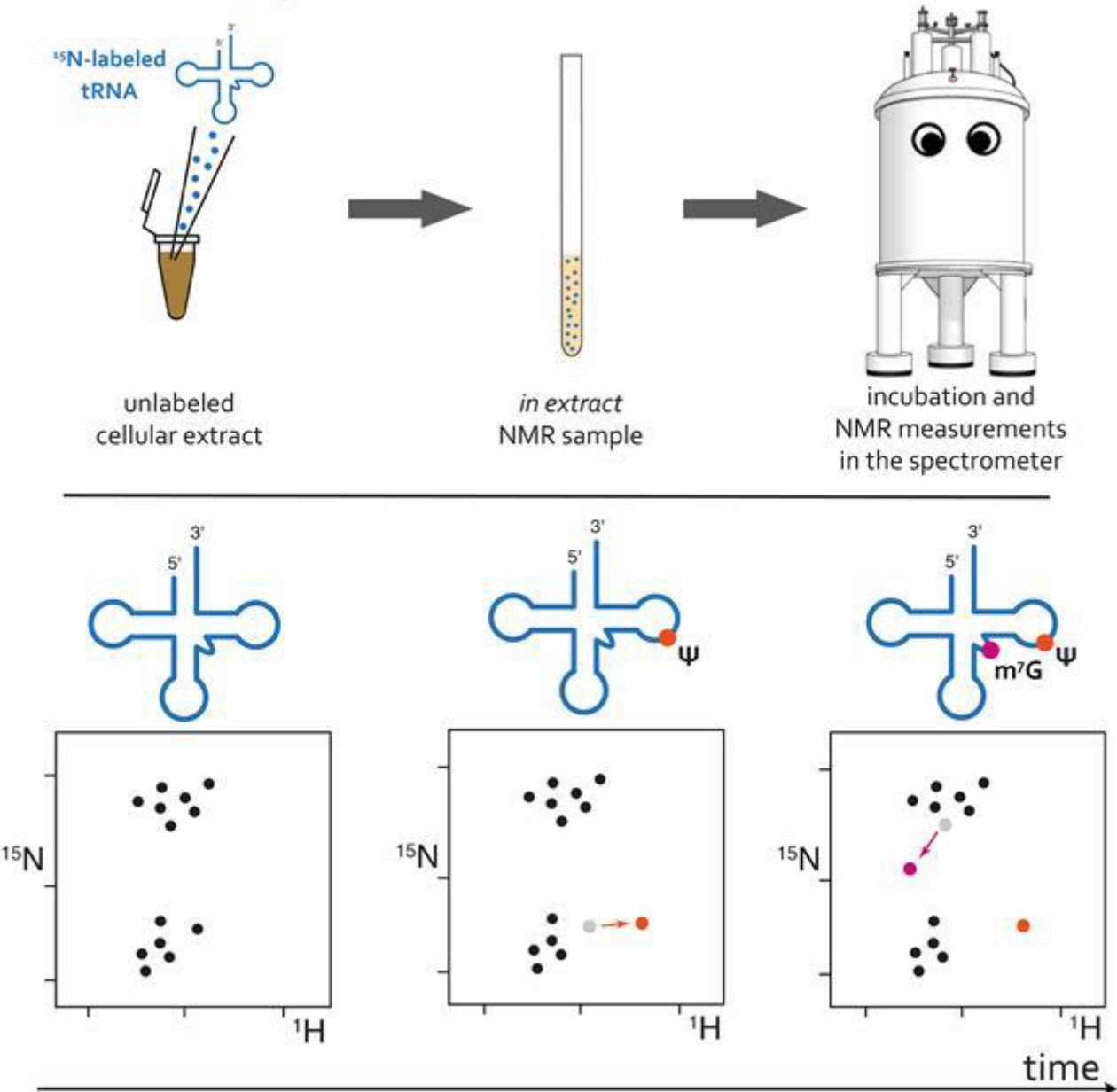
Results.

We followed modification of Bs tRNA^{Phe} produced in vitro, and identified their introduction kinetics in cell extracts. To validate our results, we developed the first system of RNA overexpression in Bs, allowing us to analyze labeled tRNAs overexpressed and modified in vivo. We also identified cross-talks between tRNA^{Phe} modifications by using Bs deleted strains. Finally, we monitored changes of tRNA modification pathways in response to various environmental stresses.

Conclusion.

The tools we developed in Bs provide the means to monitor tRNA maturation in a time-resolved manner, cross-talks between different modification events, and the adaptation of tRNA maturation in response to external stresses.

NMR monitoring of tRNA maturation



P-382 - Heteronuclear decoupling for fast MAS NMR: robustness toward $1H$ offset, RF field irradiation and RF field inhomogeneity

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

High-spectral resolution and sensitivity in solid-state NMR can be achieved by simultaneously spinning the sample about the magic-angle (MAS) and by applying heteronuclear decoupling. However, with recent developments in instrumentation, e.g., faster spinning frequencies (>100 kHz) and higher magnetic fields (up to 35.2 T), it is difficult to obtain efficient heteronuclear decoupling. The drawbacks of many current methods are achieving (i) robustness for a large chemical-shift range under low-power irradiation, (ii) independence with respect to the RF power, and (iii) robustness toward RF-field inhomogeneity.

A new heteronuclear decoupling pulse sequence for solid-state NMR and magic angle spinning (MAS) faster than 60 kHz was recently introduced [1], dubbed Rotor-Synchronized Phase-Alternated Cycles (ROSPAC). Here, we highlight the practical performances of the sequence with respect to $1H$ offset, radio-frequency (RF) power, and RF inhomogeneity. These are illustrated by representative solid-state NMR experiments and theoretical results obtained using a generalized theoretical framework based on Floquet theory. It demonstrates the robustness for a broad range of chemical shifts and low RF powers, and the almost independent behavior of the sequence with respect to the RF power. Moreover, for a flip angle in the range of 160° - 200° , the influence of the RF field inhomogeneity (or miss settings of the flip angle) can be minimized.

Implementing the ROSPAC sequence yields promising results, the theoretical results matching the experimental ones. Further improvements to the sequence are currently being investigated and will be discussed.

[1] A. Simion et al., Heteronuclear decoupling with rotor-synchronized phase-alternated cycles, J. Chem. Phys. 157, 014202 (2022); <https://doi.org/10.1063/5.0098135>

Acknowledgments:

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P-214 - ^{13}C -Enhanced 1D and 2D ^{13}C - ^{13}C and ^{13}C - ^1H Correlation Spectroscopy based on the Scalar-Coupling Based Overhauser DNP

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

DNP experiments in liquids employing dipolar coupling-based Overhauser DNP (ODNP) mechanisms are still challenging at high magnetic fields because the electron-nucleus cross-relaxation processes become inefficient: i) it requires a high-power microwave irradiation to achieve a meaningful saturation factor; ii) the spectral densities approach rapidly to zero at high magnetic fields. However, ODNP experiments via the electron-X nucleus scalar couplings are still feasible in high fields due to the relatively strong coupling factor. In this work we demonstrate 1D and 2D ^{13}C -enhanced ODNP spectra at 14.1 T detected directly or indirectly via ^1H . We have employed a large sample volume (50~100 μL) double-resonance (^1H , ^{13}C) liquid-state NMR probe using a non-microwave absorbing sample cell while employing a high-power microwave beam from a gyrotron operating at 395 GHz (14.1 T). This custom-designed HX ODNP probe enables ODNP experiments by polarizing the nuclei of heavier, electron-rich atoms ($\text{X} = ^{13}\text{C}$, ^{15}N , or ^{31}P) and subsequently transfer the X-polarization to another nuclei, either X_2 or ^1H via $\text{J}(\text{XX})$ or $\text{J}(\text{HX})$ -couplings. We have demonstrated ^{13}C - ^{13}C correlation spectra by employing COSY, TOCSY, and HORRENDOUS sequences and ^{13}C - ^1H correlation spectra by employing INEPT and HSQC sequences measured on ^{13}C -labeled quinoline, indole, phenyl acetylene, dimethyl malonate, and chloroform. Sample molecules were co-dissolved in nitroxide-based radical solutions of 10 to 20 mM TEMPO or ^{15}N , ^2H -labeled oxo-TEMPO in hexane- d_{14} or heptane- d_{16} . The samples are cooled by blowing a cold nitrogen gas stream via an FTS system to mitigate microwave heating. Experiments were optimized under conditions of obtaining high resolution by avoiding heat-generated signal broadening, resulting in a signal enhancement factor ranging from 2 to 20 depending on the molecular system.

P-374 - Ultrawide line 2D correlations among weakly interacting nuclear spins via triple-resonance, ^1H -detected, solid-state NMR

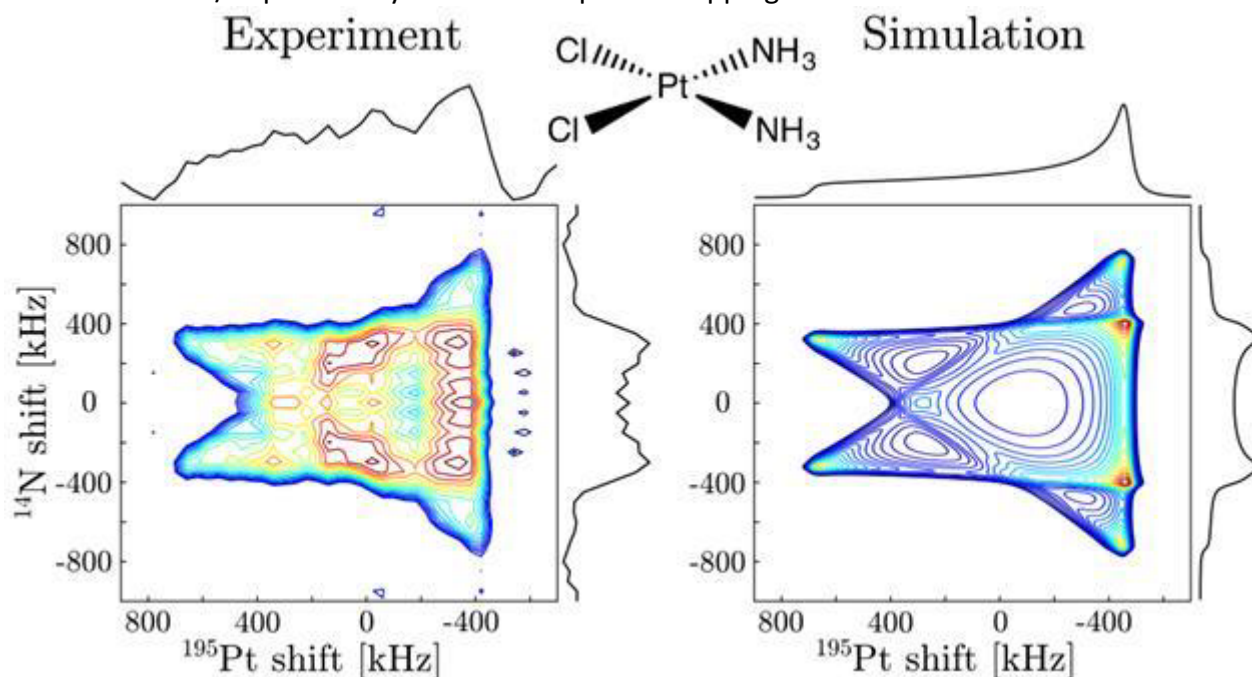
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Solid-state NMR permits the determination of the nuclear spin interaction tensor parameters, including relative tensor orientations. Although these tensor anisotropies carry valuable information about the local structure, bonding and dynamics, they may also severely broaden the resonances leading to issues in signal excitation and detection. While 1D experiments are feasible, 2D correlations of ultrawide (UW) lineshapes such as those arising from quadrupolar interactions or heavy-metal shielding anisotropies are nearly impossible due to the concomitantly low sensitivity and challenges with excitation and coherence transfer. The present study explores a new strategy that may overcome these limitations, and enable the acquisition of 2D ultrawide-ultrawide (2DUW) correlations.

The idea of these novel 2DUW experiments is to correlate heteronuclear species not by probing their direct interaction but rather their NMR proximity to a reporting ^1H . To explore this concept we rely on the recently proposed progressive saturation of the proton reservoir (PROSPR) experiment [J. Am. Chem. Soc. 143, 19778 (2021)], which repeatedly encodes UW lineshapes of insensitive nuclei, into the abundant and easily observable spin pool embodied by the ^1H s. Herein we extend PROSPR into a multidimensional experiment, which involves concurrent irradiation of the UW species to be correlated. The 2DUW method can potentially correlate weakly or altogether noninteracting spin pairs such as ^{14}N and ^{17}O , ^{14}N and ^{35}Cl , etc., in organic systems. The figure below illustrates the capacity of the new experiment with what should be a very challenging ^{14}N - ^{195}Pt 2D correlation on the chemotherapeutic drug cis-diamminedichloroplatinum. The ensuing ≈ 1 MHz lineshapes being correlated are clearly dependent on the relative orientation between the ^{14}N electric-field gradient and the ^{195}Pt chemical shift tensor. Thus, the method can extract previously inaccessible tensor parameters. Furthermore, we anticipate that the approach can be utilized to encode specific interactions and/or potentially resolve multiple overlapping resonances.



P-236 - Anomalous Nuclear Relaxation in Superconducting Endofullerides $\text{Rb}_3(\text{A}@\text{C}_{60})$

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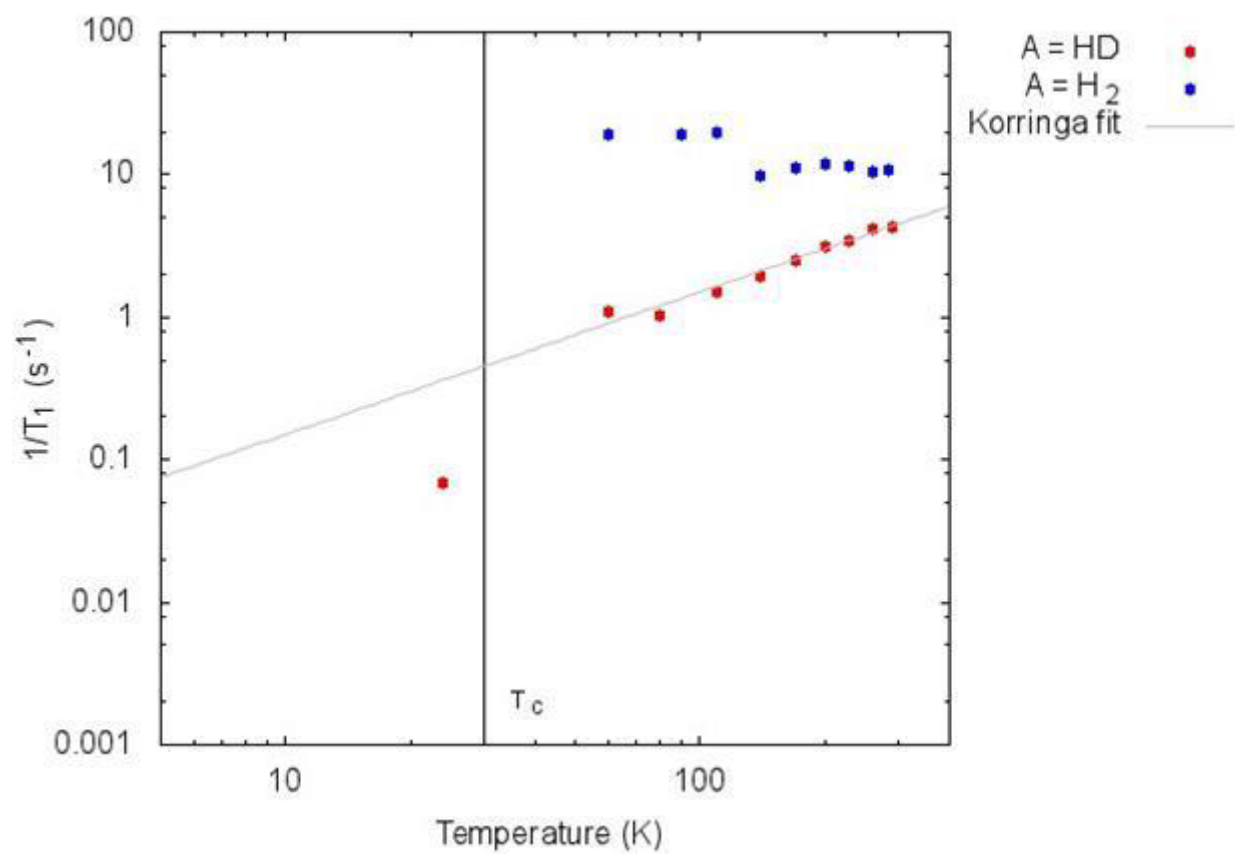
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Since the first reports of superconductivity with high critical temperatures in potassium doped C_{60} [1], many alkali metal fullerides have been studied, and TC up to 38 K along with unconventional magnetic and insulating phases have been reported [2]. NMR relaxation studies have provided insight into the metallic and superconducting states in these materials [3]. Recently, developments in the technique of “molecular surgery” have allowed for the synthesis of various endofullerenes [4][5] – C_{60} cages with endohedrally encapsulated molecules – which display unique properties due to the confining potential of the cages. Here we present NMR studies on rubidium endofullerides, in which Rb_3C_{60} is synthesised using H_2 and HD endofullerene precursors, and the unexpected deviations from relaxation behaviour that are observed.

$\text{Rb}_3(\text{HD}@\text{C}_{60})$ with 20% cage filling factor and $\text{Rb}_3(\text{H}_2@\text{C}_{60})$ with 10% filling factor were synthesised and characterised using magnetic susceptibility measurements and a TC of 30 K was observed. ^1H T_1 measurements of the HD fulleride display expected Korringa law behaviour in the normal state with a change at TC. The H_2 fulleride shows marked deviations from the Korringa law at all temperatures in the normal state. We propose a possible explanation based on a coupling of the nuclear spin to phonons via the molecular rotational state of H_2 .

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2. Y. Takabayashi and K. Prassides, Unconventional High-TC superconductivity in fullerides. *Philos. Trans. R. Soc. A* 374 20150320 (2016)
3. R. Tycko et al., Electronic properties of normal and superconducting alkali fullerides probed by ^{13}C nuclear magnetic resonance. *Phys. Rev. Lett.* 68 1912 (1992)
4. M. Murata et al., Surgery of fullerenes. *Chem. Commun.* 6083-6094 (2008)
5. S. Bloodworth and R. J. Whitby, Synthesis of endohedral fullerenes by molecular surgery. *Commun. Chem.* 5 121 (2022)

$\text{Rb}_3(\text{A}@\text{C}_{60})$ ^1H T_1 vs Temperature



P-208 - Synthesis and hyperpolarization of a 1-Allyltryptophan via para-hydrogen induced polarization for potential applications in MRI

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Introduction

Although magnetic resonance imaging (MRI) is a powerful diagnostic tool, there is much room for improvement. In fact, no more than a few parts per million of all spins in a given sample effectively contribute to the signal. Hyperpolarization techniques allow for an increase of this fraction to the order of unity, providing 10.000 – 100.000-fold signal enhancement. Here, we present an approach to hyperpolarized tryptophan, an important metabolite whose metabolism to kynurenine e.g. is a marker for meningiomas.

Results and discussion

Herein we introduce a method for the synthesis and subsequent polarization of 1-Allyltryptophan. Since not all potential starting materials for the synthesis are available in their isotopically labeled form, an emphasis was put onto the development of a synthetic route which allows for the synthesis of the perdeuterated and ¹⁵N labeled compound. This was achieved by using Toluene as a starting point for subsequent buildup of indole-3-carboxaldehyde followed by coupling to a cyclized Glycine-Alanine dipeptide. The Tryptophan obtained this way was then brought to reaction with allyl bromide to obtain 1-Allyltryptophan. The obtained compound was subsequently hyperpolarized via para-hydrogen induced polarization (PHIP) by hydrogenating the allyl function with para-hydrogen (>90%) using an in-house bubbling setup at 7 bar in a 9.4 T spectrometer to generate a signal enhancement of several orders of magnitude.

Conclusion

The herein shown results pave the way for the synthesis and hyperpolarization of ¹⁵N labeled and perdeuterated 1-Allyltryptophan. The isotopic labelling would allow for the transfer of the obtained proton polarization to ¹⁵N, leading to longer T₁ and thus longer observability of the hyperpolarized compound. The potential perdeuteration should enhance T₁ even further, making the thus hyperpolarized Tryptophan derivative an excellent candidate for the detection of e.g. meningiomas using metabolic MRI.

P-026 - Sodium Dynamics in the Cellular Environment

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Poster Session 2, Hall 1 & 2, SEC, July 11, 2023, 13:45 - 15:45

Introduction

Sodium plays a critical role in the homeostasis of electrolytes, the transduction of nerve potentials, the contraction of muscle cells and related cellular metabolisms. ^{23}Na nuclear magnetic resonance (NMR) is a non-invasive yet highly specific analytical technique that can probe the physiochemical state and map the concentration of sodium. Typically, the distinction of intra- and extracellular sodium ions is based on extraneous shift agents such as lanthanide chelates. However, the shift agents have negative biological effects and are unfavorable for in vivo studies.

Aims

We aim to characterize biological sodium based on diffusivity and relaxation which are the intrinsic properties of sodium dynamics. The relaxation of sodium in the cellular environment deviates from the bi-exponential relaxation phenomenon predicted by the Redfield theory. To date, the NMR relaxation behavior of ^{23}Na in the cellular environment has not been fully interpreted.

Methods

We study the relaxation of sodium in different protein solutions and in living cells under a 14 T ultrahigh magnetic field. The measurements of diffusivity can separate the intra- and extracellular sodium based on the translational dynamics. The measurement of relaxation as well as multi-quantum coherence can distinguish the bound and free sodium ions based on the rotational dynamics.

Results

The tri-exponential fitting and the two-compartment model are used to separate the contributions from intra- and extracellular sodium. The analysis in yeast samples shows that the simple measurement of ^{23}Na transverse relaxation can quantify the fraction of intracellular sodium and its binding strength. In addition, we show that the ^{23}Na transverse relaxation (and ^{23}Na diffusion) can be used to monitor the viability of mammalian cells in vitro.

Conclusion

Our study improves the fundamental understanding of ^{23}Na relaxation in the cellular environment and foresees the emerging capabilities of ^{23}Na NMR and MRI in biomedical applications.