OPENING SESSION

Sunday, July 23
16:20-18:30

Alexander Pines
Half a Century of Ups and Downs

Loren B. Andreas
Structure of Fully Protonated Proteins by Proton Detected Magic-Angle Spinning NMR

Björn M. Burmann
Chaperone-Client Complexes: A Dynamic Liaison

Jeffrey A. Reimer
NMR and Materials Chemistry: Where Can it Go with, or without, Hyperpolarization?
HALF A CENTURY OF UPS AND DOWNS

Alexander Pines

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My current intention is to describe my experiences in magnetic resonance and beyond, from my roots in Bulawayo, my university studies in Jerusalem and Cambridge MA, and my current resting place in Berkeley. I reserve the right to change my mind.
Structure of fully protonated proteins by proton detected magic-angle spinning NMR

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The critical factor that would allow general application of magic-angle spinning (MAS) solid-state NMR for protein structure determination is improvement in sensitivity and resolution for as many nuclear spins as possible. Such an improvement is demonstrated with detection of resolved 1H resonances in protonated proteins by increasing MAS rates to frequencies of 100 kHz and above. For large proteins and assemblies, ultrafast spinning narrows spectral resonances better than Brownian motion on which solution NMR relies, removing a fundamental barrier to the NMR study of large systems. It is now possible to record sensitive and well-resolved spectra for a variety of proteins including membrane proteins, fibrils, and large assemblies. In particular, the de novo structure of a 28-kDa protein dimer in a 2.5-MDa viral capsid assembly is presented (Fig 1). [1]

In Figure 2, aliphatic spectra of similar quality to those of AP205 are shown for a 22 kDa membrane protein of undetermined structure – a good sign for structure determination of membrane proteins.

References
Molecular chaperones are essential for maintaining a functional cellular proteome. Central aspects of chaperone function remain still poorly understood at the atomic level, including how chaperones recognize their clients, and in which conformational states these clients are kept. I will describe the research efforts used to understand these aspects, employing high-resolution NMR spectroscopy as the main method.

Initial work on the periplasmic holdase Skp with bound outer membrane proteins provided the first atomic-level description of a natural full-length chaperone–client complex [1, 2]. This work led to the description of the “fluid-globule” state for the chaperone-bound client, which is held stably by the chaperone based on avidity.

Subsequent work combining NMR spectroscopy with single-molecule force spectroscopy showed how periplasmic chaperones shape individual client folding trajectories, funneling the client polypeptide towards the native structure [3]. This study enabled us to derive different functionalities for different periplasmic chaperones for the first time.

Extending our work towards the bacterial chaperone Trigger Factor enabled us to determine its so far elusive dimeric-storage form [4]. Intriguingly, the structure reveals an unprecedented principle of protein homo-dimerization: The high-affinity dimer is formed by a dynamic, multivalent interaction between the domains, rather than by a classical well-defined single conformation. The avidity in the dimer structure explains how the dimeric state of Trigger Factor can be monomerized also by weakly interacting clients.

References
*equal contributions
One intellectual challenge for materials characterization lies in the characterization of the intermingling of chemical functional groups – their “apportionment” in space. This is an old problem, but manifests itself in several contemporary problems, such as energy storage and generation, carbon capture, and catalysis. Limited S/N poses another challenge, and despite promising and commercially available instruments that improve NMR signals, a generalized approach to nuclear hyperpolarization is needed. I surmise that the “artificial atom” may be such an approach.
PLENARY SESSION 1

Monday, July 24
08:30-09:15

Lewis E. Kay
NMR: Why Bother?
NMR: Why Bother?

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With the ever-evolving development of new biophysical tools and increasingly powerful techniques for biomolecular structural studies it is reasonable to contemplate the role of solution based NMR spectroscopy in going forward. In this talk I will describe a number of studies on molecular machines from my laboratory, emphasizing the unique role that NMR can play in providing quantitative descriptions of molecular dynamics and how such motion relates to function. The complementarity of NMR to other structural techniques is such that as they continue to advance so to will the utility of NMR. If anything NMR is far more valuable today than it was even a decade ago. This will be emphasized through a number of examples.
PLENARY SESSION 2

Monday, July 24
09:15-10:00

Malcolm H. Levitt
Nuclear Spins Far from Equilibrium
Nuclear Spins Far From Equilibrium

M. H. Levitt

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There are hundreds of nuclear magnetic resonance (NMR) methods applied today, ranging from methods for the imaging of anatomic structure (MRI) to methods for determining molecular structure and dynamics in solution and in the solid state. However most of these techniques employ nuclear spin systems which remain very close to a state of thermal equilibrium with respect to the molecular environment.

It is now possible to prepare substances in which the nuclear spin systems are very far from thermal equilibrium. Such systems may, in some cases, give rise to NMR signals which are highly enhanced with respect to signals obtained under routine conditions. The enhancement factor achieved by such hyperpolarization effects may be as large as 10^5, facilitating entirely new classes of NMR applications such as the imaging of metabolism in vivo. There are also modes of non-equilibrium nuclear spin order which are non-magnetic and do not give rise to NMR signals, but which are relatively long-lived, allowing the non-equilibrium state to be maintained for a relatively long time. Methods exist for extracting hyperpolarized spin order from such non-magnetic non-equilibrium long-lived states, on demand. In some cases, highly non-equilibrium nuclear spin order even gives rise to non-magnetic effects, such as a change in the dielectric constant of the material.

I will review the types of non-equilibrium spin order that exist, describe how they are prepared, how they may be interconverted, and how they may be applied.
SOLUTION NMR

Monday, July 24
10:30-12:00

G. Marius Clore
Uncovering Invisible Dark States of Biological Macromolecules and Their Complexes by Magnetic Resonance

Hashim M. Al-Hashimi
Nucleic Acid Excited States as Major Determinants of Spontaneous Mutations and Translational Errors

Supriya Pratihar
Supra-$\tau_c$ Protein Dynamics from High-Power Relaxation Dispersion Experiments

Luke W. Arbogast
Applications of 2D NMR for Characterization of Monoclonal Antibody Therapeutics
Uncovering invisible dark states of biological macromolecules and their complexes by magnetic resonance

G. Marius Clore*

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Sparsely-populated, transient, “dark” states of macromolecules, characterized by short lifetimes, play a key role in many biological processes including macromolecular recognition, conformational rearrangements and assembly. Yet, these species are invisible to conventional structural and biophysical techniques, including crystallography and conventional NMR. We will briefly summarize new developments involving the application of lifetime line broadening (DR2) and Dark-state Exchange Saturation Transfer (DEST) spectroscopy, combined where appropriate with relaxation dispersion and very small exchange-induced shifts, to probe exchange processes (with lifetimes ranging from about 20 µs to 10 ms) between NMR-visible and very high-molecular weight (1 to >100 MDa) NMR-invisible (“dark”) macromolecular assemblies and to elucidate atomic resolution structure and dynamics within these dark states. We will illustrate these approaches to the study of interactions of intrinsically disordered proteins and various folding intermediates with the chaperone GroEL, and to the global dynamics and exchange kinetics of proteins on the surface of lipid nanoparticles. These techniques have a wide range of applicability to many areas of current interest in both biology and materials science.
Nucleic Acid Excited States as Major Determinants of Spontaneous Mutations and Translational Errors

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Rare tautomeric and anionic nucleobases are believed to have fundamental biological roles, but their prevalence and functional importance has remained elusive because they exist transiently, in low abundance, and involve subtle movements of protons that are difficult to visualize. Using NMR relaxation dispersion, we show that wobble dG•dT and rG•rU mispairs in DNA and RNA duplexes exist in dynamic equilibrium with short-lived, low-populated Watson-Crick-like mispairs that are stabilized by rare enolic or anionic bases [1]. These mispairs can evade Watson-Crick fidelity checkpoints and form with probabilities (~10^{-3} to 10^{-5}) and kinetic rates that strongly imply a universal role in replication and translation errors [1]. The rate of inter-conversion between wobble and Watson-Crick-like mismatches can vary by several orders of magnitude depending on sequence context. Thus, a complex, highly sequence-dependent triangular kinetic network involving multiple tautomeric and anionic species underlies the earliest events leading to replication and translation errors. These results indicate that rare tautomeric and anionic bases are widespread in nucleic acids, expanding their structural and functional complexity beyond that attainable with canonical bases.

References

Protein dynamics occurring on a wide range of timescales play a crucial role in governing protein function. Particularly, motions between the globular rotational correlation time (τ_c) and 40 µs (supra-τ_c window), strongly influence molecular recognition [1, 2]. This supra-τ_c window was previously hidden, due to the lack of experimental methodology. Recently, we have developed a high power R_1ρ relaxation dispersion (RD) experiment for measuring kinetics as fast as ~2.5 µs [3]. For the first time, this method, performed under super-cooled conditions, enabled us to detect a global motion in protein GB3, which was extrapolated to 371 ± 115 ns at 310 K. This β-turn is involved in antibody binding, exhibiting the potential link of the observed supra-τ_c motion with molecular recognition (Fig. 1) [1].

Furthermore we have designed a robust 13C CT-windowless CPMG experiment that can accurately study both slow and fast timescale of motions. Using the windowless CPMG experiment, we confirm the connectivity between the population shuffling in the side chain [3] and the recently identified allosteric motion in ubiquitin (Fig. 2) [4].

Further attempts to access even faster timescale motion to make the entire supra-τ_c window detectable, will be described in the presentation.

References
APPLICATIONS OF 2D NMR FOR CHARACTERIZATION OF MONOCLONAL ANTIBODY THERAPEUTICS

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Monoclonal Antibody therapeutics (mAbs) are clinically important drugs, which as a class have experienced tremendous growth in the past two decades. Their development and manufacture however, presents many new and significant analytical challenges relative to classic small molecule drugs. In particular, methods for the characterization of higher order structure (HOS) are of critical importance, since misfolding or aggregation of mAbs can lead to loss of efficacy or cause unwanted, dangerous immune responses. Furthermore, the ability to introduce more affordable generic or biosimilar versions of mAb drugs to market requires demonstrating comparability of HOS to the originator drug substance. Two-dimensional (2D) heteronuclear \textsuperscript{1}H-\textsuperscript{15}N-amide and \textsuperscript{1}H-\textsuperscript{13}C-methyl NMR presents an excellent choice for characterization of mAb HOS, since small changes in chemical environment and structure give rise to readily observable changes in corresponding spectra. Indeed, we have previously demonstrated the feasibility of acquiring such spectra on both intact mAbs as well as protease cleaved constituent Fab and Fc domains at natural isotopic abundance.\textsuperscript{1,2} We will present on the continued development and validation of 2D NMR methods for characterization of therapeutic mAbs, including results detailing optimal performance in terms of precision and sensitivity using both conventional and non-uniform sampling methods. We will further demonstrate how systematic collections of such spectra can be statistically analyzed via multivariate approaches such as Principal Component Analysis (PCA) to correlate spectra according specific attributes of analyte HOS (figure 1). Finally, we detail the precision and robustness with which these methods can be applied in the biopharmaceutical setting.

Fig. 1. PCA scatter plot of 34 \textsuperscript{1}H-\textsuperscript{13}C methyl spectra from 3 distinct mAb species.

References
SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Monday, July 24
10:30-12:00

Clemens Glaubitz
High-Field and DNP-Enhanced Solid-State NMR on 7TM Membrane Proteins, Lipid Regulators and ABC Transporters

Philip T. F. Williamson
Molecular Insights into Biomolecular Structure and Dynamics Using 14N DNP-MAS NMR

Burkhard Bechinger
Optimization of Dynamic Nuclear Polarization Solid-State NMR for Oriented and Non-Oriented Membranes

Toshimichi Fujiwara
High-Field DNP and Quantitative Cellular Solid-State NMR under Low Temperatures
High-field and DNP-enhanced Solid-State NMR on 7TM Membrane Proteins, Lipid Regulators and ABC Transporters

Clemens Glaubitz, Johanna Becker-Baldus, Orawan Jakdetchai, Hundeep Kaur, Jagdeep Kaur, Clara Kriebel, Andrea Lakatos, Jakob Maciejko, Jiafei Mao, Kristin Möbius, Roberta Spadaccini

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The advancement in structural biology of membrane proteins mainly due to crystallography has created a great demand and offers also the opportunity of in-depth spectroscopic studies to resolve functional mechanisms. In many cases, solid-state NMR is the method of choice. MAS at high magnetic fields is essential for uniformly/extensively labelled membrane proteins, while the signal enhancement provided by DNP – despite lower fields and cryogenic conditions - has broadened its applicability towards cases, which have not been accessible before. On our experimental setup, DNP-enhancements between 40- and 100-fold are routinely achieved for membrane proteins within lipid bilayers, which enabled a range of novel applications such as trapping of photointermediate states [1], mapping of cross-protomer contacts [2] or resolving subtle mechanistic details in photoreceptors and GPCRs [3, 4]. Here, the use of $^{31}$P-$^{13}$C DNP-enhanced dipolar spectroscopy for nucleotide- and lipid-protein interactions will be explored for the ABC transporter MsbA. These data will be discussed in the context of a newly discovered mechanism, which extends the generally accepted paradigm [5]. Progress in resonance assignment using uniformly labelled samples and ‘conventional’ high-field MAS-NMR will be demonstrated for the lipid regulator diacylglycerol kinase (3x12kDa) as well as for the light-driven Na$^+$ pump KR2 (5x30kDa). The complementary use of DNP in these cases will be illustrated by studies on trapped intermediates and protomer-protomer interactions and a perspective for linking NMR parameter with structural data via quantum chemical approaches will be provided [6].

References
Molecular insights into biomolecular structure and dynamics using 14N DNP-MAS NMR

Maria Concistre¹, James A. Jarvis², Michael Jolly², Luke Evans², Sophia Lishman², Ibraheem Haies¹, Ilya Kuprov¹ Marina Carravetta¹, Philip T.F. Williamson²*

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Structural analysis of proteins and other biomaterials in the solid-state has typically required extensive labelling which has limited its application to natural materials. Over the last few years significant progress has been made towards exploiting naturally occurring nuclei including proton detection (reviewed in 1) and the analysis of 14N sites (reviewed in 2). Here we report on the use of indirect 14N detection methods for the structure analysis of proteins in the solid-state. In preliminary studies, we have exploited 13C labelling to provide resolution in the spectrum of the spy nucleus. Using DNP-MAS NMR in conjunction with rf recoupling of 14N/13C interactions³ we have been able to characterize the size of the quadrupolar interactions present in the backbone and sidechain 14N sites of a microcrystalline preparation of GB3. This data demonstrates qualitatively that the distribution in the size of quadrupolar interactions for backbone sites mirrors the types of secondary structure present in the proteins. The large range of quadrupolar couplings observed reflects their sensitivity to subtle changes in the H-bonding status of the nitrogen sites, highlighting the possibility of using these methods for the structural analysis of proteins. Currently, we are extending these methodology to exploit protons as a spy nucleus that will facilitate the analysis of natural materials as well as providing a significant increase in sensitivity. These methods allow the rapid and accurate characterization of the quadrupolar interaction in small molecules without the need to utilize DNP. Preliminary 1H/14N MAS-DNP studies of amyloid fibres have been conducted. These studies have highlighted some of the remaining challenges to employing 1H detected MAS-DNP NMR to biomolecules.

References
OPTIMIZATION OF DYNAMIC NUCLEAR POLARIZATION
SOLID-STATE NMR FOR ORIENTED AND NON-ORIENTED MEMBRANES

E.S. Salnikov¹, O. Ouari², F. Aussenac³, C. Reiter⁴, C. Aisenbrey¹, A. Purea⁴, J. Raya¹, H. Sarrouj¹, P. Tordo², I. Fedotenko², F. Engelke⁴, B. Bechinger⁴

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Dynamic Nuclear Polarization (DNP) has been introduced to overcome the sensitivity limitations of nuclear magnetic resonance (NMR) spectroscopy also of supported lipid bilayers. When investigated by solid-state NMR techniques the approach typically involves doping the samples with biradicals and their investigation at cryo-temperatures. A new NMR probe for oriented membrane systems will be presented and the specific problems associated with DNP measurements on static samples discussed [2]. The protocols, sample geometries, cooling and microwave irradiation have been optimized for such measurements and novel membrane-anchored biradicals developed, investigated in physicochemical detail and tested in phospholipid bilayer environment. Furthermore, the effects of membrane lipid deuteration and composition have been investigated. Finally the effects of temperature and membrane hydration on the topology of amphipathic and hydrophobic membrane polypeptides will be presented. Although the antimicrobial PGLa peptide in dimyristoyl phospholipids is particularly sensitive to topological alterations, the DNP conditions represent well its membrane alignment also found in bacterial lipids at ambient temperature [1]. Whereas under MAS conditions signal enhancements are up to 70-fold, for static oriented sample 17-fold, which is among the best for truly static matrix-free system. Furthermore, a membrane anchor sequence encompassing 19 hydrophobic amino acid residues was investigated. Although at cryotemperatures the transmembrane domain adjusts it membrane tilt angle by about 10 degrees, the temperature dependence of two-dimensional separated field spectra show that freezing the motions can have beneficial effects for the structural analysis of this sequence.

Figure 1: PISEMA DNP solid-state NMR spectrum of a transmembrane helical domain labelled with $^{15}\text{N}$ at five consecutive sites. The corresponding tilt angle is 10 degrees [1]. Whereas such spectra take several days of acquisition at RT they can be recorded in about 1 hour at 100K and DNP.

References
Solid state NMR has been shown to be useful for studying macromolecules in native conditions as in bilayers and aggregated states. Such analysis can also be applied to cellular systems where biomolecules are functional. Low temperature conditions provide advantageous effect for such cellular studies in the following applications.

**Quantitative NMR at low temperature for the analysis of cellular processes**

Under low temperature condition where molecular motions are suppressed, solid-state $^{13}$C magnetic resonances can be obtained equally for carbon-13 atoms in all the biomolecules. This should be compared with solution NMR which gives the signal intensities only for fast-tumbling small proteins and molecules. The thermal equilibrium NMR signal intensities give the quantitative information for various cellular molecules depending on the spectral resolution of multidimensional NMR. Thus the molecules for a single cell can be counted by combined use of optical and electron microscope analyses. We measured the quantitative NMR for studying the metabolism of *E. coli* cells under overexpression of a protein. The biosynthetic process was traced by using the $^{13}$C labeled media without the purification. The quantitative molecular information obtained is useful for the optimization of protein production systems based on systems biology. We also show the localization of biomolecules in a cell by the paramagnetic relaxation enhancement due to Gd$^{3+}$ contrast agents at the cell membranes.

**Magic-angle spinning DNP NMR at 30 K for the sensitivity enhancement**

Solid-state NMR sensitivity is enhanced by DNP at low temperatures. This is due to reduced electrons and nuclear spin relaxation and stronger spin polarization at low temperatures. However, necessity of expensive He gas for sample cooling makes the DNP experiments difficult to perform routinely. We developed a closed-cycle He-gas system for the MAS DNP NMR at 30 K and static magnetic field 16.4 T. We will show our recent results for the low temperature DNP NMR with double submillimeter wave irradiation.

Reference

SOLID-STATE NMR MATERIALS/ METHODS

Monday, July 24
10:30-12:00

Brad F. Chmelka
NMR Analyses of Order, Disorder, and Surface Interactions in Nanoscale Semiconductors

Juergen Haase
NMR Changes the View of Cuprate Superconductivity

Darren H. Brouwer
From Solid-State NMR to Zeolite Crystal Structures through Combinatorial Tiling Theory

Frédérique Pourpoint
Study of the Xenon Exchange between the Two Forms of MIL-53(Al) Using Solid-State NMR
NMR ANALYSES OF ORDER, DISORDER, AND SURFACE INTERACTIONS IN NANOSCALE SEMICONDUCTORS

B.F. Chmelka, Z.J. Berkson, M.N. Idso, R. Sangodkar, B.J. Smith

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Advances in synthesis, characterization, and modeling capabilities enable the atomic-scale compositions and structures of nanostructured semiconductors to be measured and correlated with their macroscopic physicochemical properties. Solid-state NMR spectroscopy, especially two-dimensional and dynamic-nuclear polarization (DNP)-enhancement techniques, together with X-ray scattering, electron microscopy, and molecular modelling, yield detailed insights on local bonding environments and interactions in nanostructured semiconductor materials. These include nanoscale semiconductors, such as chalcogenide, phosphide, or nitride nanocrystals and organic or hybrid photovoltaic materials, whose surface compositions and structures have important influences during their syntheses and on their subsequent macroscale opto-electronic properties. NMR analyses provide new understanding of structure-function relationships at the nanoscale, especially on molecular interactions at and complicated order-disorder near semiconductor surfaces.
NMR Changes the View of Cuprate Superconductivity

J. Haase
University of Leipzig

NMR has played an influential role for the theory of cuprate superconductivity soon after its discovery. Until very recently, apart from the interpretation of electronic and/or chemical inhomogeneity, NMR data appeared undisputed. Very recently, however, based on new NMR insight we proposed a new phase diagram of these materials, which hardly any other method could have devised. It relates cuprate properties to the chemical structure in a simple way and predicts, based on NMR parameters, a route to perhaps even reach room-temperature superconductivity. It will be shown how this information can be drawn mostly from quadrupole splittings. These results inspired, in addition, a new analysis of the magnetic shifts, which suggests a very different picture of the electronic properties compared to what was concluded years ago. Lastly, we show how the application of high-pressure NMR can make a charge density wave move in these materials, which came in the disguise of a wrongly interpreted orthorhombic splitting. This shows the power of NMR physics applications.
FROM SOLID-STATE NMR TO ZEOLITE CRYSTAL STRUCTURES THROUGH COMBINATORIAL TILING THEORY

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Solid-state NMR spectroscopy can provide a great deal of structural information about the local environments of NMR-active nuclei. When combined with other complementary techniques such as diffraction and quantum chemical calculations – an approach referred to as \textit{NMR crystallography} – powerful structure determination strategies emerge that allow for the elucidation of detailed structures that no one technique could provide on its own. We have been particularly interested in developing structure solution strategies that maximally exploit the information available in 1D and 2D solid state NMR data from network materials such as zeolites, with a view to solving structures when minimal diffraction data is available, such as layered structures \cite{1}. Based on some elegant mathematics from the field of \textit{combinatorial tiling theory} \cite{2}, we are developing a novel structure solution strategy for network materials that will efficiently generate all feasible structures that arise from the number of sites (number of peaks), relative site occupancies (peak intensities), coordination environments (chemical shifts) revealed in 1D spectra and the intersite connectivities (cross peak intensities) revealed in 2D correlation spectra. One of the key features of this approach is that no prior knowledge of the crystallographic space group or unit cell parameters is intrinsically necessary, which in principle allows for the generation of network crystal structures directly from solid-state NMR data alone. This talk will introduce and explain \textit{Delaney symbols} (mathematical structures for describing tilings of the 2D plane or 3D space), highlighting the rich amount of structural information they contain and how they allow for novel conceptual linkages between solid-state NMR and crystallography of network materials.

\textbf{Fig. 1.} Schematic of combinatorial tiling strategy to generate network crystal structures from solid-state NMR.

\textbf{References}

Study of the Xenon Exchange between the two Forms of MIL-53(Al) using Solid-State NMR

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The Metal-Organic Framework (MOF) MIL-53(Al) exhibits an interesting porous structure since a transition can occur between the large pore (lp, corresponding to the anhydrous form) and the narrow pore (np, corresponding to the low temperature hydrated form) configurations. It has already been shown that $^{129}$Xe solid-state NMR can probe these different pore openings¹ governed by the temperature, the external pressure or the presence of guest molecules. Furthermore, decreasing the temperature of the system at 230 K is required to decrease the mobility of the Xenon atoms and thus to allow efficient $^1$H→$^{129}$Xe cross-polarization transfer under MAS (CP-MAS). Therefore, only the np structure can be observed. Moreover, we investigate the xenon exchange rate between the lp and np forms by using two different NMR techniques: 2D EXSY experiments with different mixing times and a selective inversion (SI) recovery method. By modeling the experimental data, we measured the exchange rate of $^{129}$Xe nuclei between the two forms of MIL-53(Al) at room temperature. Both techniques provide a value with the same order of magnitude. Nevertheless, we prove the higher accuracy of the SI method that yields a xenon exchange rate of about (43±6) s⁻¹.

![Diagram](image)

(a-b) Inversion recovery measurement of np (blue squares) and lp (red triangles) forms of the MIL-53(Al) sample recorded with (a) Non Selective Inversion recovery experiment (NSI), and (b) SI of the np signal. The fit yields an exchange rate of (43±6) s⁻¹. (c) Example of a 2D EXSY experiment recorded with a mixing time of 50 ms.

HYPERPOLARIZATION

Monday, July 24
10:30-12:00

Mathilde H. Lerche
Quantifying Biochemical Activities in Living Cells with $^{13}$C dDNP NMR

Igor V. Koptyug
Para-H$_2$ & Heterogeneous Catalysis for Enhanced NMR

Leah Casabianca
Toward Characterization of Nanoparticle Surface Interactions by NMR

Akiva Feintuch
The Role of the Electrons in Dynamic Nuclear Polarization
Quantifying Biochemical Activities in Living Cells with $^{13}$C dDNP NMR

Pernille Rose Jensen$^1$, Magnus Karlsson$^1$, Andrea Capozzi$^1$, Jan Henrik Ardenkjær-Larsen$^1$ and Mathilde H. Lerche$^1$

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During the last decade, the development of nuclear spin polarization enhanced (hyperpolarized) molecular probes has opened up new opportunities for studying the inner workings of living cells. The hyperpolarized probes are produced ex situ, introduced into biological systems and detected with high sensitivity and contrast against background signals using high-resolution NMR spectroscopy, Fig.1.A. Experimental investigations of chemical reactions in the cell require quantitative tools for time-resolved in situ analyses in physiologically relevant settings. However, dissolution DNP (dDNP)$^{[1]}$ is not, a priori, a quantitative method when applied in studies of complex molecular systems and incomplete description of the signal evolution has until now limited dDNP to an observing tool.

The talk will focus on strategies used for the selection, design and use of hyperpolarized NMR probes in quantitative biological assays. In particular four studies will be discussed: 1) The development of a simple cellular assay from which the intracellular T1 could be measured unambiguously for the carboxylic acid moiety of four compounds. On this basis, a quantitative method when applied in studies of complex molecular systems and incomplete description of the signal evolution has until now limited dDNP to an observing tool.

2) Hyperpolarised $^{133}$Cs NMR has been developed into a sensitive real time biosensor that in seconds provides a background free measure of access to the intracellular environment, Fig.1.C. 3) Non-persistent photo-induced radicals are shown to efficiently polarise $^{133}$Cs-glucose to 50%. This type of radicals can be scavenged through fast thermalisation following DNP$^{[4]}$ leaving the bioprobe solution free of paramagnetic impurities and consequently with long T1 at appropriate conditions. The storing of frozen hyperpolarised glucose and its continuous thawing for cellular infusion over many minutes is evaluated. 4) The use of DNP to enhance metabolite extracts benefits from the high sensitivity and resolution provided by hyperpolarized samples in high-field NMR spectrometers, from the possibility to quantify metabolites by comparison with an internal standard, and from the capability to monitor slow metabolic transformations. Biological hypotheses are made using extracts from cancer cells incubated with $^{13}$C-glucose for many minutes, Fig.1.D.

Fig. 1. A. Set-up for studying dDNP hyperpolarised bioprobes in living cells.$^{[2]}$ B. Two compartment system where intra and extra cellular milieu is differentiated by the chemical shift.$^{[3]}$ C. 30% polarisation on $^{133}$Cs in 5 min allow detection of intra cellular $^{133}$Cs in membrane impaired cells. D. Metabolites (lactate and 6-phospho gluconate, 6-PG) produced over time in breast (MCF7) and prostate (PC3) cancer cells incubated with $^{13}$C-glucose.

References
Parahydrogen-induced polarization (PHIP), including its most recent variants such as SABRE, is arguably the simplest and technically least demanding hyperpolarization technique which in recent years attracts significant attention of the magnetic resonance community. Since the first unambiguous demonstration of PHIP in 1987 and until 2007, observation of PHIP effects was associated exclusively with homogeneous processes in solution, such as \( \text{H}_2 \) activation and catalytic hydrogenation of unsaturated compounds by dissolved transition metal complexes. However, during the past decade PHIP effects in liquid and gas phase hydrogenations of alkenes and alkynes have been also demonstrated with numerous heterogeneous hydrogenation catalysts, including transition metal complexes immobilized on solid supports, supported and unsupported metals, metal oxides, metal sulfides, etc. Further extension to other catalyst types such as single-site and/or molecularly defined heterogeneous hydrogenation catalysts was also demonstrated. Representative recent examples of such studies will be discussed.

The ability to produce and observe PHIP effects in heterogeneous catalytic processes (HET-PHIP) significantly expands the range of potential applications of PHIP-derived signal enhancement in NMR and MRI. We currently pursue two main directions in HET-PHIP research. One of them encompasses mechanistic studies of heterogeneous catalytic transformations of industrial importance which involve \( \text{H}_2 \) as one of the key reactants. In addition to heterogeneous hydrogenations, recent demonstrations include catalytic oligomerization and desulfurization processes. In addition, utilization of PHIP-derived signal enhancement in MRI can provide new valuable knowledge about the dynamic processes in operating reactors. Thus, combining the unique chemical specificity of NMR spectroscopy and the non-invasive nature of MRI with the strong parahydrogen-based signal enhancement can be beneficial for catalytic and chemical engineering applications of magnetic resonance.

Another major direction of HET-PHIP research is the production of hyperpolarized molecular markers for biomedical applications of MRI and MRS. Such applications require the availability of biocompatible hyperpolarized samples. Thus, if a hydrogenation catalyst contains transition metals or other toxic ingredients, it needs to be rapidly removed from the sample after reaction completion. Unlike homogeneous catalysts, the heterogeneous ones can be easily filtered out after the reaction. Furthermore, heterogeneous catalytic hydrogenation can be performed in a continuous flow mode, providing a constant stream of hyperpolarized fluid. While an ultimate catalyst with the highest HET-PHIP production efficiency is yet to be identified, we have taken decisive steps toward biomedical applications of this methodology. Examples of liquid and gas phase hydrogenations of unsaturated compounds with parahydrogen to produce catalyst-free hyperpolarized substances for potential in vivo use and to prolong polarization lifetime will be described. Current efforts to extend the methodology beyond reactions that require the use of molecular hydrogen will be also reported.

References

Nanotechnology is becoming increasingly prevalent in our everyday lives. Nanoparticles that are used as lubricants, in drug delivery, and as antibacterial agents are finding their way into the body and into the environment, where they interact with biological macromolecules such as proteins as well as small molecules. Understanding the nature of the interactions between nanoparticles and adsorbents is therefore relevant to nanoparticle toxicity. Unfortunately, the majority of techniques that are currently used to study nanoparticle surfaces are low-resolution techniques that are incapable of providing atomic-level detail into these interactions. For this reason, my lab is developing Nuclear Magnetic Resonance (NMR) techniques for studying the molecular structure of nanoparticle-surface interactions.

My group has recently[1] developed HighlY-effective Polymer/Radical Beads (HYPR-beads), which are nanoparticle-based polarization agents for dissolution Dynamic Nuclear Polarization (DNP). HYPR-beads are polystyrene latex nanospheres that have been doped with BDPA, a popular hydrophobic DNP polarizing agent. These nanospheres can be dispersed in water, allowing DNP to be carried out on water-soluble analytes without the need for a glassing co-solvent.

We are also using computational methods to examine the change in NMR chemical shift that occurs when a small molecule is located in close proximity to nanomaterials such as carbon nanotubes. Using the Gauge-Including Atomic Orbitals method, we have explored the effect of curvature on the nucleus-independent chemical shift (NICS), which represents the change in chemical shift that can be expected to be felt by a nearby nucleus, at locations near the convex and concave faces of the curved surfaces was also calculated.

References
The role of the electrons in dynamic nuclear polarization

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The term dynamic nuclear polarization (DNP) is used to describe any process in which polarization is transferred from electron spins to nuclear spins by microwave (MW) irradiation. Over the more than half a century that this phenomenon has been observed, it has been shown that this process can be driven by different mechanisms. A common approach is to classify these mechanisms by the number of spins necessary for the polarization transfer. The case of a single electron spin interacting with a single nuclear spin is termed the solid effect (SE)[1], two dipolar coupled electrons interacting with a nuclear spin can result in the cross effect(CE)[2], and an electron spin bath interacting with a nuclear spin bath are described by thermal mixing (TM)[3]. Recently it has been shown that the SE- and CE-DNP processes in samples containing radicals with an inhomogenously broadened EPR spectrum are strongly dependent on the frequency rofile of the electron polarization during MW irradiation. Such profiles are a result of the electron spectral diffusion (eSD) mechanism[4,5] and can be monitored by performing pulsed electron-electron double resonance (ELDOR) experiments. In this work we will present a large set of ELDOR spectra of TEMPOL in DMSO/H₂O measured at W-band for different radical concentrations and temperatures. These experimental results enable an evaluation of the theoretical eSD bin-model we have introduced in a previous work[4]. The data is fit to the model by changing only the value of the eSD constant. The concentration dependence of this parameter as obtained by the fits is shown in figure 1. This concentration dependence can be explained assuming the eSD process is governed by a dipolar cross-relaxation mechanism. Additionaly a strong correlation is shown between the ELDOR profiles and the frequency dependent ¹H-DNP lineshapes.

References

Fig. 1. The concentration dependence of the spectral diffusion paraeter obtained from fitting a series of ELDOR data to a theoretical model.[4]
EPR

Monday, July 24
10:30-12:00

Christoph Boehme
Spintronics Based on Spin-Selection Rules

John J. L. Morton
Silicon Donor Spins under the Spotlight and Feeling the Strain

Brendon W. Lovett
Quantum Gates with Donors in Germanium

John M. Franck
Liquid-State Overhauser DNP Interpretation
Many organic semiconductors have weak spin-orbit coupled (SOC) charge carrier states and charge transport in these materials takes place through hopping or tunneling. Spintronics applications based on spin-injection, ballistic spin-transport and SOC mediated spin-manipulation schemes therefore seem difficult to achieve for these materials [1]. However, weak SOC also implies pronounced spin-selection rules and long spin-coherence times, which do cause spin-controlled magneto-optoelectronic effects [2-4] and thus, they provide an alternative route to spintronics applications, including electron- [6] and nuclear [7]-spin memory or low-field magnetometry [5].

In this talk, a brief survey of spin-selection rules based magneto-optoelectronic effects of organic semiconductors as well as their potential applicability for spintronics is given. The physical parameters determining the dynamics of these transitions include spin-dipolar, spin-exchange, spin-orbit, as well as hyperfine interactions and the most direct way to explore these mechanisms is to carefully manipulate spin using magnetic resonance and to then observe how macroscopic observables such as conductivity or optical transition rates change. The measurement of spin-induced conductivity changes after coherent spin resonant excitation is called pulsed electrically detected magnetic resonance spectroscopy (pEDMR). Several examples for pEDMR measurements will be shown and their implications for the involved spin-dependent processes will be discussed [2]. Furthermore, conductivity signatures of exotic non-linear magnetic resonance spin-effects will be presented, including the spin-Dicke effect within charge carrier pair ensembles that can be detected through the appearance of a sudden macroscopic phase coherence between paramagnetic charge carriers under strong AC drive [8].

The work presented in this talk was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering under Award #DE-SC0000909.

References
Silicon donor spins under the spotlight and feeling the strain

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The electron and nuclear spins of (Group V) donors in silicon have been proposed as potential quantum bits, or qubits [1]. The past few years have seen several breakthroughs towards this goal, including the measurement of the spins of single donor atoms, with high fidelity, in nanoelectronic devices [2], and the measurement of spin coherence times as long as 3 seconds for Bi donors spins at magnetic field insensitive ‘clock transitions’ [3].

The spins of Group V donors in silicon can be hyperpolarized to polarizations approaching 100% using resonant optical excitation of donor-bound excitons, and this can also be used as a high-sensitivity method of performing EDMR [4] and ESR on small ensembles of donors. This technique can be used for EDMR imaging, using a scanning laser spot to identify variations in donor concentrations, or in their spin properties. The method also has applications in performing ESR on samples with low donor concentrations, enabling studies of $^{28}$Si material with a phosphorus concentration of $\sim 10^{11}$ cm$^{-3}$. At such concentrations, the effect of instantaneous diffusion is suppressed and P-donor electron spin coherence times approaching one second can be observed using a Hahn echo measurement.

Understanding the effects of strain on donor spins is also critical if they are to be incorporated into micro/nano scale devices, and we present an investigation of strain effects across Group V donors, and donor-bound optical transitions, through ESR studies on bulk crystals and near-surface ensembles measured by micro resonators, distinguishing between the effects on strain on g-factor, hyperfine coupling and quadrupole terms.

QUANTUM GATES WITH DONORS IN GERMANIUM

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Recent work has shown that electron spins in germanium (Ge) nanoscale transistors can be electrically tuned [1] and have encouraging coherence times [2]. Based on a novel and complete multi-valley effective mass theory of Ge-donor electron states, we show that Ge spin qubits could have significant advantages over silicon (Si) in the implementation of a donor-based quantum processor architecture [3]. Our theory has been validated through excellent agreement with recent experimental spin resonance measurements of Ge [4]. Our work shows that the intrinsic features of the Ge band structure enable electric and magnetic field control of the spin splitting across a range spanning several GHz (see Figure 1). This would allow for a speedup of selective (local) one-qubit gates of up to two orders of magnitude as compared to Si. Further, we find that fast, robust two-qubit gates in Ge pose less stringent fabrication constraints than in Si devices: Ge-donors spaced three times farther apart than in Si show comparable exchange couplings, allowing more space for readout and control gates. In addition, for realistic position uncertainty in donor placement, Ge:P spin couplings have a 33% chance of being within an order of magnitude of the largest coupling, compared with only 10% in Si:P. It is therefore possible that a Ge based platform would enable fast, parallel and robust architectures for quantum computing.

Fig. 1. Density plot of the modulation of the Zeeman splitting induced by a nonzero electric field $E$, aligned with the $\langle 111 \rangle$ crystallographic direction, with an angle $\phi$ between $E$ and the magnetic field $B_0$. For realistic applied voltages, the detuning can be changed over the range $\{-3$ GHz, 3 GHz$\}$, allowing for nanosecond selective manipulation of locally detuned spins. $B_0 = 0.4$ T corresponds to a donor spin coherence $T_2 \approx 1$ ms [2].

LIQUID-STATE OVERHAUSER DNP INTERPRETATION

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Over the past several years, liquid state Overhauser-Effect Dynamic Nuclear Polarization (ODNP) has experienced a renaissance, demonstrating an ability to directly observe the hydration water around biological macromolecules and soft materials. ODNP has observed the significant variability in the hydration water surrounding proteins [1,2], nucleic acids [3], and polymer systems [4,5], and it has proven superior to traditional techniques for evaluating solvent accessibility [6].

In the coming years, improved interpretation and automation of ODNP experiments will open up yet newer fields of study. For example, the lipid bilayer is known to interact with the surrounding water molecules to generate a “hydration layer” (Fig 1), which has distinct properties from bulk water. This hydration layer is key to understanding peptides and proteins that interact with, associate with, and disrupt the bilayer.

Characterizing the central role of the hydration water in driving particular systems requires (1) understanding the role of lipid and sidechain dynamics (2) exploring changes in hydration dynamics with changing experimental parameters and (3) experimentally validating simulations to develop a meaningful, atomistic picture of local water. Here, we describe our multifaceted progress towards these respective goals, by: (1) developing new processing tools to improve 2D ESR spectral resolution (2) automating and extending quantitative ODNP experiments and (3) developing methods to directly compare ODNP data and molecular dynamics trajectories.

References

SOLUTION NMR / HYPERPOLARIZATION

Monday, July 24
13:45-14:45

Sebastian Hiller
Chaperone-Client-Interactions: From Basic Principles to Roles in Health and Disease

Joshua R. Biller
Nitroxide $T_1$ and $T_2$ from 0.1 to 10 mM: Implications for Solution-State DNP Experiments at Low Magnetic Field

Meghan E. Halse
Photochemical Pump, NMR Probe Spectroscopy for Monitoring Chemical Reactivity on Micro-Millisecond Timescales
Chaperone–client-interactions: From basic principles to roles in health and disease

S. Hiller¹², B. M. Burmann¹, L. He¹, I. Burmann¹, R. Riek², S. Campioni², J. A. Gerez², P. Kumari², S. G. Rüdiger³, M. Wawrzyniuk³

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Molecular chaperones are essential in cellular protein homeostasis. Central aspects of chaperone function are still not well understood at the atomic level, including how chaperones recognize their clients, and in which conformational states clients are bound. I will describe our research efforts to understand such aspects, employing high-resolution NMR spectroscopy as the main method.

Our initial work on the periplasmic holdase Skp with bound outer membrane proteins provided the first atomic-level description of a natural full-length chaperone–client complex [1, 2]. Subsequent work combining NMR spectroscopy with single-molecule force spectroscopy showed how periplasmic chaperones shape individual client folding trajectories, funneling the client polypeptide towards the native structure [3]. Then, a characterization of the Spy–Im7 model system with intermolecular PREs revealed how the chaperone Spy selectively recognizes the flexible, locally frustrated regions of partially folded client Im7 in a highly dynamic fashion. The interaction sites are identical for further chaperones, highlighting that general principles govern client recognition [4].

We then utilize our mechanistic insights to investigate the functional role of chaperones in Parkinson’s disease. Parkinson’s is one of the most common neurodegenerative disorders, pathologically manifested by intracellular accumulation of aggregates of the intrinsically disordered protein α-Synuclein. Systematic investigations on an array of chaperones identified a general chaperone interaction motive at the α-Synuclein amino-terminus. The dominant role of chaperone interactions for cytosolic α-Synuclein was validated with in-cell mass-spectrometry and NMR spectroscopy and the functional basis for the effects of several known post-translational modifications of α-Synuclein could thus be reconstituted in vitro. The data reveal how molecular chaperones control the state and function of α-Synuclein in vivo and how the disturbance of these interactions leads to progress of pathologically relevant aggregates.

References
Nitroxide $T_1$ and $T_2$ from 0.1 to 10 mM: Implications for solution-state DNP experiments at low magnetic field

J.R. Biller$^1$*, G.R. Eaton$^2$, J. Moreland$^1$

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Dynamic nuclear polarization (DNP) describes the enhancement of a nuclear signal (usually $^1$H or $^{13}$C) by hyperpolarization using a radical molecule in close proximity to the nuclear spin under investigation. The most prevalent variety of polarizer molecules used have been the nitroxide class of radicals. DNP enhancements from nitroxide radicals in fluid solution have been investigated when varying isotopic substitution, concentration, solvent, viscosity, and the presence or absence of oxygen [1]. Despite this effort, quantitative comparison of the enhancements from nitroxide radicals remains elusive, due to the presence of three nuclear hyperfine transitions in the EPR spectrum of a nitroxide radical.

Renewed focus on the effect of sample temperature [2], collision induced exchange broadening, and the nitrogen nuclear relaxation rate [3] yield new insight into DNP with nitroxide radicals. One area not explicitly treated is the change in spin-lattice ($T_1$) and spin-spin ($T_2$) relaxation times of the nitroxide molecule at the high concentrations used for DNP experiments. Here we describe measurements of $T_1$ and $T_2$ for each transition of several $^{14}$N nitroxide radicals up to 10.3 mM at 9.5 GHz (0.3 T). Measurements were made by both three-pulse echo inversion recovery, and by two-pulse FID-detected inversion recovery for the highest concentration samples for $T_1$, and by echo decay for $T_2$.

It has been previously demonstrated that relaxation mechanisms at 9.5 GHz (0.3 T) and 250 MHz (10 mT) are similar for fast tumbling low-concentration nitroxides in aqueous solution [4]. Thus $T_1$ and $T_2$ data collected at 9.5 GHz are directly releatable to the saturation factor of DNP experiments at 10 mT. Combining the measured $T_1$ and $T_2$ values with resonator efficiency allows more explicit treatment of the saturation factor, and calculations of the coupling factor should be improved. DNP research focused on maximum obtainable enhancement makes use of very high radical concentrations, while research focused on Overhauser enhanced MRI (OMRI or PEDRI) necessitates lower concentrations. In order to effectively translate the discoveries of each application, detailed study of the $T_1$ and $T_2$ over the wide concentration range is required.

References
[1] M.D. Lingwood, S.I. Han Annu. Re. NMR. Spectrosc. 2011, 73, 96-106
Hyperpolarisation is an increasingly important area of development in magnetic resonance, where applications are often sensitivity limited. Of the many approaches to hyperpolarisation, we focus here on para-hydrogen-induced polarisation (PHIP), where the source of polarisation, \( p-H_2 \), is the singlet nuclear isomer of \( H_2 \). In PHIP experiments, the symmetry of the environments of the \( ^1H \) nuclei in \( p-H_2 \) is broken by a chemical reaction such that the NMR signals of the former \( p-H_2 \) nuclei in the product are enhanced by several orders of magnitude.

We recently introduced a pump-probe method that uses a single laser pulse to introduce \( p-H_2 \) into a metal dihydride complex and then follows the time-evolution of the \( p-H_2 \)-derived nuclear spin states by NMR (Fig. 1).[1] This time-resolved NMR experiment can be used to (a) study chemical reactivity on micro-to-millisecond timescales,[2] and (b) observe the coherent evolution of the \( p-H_2 \)-derived spin states during the pump-probe delay.[3] We demonstrate this by studying pump-probe NMR spectra of metal dihydride complexes that add hydrogen on different timescales. If the hydrogen addition is very fast on the NMR timescale (i.e. < 5 \( \mu \)s), the addition of \( p-H_2 \) to the complex is coherent and the evolution of \( p-H_2 \)-derived zero-quantum coherences during the pump-probe delay can be observed and analysed over a \( \mu \)s-ms time window.[3] If the hydrogen addition is slow on the NMR timescale (i.e. hundreds to thousands of ms), the kinetics of the chemical reactivity can be determined quantitatively in a similar manner to other time-resolve spectroscopies.[2] Finally, if the addition of \( p-H_2 \) proceeds over an NMR relevant timescale (tens to hundreds of \( \mu \)s), we find that the evolution of the zero-quantum coherences during the pump-probe delay exhibit a characteristic de-phasing that can be quantitatively analysed to extract kinetic information about the \( H_2 \) addition.

Fig. 1. (a) Synchronised in-situ photochemistry setup. (b) Pump-probe NMR pulse sequence and corresponding chemical scheme where red indicates nuclei that are hyperpolarised. (c) \( ^1H \) NMR pump-probe spectrum of \( Ru(H)_2(CO)(PPh_3)(dppp) \) in \( C_6D_6 \).

References
SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Monday, July 24
13:45-14:45

Christopher P. Jaroniec
Structural Studies of Y145Stop Prion Protein Amyloids by Solid-State NMR

Ansgar B. Siemer
Solid-State NMR on Functional and Pathological Amyloids

Kendra K. Frederick
Combining Segmental and Specific Labelling with DNP NMR to Study Amyloid Proteins
The Y145Stop prion protein mutant (PrP23–144), which has been linked to a heritable amyloid disease in humans, is a valuable in vitro model for investigating the phenomena of amyloid strains and cross-seeding barriers. Previous magic angle spinning solid-state NMR experiments show that human PrP23-144 fibrils consist of a compact ~30-residue parallel in-register beta-sheet amyloid core located near the C-terminus and a dynamically disordered N-terminal domain. I will describe our progress toward providing the structural basis of amyloid strains and seeding specificities of PrP23-144 amyloids. Our most recent studies indicate that mouse and Syrian hamster PrP23-144 fibrils also contain a compact C-terminal amyloid beta-core. The conformation of this core region for the hamster amyloid differs from that for the human and mouse proteins, which resemble each other, and these data are consistent with the observed cross-seeding barriers between the species. We also show that specific mutations of single amino acid residues in the human protein sequence that are characteristic of the mouse and hamster proteins are sufficient to alter the human amyloid core conformation into that of the corresponding species, and that these mutations appear to be responsible for the onset of polymorphism for the mouse and hamster amyloids. A preliminary structural model for human PrP23–144 amyloid provides initial insights into these observations and will also be presented.
Amyloid fibrils are found in both disease and functional contexts, and it is unclear what distinguishes these types of fibrils structurally. In addition, it is not well understood how the formation of functional amyloids is regulated and what the mechanistic reason for amyloid formation is, especially in regulatory roles. We address these questions by investigating two fibril systems, namely huntingtin exon-1 (HTT\text{ex1}), important for Huntington’s Disease, and Orb2 a functional amyloid and key regulator of long-term memory in \textit{Drosophila}.

Using a combination of solid-state NMR, EPR, and other biophysical techniques, we were able to determine the exact location of the amyloid cores of these proteins. In particular, we show that the N-terminus of Orb2 isoform A, which is critical for the aggregation of the protein and the formation of long-term memory, is able to form the in-register, parallel β-sheet core of an amyloid fibril. Surprisingly, we found that the same domain is also able to form an amphipathic helix and bind to the surface of lipid vesicles. This membrane interaction can prevent amyloid formation suggesting a possible regulatory mechanism for formation of this functional amyloid.

HTT\text{ex1}, Orb2 and many other amyloid fibrils contain large disordered domains that might contribute to their function and toxicity. These domains are sometimes so dynamic that they can be studied using triple-resonance, J-based, out-and-back type pulse sequences under MAS. We show how variations of several different solution NMR experiments can be applied for assignments and in-depth characterizations of these dynamic domains.


Combining segmental and specific labelling with DNP NMR to study amyloid proteins

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Self-propagating changes in the conformation of amyloidogenic proteins play vital roles in normal biology and disease. The yeast prion protein, Sup35NM, is a self-propagating amyloid. Despite intense study, there is no consensus on the organization of monomers within Sup35NM fibrils. Some studies point to a beta-helical arrangement while others suggest a parallel-in-register organization. Intermolecular contacts are often determined by experiments that probe long-range heteronuclear contacts for fibrils templated from a 1:1 mixture of \textsuperscript{13}C and \textsuperscript{15}N labeled monomers. However, for Sup35NM, like many large proteins, chemical shift degeneracy limits the usefulness of this approach. Segmental and specific isotopic labeling reduce degeneracy, but experiments to measure long range interactions are often too insensitive. To limit degeneracy and increase experimental sensitivity, we combined specific and segmental isotopic labeling schemes with dynamic nuclear polarization (DNP) NMR \cite{Frederick2017}. Using this combination we examined an amyloid form of Sup35NM that does not have a parallel in-register structure. The combination of a small number of specific labels with DNP NMR enables testing of structural models for systems that were previously impossible due to low experimental sensitivity.

References

SOLID-STATE NMR MATERIALS/ METHODS

Monday, July 24
13:45-14:45

Rachel Martin
Quadruple-Resonance MAS Probe for Solid-State NMR of Extensively Deuterated Biomolecules

Zhehong Gan
High-Resolution NMR of Quadrupolar Nuclei at 1.5 GHz Magnetic Field

Kurt W. Zilm
EPR and DNP in the Same Probe
Quadruple-resonance MAS probe for solid-state NMR of extensively deuterated biomolecules

The development of instrumentation that will enhance the use of $^2$H NMR in studies of structure and dynamics in biomolecules is an ongoing focus of my research group. Extensive deuteration is often used to improve resolution in solid-state NMR of proteins. Substantially deuterating the sample dramatically reduces the homonuclear ($^1$H-$^1$H) and heteronuclear ($^1$H-$^{13}$C and $^1$H-$^{15}$N) heteronuclear dipolar interactions. This improves resolution, reduces the magnitude of the RF decoupling required, and enabling effective use of $^1$H-detected experiments, even in rigid solids and at moderate MAS rates. However, this enhanced resolution is obtained at the cost of information loss due to the less abundant protons. Although it is not yet frequently applied in the context of protein structure determination, the deuterium quadrupole interaction has been used in both solid-state and solution-state NMR as a sensitive probe of local order and sample mobility. In order for $^2$H NMR to be routinely used in the context of protein structure determination, instrumentation development is required so that $^1$H, $^2$H, $^{13}$C, and $^{15}$N can all be used in a single set of multidimensional experiments, without modifying the probe in between measurements.

In this presentation, I will describe the design, construction, and testing of a $^1$H/$^{13}$C/$^2$H/$^{15}$N quadruple resonance MAS probe with high-power $^{13}$C, $^2$H, and $^{15}$N channels. The 4-channel MAS probe, which is optimized for detection on the lower-frequency channels, makes use of a cross-coil design. Because this probe is intended for use with extensively deuterated samples, high-power decoupling on the proton channel is not required, enabling the use of a slotted-tube resonator as the outer coil. The circuits for the three heteronuclei use a common triple-tuned solenoid. The probe makes use of tuning-tube circuit technology in order to make the most efficient use of space inside the magnet bore. Design parameters, benchmarks, and preliminary experimental results on protein and lipid samples will be discussed for the 4–channel MAS probe prototype.
High-Resolution NMR of Quadrupolar Nuclei at 1.5 GHz Magnetic Field

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High magnetic fields can drastically enhance NMR spectral resolution of quadrupolar nuclei in solution and solids. The National High Magnetic Field Laboratory (MagLab) is commissioning a superconducting/resistive series-connected-hybrid magnet with fields up to 36.1 T. In this early stage of magnet operation, the field homogeneity reaches better than 1 ppm over 1 cm3 DSV using both passive ferromagnetic and active shims. The temporal fluctuations of the magnetic field have been reduced from ~15 ppm to <0.2 ppm using an external Bruker field/frequency lock. The high homogeneity and stability enable high resolution 1.5 GHz NMR representing a 50% increase in field from the highest superconducting NMR magnet available today. Figure 1 shows the field regulation and initial results of 27Al and 17O magic-angle spinning NMR spectra demonstrating the gain in spectral resolution for quadrupolar nuclei. The stable magnetic field under regulation enables not only signal averaging but more importantly two-dimensional NMR experiments as illustrated by the 17O multiple-quantum magic-angle spinning (MQMAS) spectrum of benzoic-acid at ~260 K. Efforts are continuing to further suppress residual high-frequency fluctuation components for higher sub ppm resolution by combining magnetic flux stabilization and NMR field/frequency lock. More results for quadrupolar nuclei and initial results of high-resolution 13C and 15N NMR using a 2 mm HCN MAS probe and a static HX probe for aligned biological samples will also be presented. Once commissioned, the 1.5 GHz NMR facility which can also be operated at 1.0 and 1.2 GHz will open to researchers worldwide.

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**Fig. 1.** a) field regulation from ~15 ppm field fluctuations (black) to < 0.2 ppm (red) using 7Li field-frequency lock of an external LiCl solution sample doped with 750mM MnCl2, b) 27Al MAS spectra of 9Al2O3.2B2O3 with 1 scan and 512 scans signal average, c) 17O MAS spectra of p-methoxy-cinnamic acid acquired at 21.1T and 35.2 T illustrating the gain in spectral resolution, d) 17O MQMAS spectrum of benzoic acid acquired at 35.2 T with 12.5kHz MAS and 260 K sample temperature for the complete removal of quadrupolar broadening.
EPR and DNP in the Same Probe

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Combining DNP with fast MAS is an attractive proposition for addressing some of the shortcomings of both techniques. By adopting a small sample, the total power needed for DNP can be reduced if it can be focused into the sample. With the addition of DNP, the low net sensitivity that accompanies microliter size samples in fast MAS rotors can potentially be offset. In this talk we will describe our approach to addressing the challenge of delivering and focusing mm-wave power into small MAS samples by development of EPR and DNP NMR capability in the same probe. Two separate systems will be described on our 7T instrument using a 170 mW diode based mm-wave source. The first is a multinuclear solution NMR probe modified for frequency swept EPR. This probe employs a dielectric waveguide to transmit mm-waves into the sensitive volume, and is used to compare how different rotor orientations, coil geometries and materials affect mm-wave sample penetration. The second is a triple resonance MAS and EPR probe where we demonstrate the use of a dielectric antenna as an alternative to a microwave horn for delivering mm-waves into the MAS sample. Results will be presented that demonstrate how EPR can be used to make real time adjustments to focus mm-waves into the sample and thereby optimize DNP performance. The ability to record both DNP NMR and EPR in the same configuration provides many insights into DNP NMR, especially how enhancements are affected by microwave polarization and MAS. Using this capability to optimize mm-wave delivery, we have achieved DNP enhancements over 450-fold at room temperature in $^{13}$C MAS spectra of diamonds. A protocol for determining the degree of electron saturation, and therefore the size of the electron mm-wave $B_1$ field strength using reflection mode EPR will be introduced. Support for this work by the NSF Chemical Measurement and Imaging program under grant CHE-1413096 is gratefully acknowledged.

Fig. 1.Left: Transition from corrugated waveguide to Teflon dielectric antenna and lens. Right: Image of focused mm-wave beam.
EPR

Monday, July 24
13:45-14:45

Elena Bagryanskaya
Pulse Dipole EPR-Based Distance Measurements at Ambient Temperature

Stephan Pribitzer
TRIER - A New Experiment to Correlate Dipolar Frequencies in Molecules with Three Paramagnetic Centers

Thomas Schmidt
Long Distance Measurements up to 160 Å in the GroEL Tetradecamer Using Q-Band DEER EPR Spectroscopy

Sabine Van Doorslaer
Small Acceptor Oligomers as Alternatives for Fullerene-Based Acceptors - What Can We Learn from EPR Techniques?

Oleg Poluektov
ERR/DFT Approach for Characterization of Photo-Induced Charge Separation States in Organic Photovoltaic Cells

Andreas Sperlich
On the Role of Triplet Excitons in Organic Solar Cells
Pulsed dipolar (PD) EPR spectroscopy is a powerful technique allowing for distance measurements between spin labels in the range of 2.5-10.0 nm. It was proposed more than 30 years ago, and nowadays is widely used in biophysics and materials science. Until recently, PD EPR experiments were limited to cryogenic temperatures (T < 80 K). Recently, application of spin labels with long electron spin dephasing time at room temperature such as triarylmethyl radicals and nitroxides with bulky substituents at a position close to radical centers enabled measurements at room temperature and even at physiologically relevant temperatures by PD EPR as well as other approaches based on EPR (e.g., relaxation enhancement; RE). In this presentation, we review the features of PD EPR and RE at ambient temperatures, in particular, requirements on electron spin phase memory time, ways of immobilization of biomolecules, the influence of a linker between the spin probe and biomolecule, and future opportunities [1-8].

The distance measurements performed in the last three years on model proteins and nucleic acids using an EPR technique such as DQC, DEER, SIFTER, or RE, became possible due to application of spin labels with long electron spin relaxation time and proper immobilization of the biomolecule. The data obtained for model proteins and for the DNA duplex are in good agreement with the data on the same system at low temperatures, obtained by shock-freezing the samples [4-8]. Thus, in most cases, it makes sense to perform distance measurements at cryogenic temperatures, especially in the cases of a low concentration of biomolecules like large proteins or RNA where it is necessary to prevent aggregation of biomolecules. Nevertheless, distance measurements at room temperature can be useful in the cases when the dynamics of complexes of biomolecules and changes in distance at higher temperatures are expected. Thus, the development of new approaches to mild immobilization and spin labels with longer phase memory time and higher stability is still a highly relevant task for the future development of PDS distance measurements at ambient temperatures.

References

TRIER - A NEW EXPERIMENT TO CORRELATE DIPOLAR FREQUENCIES IN MOLECULES WITH THREE PARAMAGNETIC CENTERS

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A new pulse sequence is presented for correlating dipolar frequencies in molecules with more than two paramagnetic centers, the triple electron resonance (TRIER) experiment. This pulse sequence is related to double electron-electron resonance (DEER), which is used for distance determination \cite{1}. In TRIER, linear chirp pulses with smoothed edges are used to create a refocused echo, which is used for detection. We use two hyperbolic secant pulses at different excitation frequencies to excite two other subsets of spins. These are coupled to the observed spin through the dipole-dipole interaction and the echo exhibits modulation as a function of the two dipolar evolution times. By shifting the position of the pump pulses a two-dimensional data set is obtained. A two-dimensional Fourier transform of the echo integral provides a correlation plot that connects dipolar frequencies that stem from the same molecule.

![TRIER spectrum of a triradical with isosceles geometry.](image)

We expect TRIER to be used in conjunction with DEER: For structure determination DEER traces are usually recorded for several doubly labeled molecules differing in the sites of spin labeling. In the presence of two conformers each DEER trace gives two distances. Assignment of these to the individual conformers is not trivial and usually requires a time consuming trial-and-error approach \cite{2}. TRIER can provide the missing connection between distances as correlations between dipolar frequencies.

References

\cite{1} G. Jeschke \textit{Annu. Rev. Phys. Chem.} 2012, \textbf{63}, 419.

Long Distance Measurements up to 160 Å in the GroEL Tetradecamer Using Q-Band DEER EPR Spectroscopy

Thomas Schmidt
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Current distance measurements between spin-labels on multimeric protonated proteins using double electron-electron resonance (DEER) EPR spectroscopy are generally limited to the 15-60 Å range.

Here we show how DEER experiments can be extended to dipolar evolution times of ~80 µs, permitting distances up to 170 Å to be accessed in multimeric proteins. The method relies on sparse spin-labeling, supplemented by deuteration of protein and solvent, to minimize the deleterious impact of multipin effects and substantially increase the apparent spin-label phase memory relaxation time, complemented by high sensitivity afforded by measurements at Q-band.

We demonstrate the approach using the tetradecameric molecular machine GroEL as an example. Two engineered surface-exposed mutants, R268C and E315C, are used to measure pairwise distance distributions with mean values ranging from 15 to 100 Å and from 30 to 160 Å, respectively, both within and between the two heptameric rings of GroEL. The measured distance distributions are fully consistent with the known crystal structure of apo GroEL. The methodology presented here should significantly expand the use of DEER for the structural characterization of conformational changes in higher order oligomers.
SMALL ACCEPTOR OLGOMERS AS ALTERNATIVES FOR FULLERENE-BASED ACCEPTORS – WHAT CAN WE LEARN FROM EPR TECHNIQUES?

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Over the last decade, the power conversion efficiencies (PCE) of bulk heterojunction organic solar cells (OSCs) have increased steadily, demonstrating their great potential for future photovoltaic applications. One of the big challenges is to find alternative small acceptor molecules that can be used to circumvent the problems of the widely used fullerene acceptors. The latter are expensive and have poor absorption properties and limited chemical versatility. While bulk-heterojunction OSCs with these small molecule acceptors were not yet competitive in 2013 (mostly PCE <2%) [1], fullerene-free OSCs with PCEs of more than 11 % have by now been reported [2].

In this work, a combination of multi-frequency continuous-wave and pulsed EPR and ODMR is used to characterize charge transfer in blends of donor polymers (such as MDMO-PPV and P3HT) and a range of electron-accepting oligomers, such as the rhodamine-flanked acceptor molecule FBR (see scheme, [3]).

Furthermore, DFT computations are performed to identify negative polarons created upon light illumination. These results will be compared to the differences in PCE exhibited by the different blends.

A further problem that hampers large-scale applications of OSCs is their relatively limited long-term stability. Since many degradation mechanisms involve radical pathways, EPR techniques are very valuable tools to gain insight in the processes that govern OSC stability, as will be illustrated in a second part of the presentation.

References
ERR/DFT APPROACH FOR CHARACTERIZATION OF PHOTO-INDUCED CHARGE SEPARATION STATES IN ORGANIC PHOTOVOLTAIC CELLS.

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Solar-based technologies such as Organic Photovoltaic (OPV) cells could provide sufficient energy to satisfy the global economic demands in the near future. Understanding the charge separation and electronic structure at a molecular level is crucial for improving the efficiency of OPV materials. Upon illumination, two paramagnetic species are formed in these materials due to photo-induced electron transfer between the conjugated polymer and the fullerene derivative. These species are the positive, $P^+$, and the negative, $P^-$, polarons on the polymer backbone and fullerene cage, respectively, and correspond to radical cations and radical anions. To interrogate an electronic structure of these polarons, we performed high-frequency EPR (130 GHz) and pulsed ENDOR experiments which allow unambiguous determination of g-tensors and hyperfine interactions of magnetic nuclei with the unpaired electron. The g-tensors and hf-interactions are a sensitive probe of the electronic wave function and provide an excellent reference for validation of our current and future theoretical calculations, and as a result, for reconstruction of the electronic structures of the respective polarons. Our comprehensive EPR/DFT study revealed that, in all three systems, the positive polaron is distributed over distances of 40 - 60 Å along the polymer chains. This corresponds to about 15 thiophene units for P3HT, approximately three units for PCDTBT, and about three to four units for PTB7. No charge delocalization over several fullerene cages was observed in polymer–monomeric fullerene films. Delocalization of the positive polaron on the polymer donor is an important reason for the efficient charge separation in bulk heterojunction (BHJ) systems as it minimizes the wasteful process of charge recombination.

In contrast, in the novel covalently linked $C_{60}$:$C_{70}$-heterodimer fullerene it was found that the anion state is delocalized over both cages in the films, but is predominantly localized on either the $C_{60}$ or $C_{70}$ cage of the dimer in the solution. Electronic structure calculations demonstrated that different delocalization pattern in the dimers are related to the presence of two nearly iso-energetic minima, essentially the cis and trans conformers, which are separated by a thermodynamically accessible rotational barrier (Fig. 1).

For the “small” donor molecules in BHJ solar cells the combined EPR/DFT approach allows revealing not only delocalization of the positive polarons but also reconstruction of the polaron conformation states with high precision.

Fig. 1. Charge distribution in the high-performance low band gap polymer donor PTB7 (top) and on the fullerene derivative acceptors (bottom).
On the Role of Triplet Excitons in Organic Solar Cells

Andreas Sperlich, Stefan Väth, Hannes Kraus, Vladimir Dyakonov

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A strategy for increasing the conversion efficiency of organic photovoltaics has been to increase the open-circuit voltage $V_{OC}$ by tuning the energy levels of donor and acceptor components. However, this opens up a new loss pathway from an interfacial charge transfer state (CTS) to a donor triplet exciton (TE) state called electron back transfer (EBT), which is detrimental to device performance. To test this hypothesis, we study triplet formation in high performing blends of the fullerene PC$_{70}$BM with either the polymer PTB7 [1] or the soluble small molecule p-DTS(FBTTh)$_2$ [2] and determine the impact of the morphology-optimizing additive 1,8-diodooctane (DIO). Using photoluminescence and spin-sensitive optically and electrically detected magnetic resonance (ODMR, EDMR) measurements we find that TE formation does not only depend on the materials' energetics, but also on temperature and nano-morphology. Furthermore, we observe TEs in real devices under realistic working conditions even for the most efficient solar cells, which has implications not only for efficiency, but also for devices stability.

Fig. 1. EDMR at different working points of an organic solar cell: $V_{OC}$, $J_{SC}$, MPP (left). Triplet excitons on p-DTS(FBTTh)$_2$ form by Electron Back Transfer (right).

References

SOLUTION NMR / HYPERPOLARIZATION

Monday, July 24
15:15-16:30

Nate Traaseth
Elucidation of a Four-Site Allosteric Network in Fibroblast Growth Factor Receptor Tyrosine Kinases

Fabian Jähnig
Dissolution DNP at 7 T Field Using Trityl Radicals

Frederic Mentink-Vigier
One Step Closer Towards in Silico Radical Design

Frédéric Blanc
Oxygen-17 Dynamic Nuclear Polarisation Enhanced Solid-State NMR Spectroscopy at 18.8 T
Elucidation of a Four-Site Allosteric Network in Fibroblast Growth Factor Receptor Tyrosine Kinases

Nate Traaseth¹*, William Marsiglia¹, Huaibin Chen², Moosa Mohammadi²

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The single-pass receptor tyrosine kinase (RTK) class of cell surface proteins represents a fascinating example of long-range communication in cell biology where ligand-receptor engagement in the extracellular space activates the highly allosteric intracellular kinase domain to trigger a cascade of intracellular signaling pathways that modulate cell behavior including proliferation, differentiation, and metabolism. To date, the molecular determinants of allosteric connectivity within the tyrosine kinase domain are incompletely understood. Therefore, to provide insight into the long-range communication driving the activation of the enzyme, we employed a combination of structural methods (NMR and X-ray) and functional characterization of pathogenic gain-of-function mutations affecting the FGF receptor (FGFR) tyrosine kinase domain. The abundance of naturally occurring gain-of-function mutations in fibroblast growth factor receptor (FGFR1-4) kinases renders this RTK subfamily an ideal model system for elucidating the determinants of tyrosine kinase allostery in a physiologically relevant fashion. In this talk, solution NMR results will be presented for wild-type and pathogenic kinases that revealed a long-distance allosteric network composed of four interconnected sites termed the ‘molecular brake’, ‘DFG latch’, ‘A-loop plug’, and ‘αC tether’. The first three sites repress the kinase from adopting an active conformation, whereas the αC tether promotes the active conformation. The skewed design of this four-site allosteric network imposes tight autoinhibition and accounts for the incomplete mimicry of the activated conformation by pathogenic mutations targeting a single site. Based on the structural similarity shared among RTKs, we propose that this allosteric model for FGFR kinases is applicable to other RTKs.

Fig. 1. Model of the proposed allosteric network for the kinase domain of FGFR in the autoinhibited (A) and activated forms (B).
Dissolution DNP at 7 T field using trityl radicals

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Recent progress in dissolution DNP has seen a trend for developing polarizers at higher static magnetic fields to enhance the maximum level of polarization that can be obtained [1,2,3]. We have designed and implemented a new polarizer operating at a static magnetic field of 7 T (197 GHz) following the same design principles as realized in our home-built 3.35 T (94.5 GHz) systems [4,5]. We will discuss the changes and improvements made in the design of the polarizer compared to the previously published versions.

The main focus will be on polarization data obtained for samples of neat [1-13C]-pyruvic acid doped with two different trityl radicals (AH111501 and OX063) and trace amounts of Gd3+. Such samples are typically used for direct 13C DNP applied in in-vivo MRI of small animals, which requires as much polarization as possible for optimum resolution and signal-to-noise. While the maximum polarization (50-55%) and the build-up time constants (1500-2000 s) obtained for the two radicals in the solid state are quite similar, the effect of adding Gd3+ is surprisingly different. Additionally, we discuss the problem of reproducibility of obtained polarization levels as a function of the freezing protocol with the purpose of comparison with polarization values reported in the literature [1,2,3,4,6].

We also present how achievable proton polarization levels at 7 T for TEMPO-based samples change as a function of the deuteration of the glassing matrix. Such samples can be used in combination with cross polarization to rare nuclei (e.g. 13C) to benefit from the faster polarization build-up times of protons. The time dependence of the polarization is analyzed in the context of an extended spin-thermodynamic model that has been previously used with data obtained at lower field [5].

References
One step closer towards in silico radical design

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Nitroxide biradicals are very effective polarizing agents under Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) condition. The most effective biradicals were obtained empirically by controlling the nitroxides’ relative orientation (i.e. their g-tensors), the electron-electron interaction strength and the electronic relaxation times. In parallel to these improvements, numerical simulations on small spin systems have helped understanding in details the DNP mechanism at stake under MAS. These simulations have shed light on a complex DNP process and explained qualitatively many experimental observations, such as the spinning induced signal losses (depolarization), the MAS dependence of the DNP efficiency, the role of the g-tensor’s relative orientation/interaction strengths and the relaxations times... However, further developments are still mandatory in order to build a predictive tool to design more efficient polarizing agents.

In the first part, we will briefly present an alternative approach to existing Cross-Effect MAS-DNP codes that yields fast and accurate simulations. This model computes MAS-DNP process with unprecedented time savings as a result scanning through multiple parameters is easier and allows disentangling their mutual influences. It can also probe more realistic scenarios by handling multiple electrons/and protons. Some experimental observations in terms of polarization/depolarization gain and buildup times will be discussed by analyzing the impact of the polarizing agent’s structure (hyperfine, dipolar/J couplings) and concentration, the nuclear and electron relaxation times, the microwave irradiation strength and the main magnetic field.

In a second part we will describe our effort to combine High-Field EPR, Quantum Calculation (DFT), and MAS-DNP simulations to build a quasi-predictive tool. More precisely DFT calculations are used to generate an initial biradical structure (g and dipolar tensors). These inputs serve to fit High Field EPR spectra to refine the biradical structure while Pulsed EPR is used to determine the electron relaxation times. The obtained structure and relaxation times are the inputs for the MAS-DNP simulations which are compared to experiments. The efficiency of this approach will be demonstrated and discussed in the case of AMUPol.

References
Oxygen-17 Dynamic Nuclear Polarisation Enhanced Solid-State NMR Spectroscopy at 18.8 T

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Dynamic nuclear polarization (DNP) offers a spectacular approach for dramatic signal enhancement of multiple orders of magnitude in solid-state NMR¹ and is being exploited for atomic scale structure determination of materials. In particular, DNP NMR permits the fast detection of the NMR signals of insensitive nuclei such as the ones with very low natural abundance²,³ or with small magnetic moments.⁴

Natural abundance ¹⁷O NMR of oxides and hydroxides could be obtained in minutes at 9.4 T using cross effect DNP with biradicals (bCTbK, TEKPol)⁵ at low temperature under both static and MAS conditions.³ The results open up a powerful method to rapidly acquire high signal-to-noise ratio ¹⁷O NMR spectra and probe sites on or near the surface.

At a higher magnetic field of 18.8 T, ¹⁷O Overhauser DNP⁶,⁷ on hydroxides incorporated in a glassy solid matrix doped with narrow line radical gives the largest signal enhancement factor (Fig. 1a) whilst cross effect DNP with biradicals yields a slightly lower enhancement but more time efficient data acquisition (Fig 1b).⁸ This paves the way to a wider application of DNP NMR at very high magnetic field and its use on quadrupolar nuclei with strong second-order quadrupolar broadenings which hamper spectral resolution at low field.

References
Gianluigi Veglia
Solid-State NMR Spectroscopy Maps the Allosteric Regulation of the SR Ca\textsuperscript{2+}-ATPase (SERCA) by Phospholamban and its Lethal Mutants

Stefan Elrington
Increasing \textsuperscript{1}H→\textsuperscript{31}P Cross-Polarization Efficiency in Bone Using a Multiple Step Cross-Polarization Sequence, StepCP\textsuperscript{n}

Ieva Goldberga
Insight into Collagen Structure from \textsuperscript{15}N-Labelled Synthetic Model Peptides and Mouse Bone

Wing Ying Chow
Probing Physiological and Pathological Modifications of Collagen Fibrils in the Extracellular Matrix by DNP-Enhanced Solid-State NMR
Solid-State NMR Spectroscopy Maps the Allosteric Regulation of the SR Ca\(^{2+}\)-ATPase (SERCA) by Phospholamban and its Lethal Mutants

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The membrane protein complex between the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and phospholamban (PLN) controls Ca\(^{2+}\) transport in cardiomyocytes, thereby modulating cardiac muscle contractility. Upon β-adrenergic stimulation, PLN is phosphorylated at Ser16 by cAMP-dependent protein kinase A (PKA), which reverses its inhibitory action via an unknown mechanism. Single-site mutants in close proximity to the phosphorylation site prevent PKA recognition and PLN phosphorylation, causing the development of dilated cardiomyopathy.

Using solid-state NMR spectroscopy, we mapped the interactions between SERCA and PLN in membrane bilayers. We found that the allosteric regulation of the ATPase depends on the conformational equilibria of this endogenous regulator that maintains SERCA’s apparent Ca\(^{2+}\) affinity within a physiological window. Here, we present a new regulatory model for PLN that represent a paradigm-shift for understanding SERCA function. In addition, we show the analysis of SERCA’s interactions with deadly mutants of PLN (PLN\(^{R9C}\) and PLN\(^{R145}\)), providing additional insights on the molecular etiology of DCM.
Increasing $^1$H→$^{31}$P cross-polarization efficiency in bone using a multiple step cross-polarization sequence, StepCP$^n$

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Cross-polarization is widely used in solid state NMR to improve the sensitivity of rare nuclei, (the S-spins)[1]. Ideally, a cold bath of abundant I-spins cross-relaxes with a small number of warm S-spins, at a rate that is much faster than relaxation to the environment. A maximum enhancement of $\gamma_I/\gamma_S$ is expected under ideal conditions (ie. abundant I-spin bath and fast cross-relaxation). Bone mineral is far from this ideal case, since each $^1$H is proximal to several $^{31}$P spins, and thus $^1$H→$^{31}$P CP sensitivity is only 9% of $\gamma_I/\gamma_P$ [2]. Using a spin thermodynamics approach, we demonstrate that a multiple step CP-[Store/Equilibrate-CP]$^n$ sequence, StepCP$^n$, can i) improve the sensitivity of $^1$H→$^{31}$P CP from 9% to 63% of $\gamma_I/\gamma_P$ and ii) exceed the sensitivity of the Bloch decay experiment in less time (Figure 1).

By modeling the inverse spin temperatures of the $^{31}$P and $^1$H spin baths to the I-I*-S and I-S CP dynamics, we show that in bone, the theoretical maximum enhancement of the StepCP$^n$ experiment is 80% of $\gamma_I/\gamma_P$ due primarily to the effects of finite $T_{1P}$. Our work shows that CP efficiency can be improved in non-ideal CP samples and thus suggests a broader use of CP to successfully increase sensitivity in solid state nuclear magnetic resonance.

References
INSIGHT INTO COLLAGEN STRUCTURE FROM $^{15}$N-LABELLED SYNTHETIC MODEL PEPTIDES AND MOUSE BONE.

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Collagens are the most abundant components of the extracellular matrix. Due to their diverse structures and compositions collagens serve many functions, providing structural and mechanical support for surrounding cells, and playing important roles in cell-to-cell communication [1]. Nonetheless, despite being at first glance a simple protein formed by three homologous polypeptide chains of repeating three-amino-acid triads trimerized into a triple helix, it is actually a highly versatile and complex system. With over 3000 amino acids per triple helix, it is insoluble and does not crystallize. Due to its complexity and size, and in spite of technological advances, there is still poor understanding of collagen structure, flexibility, and dynamics at the atomic level [2]. Solid state NMR is a powerful probe of these properties.

This work focuses on $^{15}$N spectral assignment in synthetic collagen model peptides and $^{15}$N-labelled mouse bone. As nitrogen is a component of peptide bonds, $^{15}$N relaxation is a sensitive probe of collagen protein backbone dynamics. Interpretation is assisted by selectively labelled amino acids in model collagen peptides which allow characterization of sequence and neighbour effects on $^{15}$N relaxation.

Besides backbone dynamics, $^{15}$N can be used to study amino acid side-chains and their modifications. While $^{13}$C NMR of mouse bone shows only the predicted signals for collagen, additional, unexpected resonances are observed in $^{15}$N spectra (Fig.1.). We hypothesize that these correspond to glycosylation or glycation products that are formed from hydroxylysines and/or lysines side-chains in collagen reacting with sugars [3]. These product signals are well resolved in $^{15}$N NMR, whereas the corresponding $^{13}$C signals likely overlap those of other amino acids, hindering analysis. Free amino groups, as in arginine and lysine, undergo glycation, and glycosylation processes and $^{15}$N NMR could be a powerful tool for detailed characterization of these processes crucial to collagen ordering and mechanical properties.

References:

Fig. 1. 2D $^{13}$C-$^{15}$N DCP correlation spectra of mouse limb bone at 13.2 kHz MAS. Spectra was obtained during iNEXT visit in Berlin.
PROBING PHYSIOLOGICAL AND PATHOLOGICAL MODIFICATIONS OF COLLAGEN FIBRILS IN THE EXTRACELLULAR MATRIX BY DNP-ENHANCED SOLID-STATE NMR

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Main text: The extracellular matrix is a heterogeneous and insoluble biomaterial mostly based on collagen, the most abundant proteins in the body by weight. As an essential part of its biosynthesis and function, collagen undergoes extensive post-translational modifications. In the biochemical environment of the extracellular matrix, collagens accumulate irreversible changes that can affect the mechanical integrity of the tissue and also affect cell behaviour. Solid-state NMR offers the possibility to extract information on post-translational modifications and subsequent glycation \cite{1} and crosslinking of the collagen fibril directly from the solid tissues without extensive processing or purification.

However, with 3 chains each consisting of over 1000 residues in collagen type I (the predominant type of collagen in bone, skin and tendon), some of these modifications that we are interested in studying are relatively rare, occurring at a handful of sites out of 3000 residues (<0.5%). We demonstrated that dynamic nuclear polarisation (DNP) offers a feasible strategy for overcoming this inherent sensitivity challenge, and can be applied on samples obtained from different sources, including from cell culture and mammalian tissue for which various labelling regimes has been previously developed \cite{2,3}.

In addition, we can also demonstrate the potential of DNP for studying samples obtained from patients suffering from alkaptonuria (AKU), a rare metabolic disease which leads to striking symptoms including very dark pigmentation of cartilage (ochronosis) and joint degradation in mid-adulthood. The chemical structure of the dark pigment in AKU is yet uncertain. DNP enabled the acquisition of 1H-13C HETCOR spectra on pigmented and non-pigmented patient samples, providing further insight on the previously observed disorder in pigmented cartilage collagen \cite{4} and sheds light on possible structures of the pigment.

References
Gillian R. Goward
*In Situ* NMR Imaging of Ion Transport in Li-Ion Batteries

Dominik J. Kubicki
A Link between Photovoltaic Performance, Phase Composition and Cation Dynamics in Mixed Double A-Cation Lead Halide Perovskites Unveiled by Solid-State NMR

Christophe Odin
$^{45}$Sc and $^{67}$Zn NMR Studies in Tsai-Type Quasicrystal and Approximant

Christopher A. Klug
Real-Time Control of NMR Relaxation for Improved Sensitivity and Resolution
In situ NMR Imaging of Ion Transport in Li-Ion Batteries

Sergey A. Krachkovskiy, J. David Bazak, Bruce J. Balcom, Ion C. Halalay, and Gillian R. Goward*

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Effective modeling of the performance of automotive Li-ion batteries requires the knowledge of electrolyte transport properties: specific conductivity, Li\(^+\) transference number and salt diffusion coefficient, as functions of temperature and salt concentration. Determination of these parameters is not a trivial task, due to the fact that the values of ionic diffusion coefficients in an electrolyte solution depend on the salt concentration. At the same time, the salt concentration inside a battery is neither stationary nor homogeneous at high charging or discharging rates, as experienced during vehicle acceleration or braking. In contrast to commonly used methods, such as PFG-NMR and electrochemical techniques, MRI can provide spatially resolved details about the chemical and dynamic features of solution species.

Using in situ MRI, we have unambiguously demonstrated that the electrolyte solutions can experience large concentration polarizations during battery operation. Because of this situation, the difference in ionic diffusivities at opposite ends of the cell can be as large as 60% (Fig. 1b), which must be taken into account in the description of mass transport. One can directly determine both the salt diffusivity and the Li\(^+\) transference number, by combining the PFG-NMR diffusion measurement with in situ MRI techniques into single pseudo-3D experiment, [1]. Furthermore, implementation of pure phase-encoding MRI methods, e.g. centric scan SPRITE [2], allows the mapping of not only the transport of ions through the electrolyte solution, but also the lithiation of electrodes (Fig. 1c), which leads to the next level of battery performance characterization by nuclear magnetic resonance techniques.

Fig. 1. (a) In-situ electrophoretic NMR cell design (dimensions in mm). (b) Steady-state lithium salt concentration (blue) and diffusivity (maroon) profiles in 1M LiPF\(_6\)/EC:DMC (1:1 v/v) at a current density of 9 A/m\(^2\). (c) Lithium profile in a graphite electrode charged to a voltage of 0.03 V at current density of 1.5 A/m\(^2\).

References

A link between photovoltaic performance, phase composition and cation dynamics in mixed double cation lead halide perovskites unveiled by solid-state NMR

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Mixed cation organic lead halide perovskites posses excellent photovoltaic properties. At the time of writing, one of the best performing materials contains 4 cations and provides power conversion efficiencies up to around 22%. Here, we report the first quantitative, cation-specific data on cation reorientation dynamics in hybrid double-cation formamidinium (FA)/methylammonium (MA) lead halide perovskites. We use 14N, 2H, 13C and 1H solid-state MAS NMR to elucidate cation reorientation dynamics, microscopic phase composition and to quantify the MA/FA ratio, in bulk FA/MA lead iodide between 100 and 330 K. We compare the cation reorientation rates obtained by NMR with values previously reported for single-cation perovskites from other techniques including ultrafast 2D IR spectroscopy, quasielastic neutron scattering, and molecular dynamics. Our findings correlate in a striking manner with the charge carrier lifetimes previously reported for these materials.

Fig. 1. A) A cartoon showing the structure of the double-cation three-dimensional perovskite phase of FA0.67MA0.33PbI3, B) and the corresponding solid-state 14N echo-detected variable-temperature 5 kHz MAS NMR spectra. The top insets (red) show a close-up of the isotropic peak at 333 K and 20 kHz MAS conditions under which a characteristic splitting due to the J-coupling between the nitrogen and the proton is resolved, making the signal assignment straightforward. The arrows indicate the corresponding SSB manifolds.

References
45Sc and 67Zn NMR studies in Tsai-type quasicrystal and approximant.

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Quasicrystals belongs to a new state of matter, whose discovery was recently awarded by the Nobel prize, which presents a long range order without translational symmetry. Major questions concern the location of the atoms, their dynamics and possible structural phase transitions. The family of icosahedral quasicrystals of Tsai type [1,2] contains binary stable i-QC with low chemical disorder that offer the opportunity of precise structural analysis [3]. Neutron scattering [4] showed evidences of a dynamics of the central symmetry-breaking tetrahedron in the periodic approximant, and the phase transition near 160K was explained by an ordering of the tetrahedra. These results raise fundamental questions about the origin of the phase transition, and the coupling between the different shells of the Tsai cluster which is the main building unit of the approximant and i-QC (Fig.1).

In this presentation, we will report results of 45Sc (I=7/2) and 67Zn (I=5/2) solid-state NMR experiments at different fields, and temperatures, in the 1/1 approximant Zn6Sc and quasicrystal ZnScAg. In particular, 67Zn spectra of Zn6Sc approximant were obtained at fields up to 18.8T (Lille High Field NMR platform). Observing such a nucleus was a challenge in such metallic samples, because of its very low sensitivity (low nuclear gyromagnetic ratio, natural abundance of 4.1%, medium quadrupolar coupling). Both nuclei carry complementary information within the icosahedral cluster, composed of five successive shells of increasing radius : tetrahedron (4Zn), dodecahedron (20Zn), icosahedron (12Sc), icosidodecahedron (30Zn) and rhombic triancontahedrom (92Zn). Scandium that belongs only to the icosahedron shell are particularly well suited to probe intershell coupling and tetrahedron induced distortions. We will discuss how NMR provides insight in the structure and dynamics of these compounds.

Fig. 1. Four first shells of a Tsai cluster : tetrahedra, dodecahedra and Sc icosahedron (pink), icosidodecahedron.

Acknowledgements: We acknowledge IRRMN FR3050 CNRS for support of High Field NMR experiments in Lille. This research received FEDER financial support (FEDER 34722-Prin2Tan) for funding NMR spectrometers in Rennes.

References
Real-Time Control of NMR Relaxation for Improved Sensitivity and Resolution

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Many methods are known for enhancing NMR sensitivity. We propose a method which combines the increase in sensitivity per scan due to the large differences in Boltzman populations at low temperatures with the increased relaxation rate at low temperatures due to the presence of paramagnetic species. While paramagnetic relaxation enhancement NMR experiments have a long history, our key innovation is to use paramagnetic species created via photoexcitation. In this way one avoids the spectral broadening associated with paramagnetic species. The method is shown schematically in Fig 1.

Our initial experiments have focused on exploiting the Light-Induced Excited Spin State Trapping (LIESST) phenomenon associated with iron spin-crossover compounds, where spin-state transitions occur as a function of both temperature and optical excitation. We will present results which confirm reproducible spin-state transitions in a solid matrix where the spin-crossover compound is blended with the polymer polystyrene. Theoretical models are developed to describe spin-diffusion and the polarization distribution within the matrix.

References
EPR
Monday, July 24
15:15-16:30

Hassane S. Mchaourab
Transporter Conformational Dynamics from Spin Labeling EPR Spectroscopy

Denise Schuetz
Effect of Ubiquitin-Binding Domains on the Conformational Flexibility of K48-Linked Ubiquitin Chains Studied by Pulsed EPR Spectroscopy

Anna Weyrauch
EPR Spectroscopy of Peptides and Proteins with Intrinsic Disorder

Donald J. Hirsh
Sensitive Detection of Nitric Oxide Using a Liposome-Encapsulated Spin Trap

Mary E. Zvanut
Time-Dependent Photo-EPR Applied to Point Defects in Crystals

Klaus-Peter Dinse
Investigation of Catalysts: Can EPR Deliver?

Chiara Gionco
Oxygen Carriers: The Example of the Ceria-Titania Mixed System

Ana-Maria Ariciu
Actinide Covalency Measured by Pulsed Electron Paramagnetic Resonance Spectroscopy
Understanding the mechanisms of membrane proteins entails complementing static structures with the conformational changes in the structure. Spin labeling in conjunction with electron paramagnetic resonance (EPR) spectroscopy offers an exquisite window into membrane protein dynamics in the native–like environment of a lipid bilayer. Recent advances in Double Electron-Electron Resonance (DEER) spectroscopy along with computational methods to generate restrained models of proteins are enabling unprecedented insights into the conformational dynamics of active transporters. My laboratory use EPR methods to define conformational equilibria that mediate the transport cycle of ion-coupled symports and antiports. Specific examples in the LeuT transporter structural classes will illustrate the importance of defining the mechanistic identity of membrane protein crystal structures. I will also show comparative analysis of ATP-binding cassette (ABC) exporters to reveal the role of dynamics in their transport cycles. Together, these studies are illuminating the mechanistic commonalities and differences in transporter families in the absence of conformational selectivity imposed by the crystal lattice.
Effect of ubiquitin-binding domains on the conformational flexibility of K48-linked ubiquitin chains studied by pulsed EPR spectroscopy

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Polyubiquitination is the most versatile posttranslational modification. The information content is encoded by linkage type and chain length, which determine the conformational space of ubiquitin chains. Translation of the encoded signal into a cellular response is achieved by ubiquitin binding domains (UBDs) or deubiquitinating enzymes (DUBs). This study investigates the conformational flexibility of K48-linked ubiquitin chains by Pulsed Electron-Electron Double Resonance (PELDOR/DEER) [1] spectroscopy, revealing a high intrinsic flexibility of these chains. Besides, the conventional 4-pulse DEER sequence [2], which is able to determine distances from 1.8 to 8 nm, we applied the recently developed 7-pulse Carr-Purcell PELDOR sequence [3]. This provides an increased accuracy of the observable distance distributions by an extended time window, especially important for longer chains. In addition, we examine the effects of an UBD and a linkage specific DUB. The coupling of ubiquitin conjugation to endoplasmatic reticulum degradation (CUE) domain of yeast Cue1 [4] is found to bind under conformational selection. While the K48-linkage specific DUB, OTUB1 [5], binds under conformational remodeling. (Fig.1) These effects are visualized by applying the obtained distance restraints for structural modeling using the software combined assignment and dynamics algorithm for NMR applications (CYANA) [6].

Fig. 1. The effect of the CUE domain of Cue1 and OTUB1 on the conformational flexibility of diubiquitin chains. Experimental PELDOR data of doubly spin-labeled K48-linked diubiquitin chains in complex with Cue1 and OTUB1 (Left and Right). The conformational ensemble of the distal ubiquitin (blue) with respect to the proximal ubiquitin grey. In comparison to the Cue1 bound (red) and OTUB1 (green) bound states.

References
EPR spectroscopy of peptides and proteins with intrinsic disorder

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Intrinsically disordered proteins (IDPs) and proteins with intrinsically disordered regions (IDRs) are a class of proteins that lack a well-defined three-dimensional structure. Often they adopt a more ordered structure upon binding to their respective targets, i.e. folding upon binding. Due to their high dynamic flexibility it is not possible to characterize these proteins by means of X-ray crystallography. However it is possible to perform EPR measurements if either the protein is spin-labelled or EPR-active spin probes are used.

One of the most studied IDPs is α-Synuclein, composed of 140 amino acids, that forms a major constituent of the Lewy bodies characteristic of Parkinson’s disease. Although no precise function has yet been determined, binding to membranes seems to the important for its physiological role. The membrane-bound state was studied by using single cysteine-mutants labelled with MTS. Moreover the binding affinity to different lipids was studied¹.[1]

Histidine-Proline-rich Glycoprotein (HPRG) is a modular protein of vertebrate plasma that contains a central histidine-rich region flanked by two proline-rich regions. These regions are assumed to be intrinsically disordered. An exact physiological role has not yet been discovered but it can interact with a variety of ligands, such as divalent metal ions and heme, that can be used as spin probes. Via EPR measurements it is possible to determine possible conformational changes and the amount of ligands that can be bound. First measurements were conducted by using synthesized peptides based on the sequences of these IDRs².[2]

References
[1] A. Weyrauch et al. (in preparation)
SENSITIVE DETECTION OF NITRIC OXIDE USING A LIPOSONE-ENCAPSULATED SPIN TRAP

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Nitric oxide (NO), a diatomic radical, is produced by all three domains of life. Even now, thirty years after identifying "endothelium-derived relaxing factor" as NO, the scientific community is still learning about nitric oxide's enzymatic production and biological significance. We have developed a liposome-encapsulated spin trap (LEST) to capture NO and detect it by electron paramagnetic resonance (EPR) spectroscopy. [1] Since biochemical NO production can be followed in real time with LEST, we are using it to investigate the enzyme kinetics of inducible nitric oxide synthase (iNOS). The cumulative nature of NO trapping in LEST, combined with its quantitative recovery from large incubation volumes, allows for the detection of steady state NO concentrations below 1 nM. Such high sensitivity has allowed us to study NO production/signaling in marine algal cell cultures containing commensal bacteria. The versatility and sensitivity of LEST provide new opportunities to explore NO biochemistry and ecology by EPR.

Fig. 1. (a) A schematic of the liposome-encapsulated spin trap. The phospholipid bilayer forms a gas-permeable barrier, separating the iron dithiocarbamate spin trap (green) from enzymes like nitric oxide synthase (NOS) and nitrate reductase (NR) that produce NO. NO easily diffuses through the bilayer, binding to the spin-trap and forming EPR-detectable M₂Fe(II)NO. (b) Kinetics of NO production by iNOS in the presence of LEST. Inset: Free spin trap is a potent inhibitor of the enzyme. (c) The cooperative nature of NO production by iNOS. As enzyme concentration increases, so too does enzyme activity. Inset: The observed cooperativity suggests that the activity of iNOS is modulated by the formation of higher order assemblies, such as a dimer of dimers.

References

Time-dependent Photo-EPR applied to point defects in crystals
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Optical excitation is commonly used in concert with EPR to create the paramagnetic state of centers in a wide range of systems, from randomly ordered solutions to highly ordered crystals. However, except for some pioneering work in the early 80’s, there are few reports of time dependent photo-EPR of point defects in crystalline semiconductors. The scant use is caused, in part, by the limited time response imposed by the commonly used 100 kHz magnetic field modulation and the small number of EPR-active centers inherent to technologically relevant semiconductors. Recently, however, we have analyzed time-dependent data in several types of semiconductors using standard phase-sensitive detection. The results of one such study will be summarized here, emphasizing both the utility and limitations of the technique. The systems to be discussed are Be- and C- doped GaN, in which the impurity acts as a deep acceptor providing electrical compensation and, in some cases, yellow luminescence. Charge trapping parameters such as defect level (ionization energy) and capture coefficients are critical to understanding the effectiveness of the dopants. The samples studied are 0.1-0.3 um thick, 0.5 cm² GaN platelets doped with $10^{17}$-$10^{19}$ cm⁻³ Be or C, and are measured at 3.5 K. The time-dependent photo-EPR data is analyzed using a set of coupled differential equations based on charge transfer among the acceptor dopant, unintentionally added donors, and the conduction and valence bands. Analysis of the results obtained during illumination with selected photon energies yields optical thresholds for Be (C) of 2.8 (~2) eV, which accounts for the effectiveness of the dopant as a compensating center. Further study shows that Be undergoes a structural relaxation of at least 0.5 eV, consistent with theoretical predictions and observation of the 2.2 eV optical emission. The significance of these results and the complications associated with solid state systems will be reviewed in context of systems more commonly addressed with photo-EPR such as organic crystals or polymers.
Investigation of catalysts: Can EPR deliver?
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Physics department, FU Berlin, Germany

In contrast to the well-defined structures found in biological samples, the topology of technical catalysts is much less defined, and it is difficult to deduce information about the functionality of such surface active catalysts, even if surface plots offering atomic identification are available. Under such conditions it is mandatory to gather additional information about the oxidation state of atoms, because their involvement in the catalytic cycle is usually connected with the transfer of electrons. Although in most cases the experiment being limited to “non operando” conditions, EPR techniques can provide important information, because changes in the oxidation state of atoms involved in the catalytic cycle are combined with a change of the effective spin of the probed ions. By measuring the transition moment of paramagnetic centers it is also possible to search for coupled spin clusters, which might be involved in the catalytic cycle. Furthermore, advanced EPR techniques can supply information about the local topology of “active” sites, if hyperfine interaction with adjacent nuclei can be observed, or if higher rank tensor parameters can be identified. Examples of recent studies of catalysts used for oxidative dehydrogenation (ODP) and oxidative coupling of methane (OCM) will be presented.
The interaction of oxygen with the surface of CeO$_2$–TiO$_2$ mixed oxides prepared via sol–gel was investigated by means of electron paramagnetic resonance (EPR). Upon admission of molecular oxygen onto the surface of the materials (which underwent final oxidative calcination) the formation of superoxide O$_2^-$ ions is observed.

The superoxide species is symmetrically adsorbed (“side-on” structure) on the top of a Ce$^{4+}$ ion as confirmed by the experiments performed using $^{17}$O$_2$.

Surprisingly the electron transfer is fully reversible at room temperature, showing the typical behavior observed by molecular oxygen carriers, which, however, are linked to the oxygen in a completely different manner (“end-on” structure). Following the values of the integrated intensity of the EPR signal it was possible to create an adsorption isotherm and to find out the number of active sites present at the surface.

We suggest that the active sites are Ce$^{3+}$ ions present in the stoichiometric cerium titanate which forms during the synthesis. The features of these Ce$^{3+}$ ions must be different from those of the same ions formed in CeO$_2$ by reductive treatments (thermal annealing), which show a different reactivity towards O$_2$. The observation reported here opens up innovative perspectives in the field of heterogeneous catalysis and in that of sensors as the total reversibility of the electron transfer is observed in a significant range of oxygen pressure [1].

![Graph](image)

**Fig. 1.** Panel A: EPR spectra (recorded at 77 K) describing the effect of oxygen adsorption on as prepared CT50: (a) baseline recorded under vacuum; (b) after admission of 25 mbar O$_2$ at room temperature, experimental (b) and simulated (b') traces. Panel B: Plot of the integrated area of the EPR signals for different cycles of adsorption/evacuation of 10 mbar O$_2$ over CT50. Panel C: adsorption isotherm of O$_2$ on CT50.

**References**

ACTINIDE COVALENCY MEASURED BY PULSED ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

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The concept of oxidation states is consistently used in order to rationalise the reactivity and the bonding regimes of atoms in different environments. Whereas the approach of formal oxidation states, where electron density is assigned to individual elements in compounds, gives a relevant approximation of atomic behaviour, the actual spin densities and electronic studies can be solved through comprehensive physical studies which are sparse for elements in uncommon formal oxidation states in the actinide series.

Although the first structurally characterized Th(III) complex was isolated decades ago, the oxidation state is not thoroughly understood. Here we focus on describing a novel Th(III) complex for comparison with a U(III) homologue and Th(IV) and U(IV) complexes in similar ligand environments.\textsuperscript{[1-3]} The bonding in these complexes has been studied using XRD and NIR/Vis/UV spectroscopies together with EPR and EXAFS spectroscopies and SQUID measurements to probe the electronic structures and valence orbital spin densities of these systems. Using a combination of pulse EPR techniques (ESEEM, HYSCORE, ENDOR), we were able to quantify the hyperfine interactions to the \textsuperscript{1}H and \textsuperscript{13}C nuclei of the Cp ligands. These results for the first time provide an experimental measure of the varying extend of covalency in related systems containing Th(III) and U(III) oxidation states. \textsuperscript{[4]}

References
\textsuperscript{[1]} S. T. Liddle. Angew. Chem. Int. Ed., 2015, 54, 30, 8604;
\textsuperscript{[2]} H. S. La Pierre, K. Meyer, Prog. Inorg. Chem. 2014, 58, 303;
PLENARY SESSION 3

Tuesday, July 25
08:30-9:15

Hanudatta S. Atreya
NMR Spectroscopy with High Speed and Resolution for Metabolomics
NMR-based metabolomics faces the challenge of low resolution and sensitivity, which slows down the process due to large amount of time of spent in data collection and analysis. We have developed new methods, which addresses these problems and breaks the barrier for application of NMR to challenging systems. For accelerating data collection, our method is based on the dual receiver system, which facilitates the acquisition of multiple two-dimensional spectra in a single data set. The analysis of the spectra thus obtained can be speeded up using a calibration-free pattern recognition based approach. Finally, the mapping of the metabolites to their respective biological pathways in the system can be rapidly done using an approach that utilizes a single two dimensional NMR spectrum. We have recently devised means to further optimize the sensitivity of these methods and achieve super-resolution using novel signal processing methods. The applications of these methods to different systems in general and human in-vitro fertilization, in particular, will be discussed.
PLENARY SESSION 4

Tuesday, July 25
09:15-10:00

Enrica Bordignon
Protein Conformational Changes and Local Hydration Dynamics
PROTEIN CONFORMATIONAL CHANGES AND LOCAL HYDRATION DYNAMICS

E. Bordignon\textsuperscript{1,*}, T. E. Assafa\textsuperscript{1}, S. Bleicken\textsuperscript{1}, S. Nandi\textsuperscript{1}, M. Teucher\textsuperscript{1}, M. H. Timachi\textsuperscript{1}.

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Site-directed spin labeling EPR enables detection of conformational changes in proteins with almost no restriction in the environmental conditions. Key information for structural analysis is provided by changes in the dynamics of spin-labeled sites and by interspin distances between selected pairs of labels. The long-range EPR distance constraints, combined with existing structural data at atomic level for one state of the protein, enable the creation of coarse-grained models of complex protein rearrangements in their physiological milieu.

Each protein conformational transition due to oligomerization, ligand binding, transfer from a water to a membrane environment, etc. is tightly coupled to rearrangements in the hydration water surrounding the different protein interfaces. Changes in local hydration dynamics and water accessibility can be monitored directly and with high precision at physiological temperature using X-band ODNP, which has several advantages with respect to other EPR techniques which provide water accessibility data.

We will show examples of structural studies in which the changes in the protein and its surrounding water environment are observed. Examples will be given on ABC transporters\textsuperscript{[1]}, Bcl-2 proteins\textsuperscript{[2]}, and light receptors\textsuperscript{[3]}.

References
**SOLUTION NMR**

Tuesday, July 25
10:30-12:00

Stephan Grzesiek
Insights into Allosteric Regulation of GPCRs and Abelson Kinase by Solution NMR

Ramakrishna V. Hosur
New Methods for NMR of Complex Mixtures and Complex Systems

Nicolas Giraud
Paramagnetic NMR: An Accurate Tool for Probing Supramolecular Interactions between Proteins and Lanthanide Complexes

Yunyi Wang
Structural Constraints in Protein-Ligand Interaction by Polarization Transfer from Hyperpolarized Ligand
INSIGHTS INTO ALLOSTERIC REGULATION OF GPCRS AND ABELSON KINASE BY SOLUTION NMR

Stephan Grzesiek1,*; Anne Grahl1; Shin Isogai1; Christian Opitz1; Rajesh Sonti1; Xavier Deupi2; Gebhard Schertler2; Dmitry Veprintsev2

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The function of many proteins is regulated via intricate, allostERIC interactions. These are not visible in static structures, but can only be derived from additional dynamical information. NMR plays an important role to provide this missing link. In recent years, we have sought to contribute to the understanding of the allosteric regulation of several G protein-coupled receptors (GPCRs) and Abelson kinase by solution NMR methods. Both systems are challenging due to their large size and since they are difficult to express in functional isotope-labeled form in E. coli.

The beta1-adrenergic GPCR (b1AR) is one of targets of beta-blockers in the heart muscle. We could recently show that allosteric receptor motions in response to different agonist and antagonist ligands can be followed at virtually any backbone site via $^1$H-$^15$N chemical shifts in a detergent-solubilized thermostabilized mutant of the turkey b1AR [1], which was produced in the insect cell system using selectively isotope-labeled amino acids. Complementary $^15$N relaxation data give now a more quantitative description of overall and internal receptor motions in response to the different ligands. Similar information was also obtained on the human chemokine GPCR and HIV1-coreceptor CCR5.

Abelson (Abl) kinase is a prime drug target in the treatment of chronic myelogenous leukemia and presents a further example of a multidomain protein regulated by allosteric interactions within its core region consisting of the sequential SH3, SH2 and kinase (KD) domains. Whereas the regulated regulated core of c-Abl can be expressed in E. coli, the isolated KD can so far only be expressed well in the insect cell system. We have previously studied conformations of the activation loop of the isolated KD [2] as well as of the regulated core [3]. In particular, the regulated core adopts a compact (assembled) conformation in its apo form and an open, flexible (disassembled) conformation in complex with the ATP-site inhibitor imatinib (Gleevec). We have now extended this analysis to complexes with 12 of the most important ATP-site ligands. The complexes fall into distinct classes of assembled or disassembled SH3-SH2-KD conformations. These findings provide a direct allosteric link between the SH3 and SH2 domains to the conformation of the ATP-binding pocket.

Time permitting, we will also discuss recently developed strategies for economic isotope labeling in insect cells based on isotope-labeled yeast extracts, which provide up to 90 % $^15$N and $^{13}$C as well as 60 % $^2$H incorporation [4].

References
New Methods for NMR of Complex Mixtures and Complex Systems

Ramakrishna V. Hosur$^{1,2,*}$

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Nuclear Magnetic Resonance (NMR) spectroscopy has come a long way since its discovery seven decades ago and continues to evolve unabated with new applications emerging in many areas of biology and chemistry. Our group has been engaged for the past several years on enhancing the speed of protein structure determination, elucidation of equilibrium folding transitions and self-association pathways, characterization of intrinsically disordered proteins, on one hand and on obtaining high resolution spectra of complex organic mixtures in rapid manner, on the other. These enable efficient interaction studies between proteins and small molecules which are often substrates or inhibitors. Encompassing the above, this talk will summarize the methodological advances from our laboratory that includes design of new pulse sequences, use of dual receivers, pure shift spectroscopy and Hadamard NMR.
PARAMAGNETIC NMR: AN ACCURATE TOOL FOR PROBING SUPRAMOLECULAR INTERACTIONS BETWEEN PROTEINS AND LANTHANIDE COMPLEXES

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Non-covalent incorporation of lanthanide derivatives into protein crystals has shown to be of prime interest both for NMR spectroscopy and X-ray crystallography, in so far as these versatile compounds can interact through supramolecular interactions with proteins, and even co-crystallize with them. Lanthanide complexes were thus successfully used as promising auxiliaries for protein structure determination, either as strong anomalous scatterers for anomalous-based diffraction techniques, or more recently, as non covalent paramagnetic tags for NMR applications.[1]

Here we will show how techniques based on paramagnetic NMR can be used together with a panel of other methods (X-ray diffraction, quantum chemistry) to decipher the selective affinity of tris-dipicolinate lanthanide complexes for cationic amino-acid residues in proteins that underly the supramolecular interaction mechanism.[2]

We will also report the implementation of paramagnetic DOSY, allowing to evaluate diffusion coefficients of paramagnetic adducts with high precision and accuracy. We show that the interaction between lanthanide complexes and a model protein can then be probed directly by monitoring the evolution of the diffusion coefficient of the paramagnetic species.[3]

References

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Structural Constraints in Protein-ligand Interaction by Polarization Transfer from Hyperpolarized Ligand

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Observation of polarization transfer by intermolecular nuclear Overhauser effect (NOE) is one of the most common methods to determine intermolecular interactions, including between proteins and ligands. Using a hyperpolarized source spin in such experiments provides signal enhancements of orders of magnitude and allows the detection of the NOE even in a single scan. Here, we use dissolution dynamic nuclear polarization (D-DNP) to hyperpolarize ¹H spins of a ligand, folic acid, and observe polarization transfer to a protein, dihydrofolate reductase (DHFR) [1]. Signals of protein ¹H spins in spatial proximity to the hyperpolarized ligand are selectively enhanced and become visible in one-dimensional spectra of the mixture (Fig. 1a). Introducing a single-quantum coherence filter for ¹³C chemical shift selection leads to resolved protein methyl proton peaks. The origins of individual NOE signals can be determined by applying selective inversion pulses on ligand ¹H resonances (Fig. 1b-red). These NOE signals are correlated with short intermolecular distances between protein and ligand protons in the crystal structure of the folic acid-DHFR complex. Simulations of 1D DNP NMR spectra based on spin relaxation and exchange (Fig. 1b-black) show a good match with the experimentally observed spectra. These simulations consider a set of ¹H ligand spins and protein spins near the binding site, using the known crystal structure. Comparing simulated spectra based on poses obtained using computational docking with experimental spectra further allows ranking of ligand poses (Fig. 1c). These experiments demonstrate that structural constraints in protein-ligand binding can be obtained by acquiring single scans of ¹H DNP NMR spectra, using spin polarization transfer combined with chemical shift selective encoding on both the sides of protein and hyperpolarized ligand.

Fig. 1. a) Comparison of DNP and thermal spectra of DHFR with hyperpolarized folic acid. b) DNP spectra of DHFR with hyperpolarized ligand acquired with a single-quantum filter for ¹³C chemical shift and selective inversion on ligand ¹H resonances (red). Simulated spectra based on the crystal structure (black). c) Crystal structure of DHFR-folic acid complex (PDB:1RE7 [2]), showing the best docked ligand pose.

References
SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Tuesday, July 25
10:30-12:00

Vladimir Ladizhansky
Insights into Structure and Dynamics of Membrane Proteins

Leonard J. Mueller
NMR Crystallography in Tryptophan Synthase: Proton Positions, Stable Intermediates, and Transition States

Meaghan E. Ward
Deciphering the Organization of Dynamic Protein Assemblies by Nuclear Magnetic Resonance: Applications to Axin 1 of the Wnt Signaling Pathway

Timothy A. Cross
Membrane Protein Biophysics: Revelations from Solid State NMR
Insights into structure and dynamics of membrane proteins

Shenlin Wang,1 Daryl Good,1 Sanaz Emami,1 Christopher Ing,2 Yunjiang Jiang,3 Hongjun Liang,3 Regis Pomes,2 Leonid Brown,1 Vladimir Ladizhansky1

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Abstract

Magic angle spinning solid-state NMR shows great potential for studying structure and dynamics of membrane proteins, which are not readily amenable to X-ray crystallography and solution NMR. In this abstract we describe our recent efforts towards elucidation of membrane protein structure and dynamics by solid state NMR. In the first part we discuss conformational dynamics in a seven-helical transmembrane photoreceptor Anabaena Sensory Rhodopsin (ASR). Relaxation and order parameter measurements performed at two temperatures and two magnetic fields provide evidence for internal motions occurring on at least two time scales. Faster picosecond local motions occur throughout the protein and are dominant in the middle portions of helices. In contrast, the amplitudes of the slower collective motions occurring on the nanosecond timescale, are smaller in the central parts of helices, but increase towards their cytoplasmic sides as well as in the interhelical loops. ASR interacts with a soluble transducer protein on its cytoplasmic surface, and its binding affinity is modulated by light. The larger amplitudes of motions on the cytoplasmic sides of the TM helices correlate with the ability of ASR to undergo large conformational changes in the process of binding/unbinding its soluble transducer.

In the second part, we will our discuss progress towards structural characterization of a human water channel Aquaporin 1 (hAQP1), focusing on the conformation of extracellular loops. Solid-state NMR chemical shift analysis of secondary structure agrees with the crystallographic data for transmembrane regions, but reveals pronounced differences in the extracellular loops A and C. Loop C appears to be structured and more rigid than suggested by the B-factors in the crystallographic structures. Site-specific hydrogen deuterium exchange measurements identified a number of protected backbone amide groups and nitrogen-bearing side chains that are likely involved in stabilizing the loops. To gain additional insight into a structural organization of these loops, we performed molecular dynamics simulations with NMR-derived restraints and filtering based on solvent accessibility. The simulations suggested loop-stabilizing interactions that alter the extracellular surface of hAQP1, with possible implications for water transport regulation through the channel.
NMR Crystallography in Tryptophan Synthase: Proton Positions, Stable Intermediates, and Transition States

Bethany G. Caulkins, Robert P. Young, Michael F. Dunn, and Leonard J. Mueller

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NMR-assisted crystallography – the synergistic combination of solid-state NMR, X-ray crystallography, and first-principles computational chemistry – holds remarkable promise for mechanistic enzymology; by providing atomic-resolution characterization of stable intermediates in the enzyme active site – including hydrogen atom locations and tautomeric equilibria – it offers insight into structure, dynamics, and function. Here, we make use of this combined approach to characterize the aminoacylate intermediate in tryptophan synthase, a defining species for pyridoxal-5′-phosphate-dependent enzymes on the β-elimination and replacement pathway. By uniquely identifying the protonation states of ionizable sites on the cofactor, substrates, and catalytic side chains, as well as the location and orientation of structural waters in the active site, a remarkably clear picture of structure and reactivity emerges. Most incredibly, this intermediate appears to be mere tenths of angstroms away from the preceding transition state in which the β-hydroxyl of the serine substrate is lost. The position and orientation of the structural water immediately adjacent to the substrate β-carbon suggests not only the fate of that hydroxyl group, but also the pathway back to the transition state and the identity of the active site acid-base catalytic residue. Enabling this analysis is the ability to measure active-site isotropic and anisotropic NMR chemical shifts under conditions of active catalysis, and the development of fully quantum mechanical computational models of the enzyme active site that allow the accurate prediction of NMR spectral parameters.

DECIPHERING THE ORGANIZATION OF DYNAMIC PROTEIN ASSEMBLIES BY NUCLEAR MAGNETIC RESONANCE: APPLICATIONS TO AXIN 1 OF THE WNT SIGNALING PATHWAY

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The Wnt signaling pathway plays crucial roles during embryonic development, and aberration of this pathway is implicated in a variety of human cancers. Axin (Fig. 1A), a scaffold protein in a multiprotein destruction complex which moderates Wnt signaling, is composed of an N-terminal RGS domain and a C-terminal DIX domain which are connected by a natively disordered central region. In cells, the DIX domain self-associates, leading to the formation of highly dynamic polymeric protein assemblies, known as puncta. Cancer mutations to the RGS domain have been shown to lead to an inability to form puncta and the formation of nano-sized aggregates mediated by the mutated RGS domain [1].

Due to the size, as well as the dynamic and heterogeneous nature, of the structures formed by Axin, obtaining high-resolution structural information is challenging. Solid-state nuclear magnetic resonance (SSNMR) is a powerful method for the structural investigation of large, dynamic complexes. Here, we initiate such studies on these assemblies. To begin, the isolated DIX and mutated RGS domains are studied in order to gain a deeper understanding of the mechanisms of self-association in these domains. Initial in vitro experiments on the DIX domain (Fig. 1C) indicate that the protein is well-folded and reveals the existence of a co-purifying molecule. Three-dimensional spectroscopy allows for the assignment of many residues and detailed structural analysis (Fig. 1B).

The information gained from the SSNMR experiments performed on the isolated domains of Axin will be combined with studies of NMR-labeled variants of the DIX and RGS domains in cellular environments ranging from cell lysates to whole cells. The methods developed on Axin will provide novel opportunities for the study of similar liquid-liquid phase separation (LLPS) structures, many which contain proteins which have been implicated in a range of human diseases.

References
Membrane Protein Biophysics: Revelations from Solid State NMR

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Membrane proteins exist in a very complex environment involving remarkably steep gradients in water concentration and dielectric constant that influences their structure, dynamics and how they function. While the transmembrane domain in the very low dielectric environment of the membrane intersticies has been discussed extensively in the literature, the interfacial region with the steep gradients has been less studied and less appreciated from a perspective of the membrane proteins that function in this environment. A variety of oriented sample (OS) and MAS spectroscopy has been used to characterize these protein domains, as well as some solution NMR.

We have been studying multiple proteins with different structural features in this environment. Two proteins from the \textit{Mycobacterium tuberculosis} cell division apparatus, ChiZ and CrgA have N-terminal domains on the cytoplasmic side of the cellular membrane that are intrinsically disordered regions. CrgA which has two transmembrane helices has been found to be a dimer and yet the multiple glycine residues that would be anticipated to stabilize the dimeric structure, in fact do not. Instead, in the N-terminal region a short \(\beta\)-strand tethered to the interfacial lipid environment via lysine residues is responsible for stabilizing the dimeric structure.

Biochemical assays have been used to suggest that the N-terminus of ChiZ has peptidoglycan hydrolase activity consistent with it’s known functional role in cell division. Now, we have shown that the assays that are widely used are flawed and there is no hydrolase activity in the intrinsically disordered N-terminal domain, which is also tethered to the membrane interface by the domains excessive positive charge. The INEPT experiments shown illustrate the extensive dynamics of N-terminus in the full length protein and the influence of the paramagnetic labeled lipid on residues far removed from the transmembrane helical sequence.

The M2 protein from Influenza A virus has an amphipathic helix that is stabilized in the interfacial region of the lipid bilayers through binding to cholesterol. Mixed labeling of monomers that form the tetrameric structure have been used to characterize the conditions under which the amphipathic helix is stabilized in the lipid bilayer forming a pyramidal shape that induces curvature of the lipid bilayer responsible for facilitating viral budding. The cholesterol binding site is consistent with many similar binding sites in GPCRs.

Fig. 1. 2D $^1$H–$^{13}$C INEPT PRE based spectra of full length ChiZ were collected in POPG:POPE liposomes (1:80 protein to lipid ratio) in the presence (Red) and absence (Black) of 1\% Gd\textsuperscript{3+} labeled POPE. Experiments were carried out at 25°C and 12.2 kHz in a 600 MHz instrument.
MRI METHODS, MEDICINE, MATERIALS / SOLID-STATE NMR MATERIALS/METHODS

Tuesday, July 25
10:30-12:00

Lucio Frydman
Single- and Multi-Shot Spatiotemporally Encoded MRI

Klaas P. Pruessmann
NMR Field Sensing: Recent Progress and MRI Applications

E. S. (Merijn) Blaakmeer
Advanced Solid State NMR Methods for the Study of Ziegler-Natta Catalysts

Hubert Koller
Resolving $^{27}\text{Al}$ Lineshapes and Spin States in $^1\text{H}$ MAS NMR and $^1\text{H}^{\{^{27}\text{Al}\}}$ REAPDOR of Zeolite Acid Sites
**Single- and Multi-shot Spatiotemporally Encoded MRI**  
Lucio Frydman, Weizmann Institute, 76100 Rehovot, Israel

Over the last decade we and others have introduced and perfected a so-called spatiotemporal encoding (SPEN) methodology to collect multidimensional NMR spectra and images in a single scan. This talk will focus on introducing this technique and showing its potential to deliver superior imaging information, particularly in comparison with established methods such as spin-echo EPI in the realm of single-shot MRI, and fast-spin-echo/RARE in multi-shot anatomical MRI. The figure below illustrates some of our ongoing achievements in the former area including (a) Breast-cancer diffusion-based studies executed on a patient with both cysts and a lesion. In the brain cases the SPEN/PE axes run along the vertical (RO horizontal), whereas in the breast scan SPEN/PE was imparted horizontally. (b) Single-shot diffusion brain characterizations. (c) Brain images collected at 7T. (d) Single-shot 3T data collected on a volunteer’s head with non-ferromagnetic dentures. Notice in (a) the difficulties to deal with fat-derived replicas that complicate the identification of cysts and lesions, in (b) the much better resolution, in (c) the weaker distortions, and in (d) EPI’s complication to deal with the implant-related field inhomogeneities.
NMR FIELD SENSING: RECENT PROGRESS AND MRI APPLICATIONS

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MRI relies on the use of static and dynamic magnetic fields of high strength, stability, rate of change, and accuracy. While present-day hardware achieves high levels of fidelity, remaining imperfections of spatiotemporal field behavior continue to limit the performance of demanding imaging techniques. Relevant errors and perturbation arise, e.g., from magnet drift, eddy currents, mechanical resonances, and dynamic susceptibility effects of physiological origin.

In efforts to address field imperfections it is instrumental to measure magnetic field distributions with suitable spatial and temporal resolution. A versatile mechanism for this purpose is NMR itself (1). Field information of remarkable precision can be obtained by recording and analyzing NMR signals from droplets of sample liquid contained in small probeheads (2). Advances in susceptibility matching, detector geometry, and receiver electronics have recently pushed the sensitivity of NMR field sensing in high backgrounds to the range of parts per trillion (3).

The most versatile implementation of field sensing in MRI is to incorporate an array of field probes around the volume of interest and perform field monitoring concurrently with imaging procedures. Field readouts can then be deployed for a range of purposes including hardware characterization (4), software compensation (5), run-time field corrections (6), and retrospective correction using expanded signal models and algebraic inversion (7). The presentation will highlight these applications by examples of high-resolution and high-speed neuroimaging, including single-shot functional brain imaging at sub-millimeter resolution.

Besides its immediate uses in MR image encoding and reconstruction, the ability to measure small changes in strong magnetic fields opens up a number of further applications such as motion tracking and correction in MRI (8,9) and sensing of magnetic field dynamics that arise from breathing and cardiac action (3,10). For NMR in general, sub-nanotesla field sensing offers a direct means of observing longitudinal nuclear magnetization at high field, enabling, e.g., relaxation studies and the characterization of contrast agents (3).

References
Advanced Solid State NMR Methods for the Study of Ziegler-Natta catalysts

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Heterogeneous Ziegler-Natta catalysts (ZNCs) are complex multicomponent systems which, besides the MgCl\textsubscript{2} support and the active titanium centers, contain electron donors and aluminum alkyls (co-catalysts). Despite the large-scale use of Ziegler-Natta catalysts for over 40 years, they are still subject of a controversial debate on the nature and structure of the active surfaces.

To gain an in-depth knowledge of Ziegler-Natta catalysts we tackle the elucidation of relevant structures in a built-up fashion using solid state NMR spectroscopy on model systems. We have shown how \textsuperscript{25}Mg and \textsuperscript{35}Cl NMR, using QCPMG-ssDFS sequences, can be used to study the MgCl\textsubscript{2} support\textsuperscript{2}. The coordination of organic donors to the surface of the MgCl\textsubscript{2}-support is investigated by \textsuperscript{13}C NMR. Quantum chemical calculations are employed to correlate the carbon chemical shifts to potential surface structures. Currently we are investigating the potential of DNP SENS techniques for the study of surface species.

![Fig. 1](image)

\textbf{Fig. 1}: Static \textsuperscript{35}Cl spectra of MgCl\textsubscript{2} showing the effect of ball-milling to generate large surface areas.

Secondly, we present a new application of the recently revived technique of nutation NMR for the identification of broad resonances. Especially in disordered systems, like catalysts, it can happen that several sites are present with quadrupole parameters differing by orders of magnitude. This is exactly what is expected for the surfaces of MgCl\textsubscript{2}-supported nanoparticles. Nutation NMR of the satellite transitions shows a characteristic pattern. This can be used to set satellite transitions from a well-defined bulk sites apart from an extensively broadened central transition from a surface site.

\textbf{References}

Resolving $^{27}\text{Al}$ Lineshapes and Spin States in $^1\text{H}$ MAS NMR and $^1\text{H}\{^{27}\text{Al}\}$ REAPDOR of Zeolite Acid Sites

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Zeolite acid sites were investigated by $^1\text{H}\{^{27}\text{Al}\}$ REAPDOR evolution and Offset REAPDOR with variable $^{27}\text{Al}$ irradiation frequency at constant evolution time. Experimental data were fitted to structural models with full density matrix simulations of the spin system using the SIMPSON software [1].

High-resolution line fitting yields two $^1\text{H}$ components at 3.7 and 4.0 ppm for Brønsted acid sites (Fig 1a). The $^{27}\text{Al}$ offset mapping in $^1\text{H}\{^{27}\text{Al}\}$ REAPDOR of the line at 3.7 ppm shows maxima located at $^{27}\text{Al}$ resonance offsets close to the singularities of the $|\pm 3/2> \leftrightarrow |\pm 5/2>$ transitions (Fig. 1b). The component at 4.0 ppm shows a stronger dephasing for on-resonance $^{27}\text{Al}$ irradiation. These findings are clear indicators that the line at 3.7 ppm is due to protons, whose $^{27}\text{Al}$ neighbors are in spin states $|\pm 5/2>$, whereas the protons at 4.0 ppm are near $^{27}\text{Al}$ nuclei in the inner spin states. This is direct evidence of the expected [2] frequency shift in $^1\text{H}$ NMR induced by different $^{27}\text{Al}$ spin states in zeolite acid sites. This effect is only significant for large quadrupolar coupling constants. Quantitative simulations of the REAPDOR evolution curves of this zeolite yield $^1\text{H}-^{27}\text{Al}$ distances with accuracies as high as 2 %.

Fig. 1. a) experimental spectra of zeolite beta at 12 rotor cycles (12 kHz rotation, on-resonance $^{27}\text{Al}$ irradiation), b) Offset REAPDOR dephasing for components along with simulated $^{27}\text{Al}$ NMR lineshape.

Further work on zeolite beta and zeolite A, using different dephasing pulse lengths, illustrates the information that can be extracted from such Offset REAPDOR experiments.

References
HYPERPOLARIZATION / UNUSUAL

Tuesday, July 25
10:30-12:00

Lyndon Emsley
Advances in DNP Enhanced Solid-State NMR

David G. Cory
Electron Spin Control with High Q Superconducting Resonators

Mark D. Bird
The 1.5 GHz, 1 ppm NMR Magnet at the National High Magnetic Field Lab

Hugo van Ingen
Weak Coupling between Magnetically Inequivalent Spins
Dynamic Nuclear Polarization has recently emerged as a tool to enhance the sensitivity of solid-state NMR experiments. However, so far high enhancements (>100) are limited to relatively low magnetic fields, and DNP at fields higher than 9.4 T significantly drops in efficiency. Here we report solid-state Overhauser effect DNP enhancements of over 100 at 18.8 T. This is achieved through the unexpected discovery that enhancements increase rapidly with increasing magic angle spinning rates. The measurements are made using 1,3-bisdiphenylene-2-phenylallyl (BDPA) dissolved in ortho-terphenyl (OTP) at 40 kHz MAS. We introduce a source-sink diffusion model for polarization transfer which is capable of explaining the experimental observations. The advantage of this approach is demonstrated on mesoporous alumina with the acquisition of well-resolved DNP surface enhanced $^{27}$Al CP spectra.

We then go on to show how the same models for DNP polarization dynamics can be used to determine the domain sizes in complex materials. By selectively doping a source component with radicals, and leaving the target undoped, we can measure experimental polarization build-up curves which can be compared with simulations based on heterogeneous distributions of polarization within the sample. The variation of the integrated DNP enhancement as a function of the polarization time is found to be characteristic of the geometry. We demonstrate the method experimentally in several different systems where we successfully determine domain sizes between 200 and 20 000 nanometers, specifically in: powdered histidine hydrochloride monohydrate, pore lengths of mesporous silica materials, domain sizes in two-component polymer film coatings, and cargo distributions in lipid nanoparticles.
Electron Spin Control with High Q Superconducting Resonators

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High Q resonators provide a path toward higher sensitivity devices. Achieving high Q is both a materials science and device physics challenge. Recent advances in the fabrication of superconducting devices for quantum information studies present an opportunity to employ this technology for a wide variety of applications. In particular, resonators made from thin film superconducting materials may be engineered for low loss, leading to high Q devices that significantly enhance signal-to-noise ratio for magnetic resonance measurements \cite{Bienfait2016}. We present high Q planar superconducting cavities that are designed especially for high sensitivity ESR measurements; the mode volume is optimized for microscale samples and the high Q (> \textit{10}^4 at 4 K) is maintained at fields appropriate for X-band studies (~ 0.4 T).

We will discuss the challenges associated with controlling electron spins with high Q superconducting resonators and demonstrate robust solutions to those challenges. In particular, our control schemes account for both the finite circuit reactance associated with high Q devices \cite{Borneman2012} and the kinetic inductance \cite{Hincks2015} that emerges in superconductors due to the energy cost associated with changing the momentum of cooper pairs under a strong microwave drive. The kinetic inductance manifests as a nonlinear response of the microwave field to an applied time-dependent voltage. A perhaps surprising result is that solutions may be found that are inherently robust to a range of nonlinearity.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{(A) Planar thin film Nb superconducting microstrip resonator \cite{Mohebbi2014} operating at 10 GHz. (B) Bloch sphere trajectories of a square inversion pulse subject to kinetic inductance nonlinearity and (C) an optimized pulse of the same length robust to a range of nonlinearity (multiple curves represent varying nonlinearity).}
\end{figure}

\textbf{References}

\begin{thebibliography}{9}
\end{thebibliography}
The 1.5 GHz, 1 ppm NMR Magnet at the National High Magnetic Field Lab

M.D. Bird1*, W.W. Brey1, I.R. Dixon1, Z. Gan1, A. Griffin2, S.T. Hannahs1, J. Kynoch1, I. Litvak1, J.L. Schiano3, J. Toth1

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The National High Magnetic Field Laboratory (MagLab) is commissioning a 36.1 T magnet with homogeneity and stability of 1 ppm over a 10 mm diameter spherical volume [1]. Most NMR magnets use single strands of superconducting wire carrying a few hundred amps, and persistent joints and switches. In contrast, this magnet incorporates a 20 kA superconducting cable in a steel conduit for the outer part of the magnet and copper-alloy sheet metal for the inner part of the magnet.

While >15 hybrid magnets have been built worldwide [2], they typically have a field uniformity of ~250 ppm/cm DSV and stability might be no better than 10 ppm. To attain 1 ppm uniformity, current density grading was employed in the resistive coils, ferroshims and resistive shims developed by Oxford NMR were employed [3]. While most hybrids use separate power supplies for the resistive and superconducting magnets, here a single 14 MW power supply provides 20 kA to the two magnets connected electrically in series. The voltage across the resistive magnet is 640 V, which means persistent operation is impossible. A stabilization system using a pickup coil and an NMR lock developed by J. Schiano at Penn State University improved the stability to 0.2 ppm [3].

Fig. 1 shows magic angle spinning spectra obtained in a 19.6 T superconducting magnet, a 25 T resistive magnet, the 36 T hybrid magnet described herein, and our 45 T hybrid magnet.

The highest quality spectra are the 35.2T spectra (1.5 GHz for 1H) obtained from the new hybrid with the lock unit operating.

In addition to a 1Hz distributed control system, there is a 1 kHz system that monitors the resistive and superconducting coils and can interrupt the 20 kA of current and discharge the 47.5 MJ of magnetic energy in an external resistor at a voltage of up to 2 kV. The magnet system also requires a steady supply of 10 g/s of supercritical helium.

References
WEAK COUPLING BETWEEN MAGNETICALLY INEQUIVALENT SPINS

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Magnetic inequivalence of nuclear spins is well known to cause additional splittings that complicate spectral analysis [1]. Here, we present an extreme case of magnetic inequivalence, manifested in the 13C,15N-labeled trimethyl amine (Fig. 1A) [2]. The protons of the three methyl groups are chemically equivalent due to the molecular symmetry, but are not magnetically equivalent as they have different $^{1}J_{CH}$ and $^{2}J_{CH}$ couplings. As a result, the $^1$H spectrum shows additional splittings due to (small) $^{4}J_{HH}$ and $^{2}J_{CC}$ couplings (Fig. 1B).

We show that due to a 'perfect storm' of coupling constants, the spectrum can be perfectly described as a sum of first-order "ax"-type spectra (Fig. 1C). The two sides of the main $^{1}J_{CH}$ doublet are essentially weakly coupled to each other with coupling constant $^{4}J_{HH}$. We demonstrate that spin-state selective homo-decoupling of the $^{1}J_{CH}$ doublet removes these additional splittings and transforms the spin system to a set of fully equivalent spins (Fig 1D).

We believe this curious case is a textbook-worthy, instructive example of magnetic inequivalence. The spectra may be considered deceptively simple [5] as fewer lines are observed as one would anticipate. At the same time, the spectra are deceptively complicated as they can be very well approximated by intuitive reasoning.

![Fig. 1.](image)

References
EPR

Tuesday, July 25
10:30-12:00

K. V. Lakshmi
High-Resolution Pulsed EPR Spectroscopy of the Solar Water Oxidation Reaction of Photosystem II

R. David Britt
Mechanisms of Solar Fuel Reactions as Probed by Advanced EPR Spectroscopy

Müge Kasanmascheff
The Radical Transfer Mechanism across the Subunit Interface of E. Coli Ribonucleotide Reductase Studied by Advanced EPR Spectroscopy

Aleksei Litvinov
Hyperfine EPR Spectroscopic Techniques: A New Angle on the Mn$^{2+}$-ATP Complex
High-resolution Pulsed EPR Spectroscopy of the Solar Water Oxidation Reaction of Photosystem II†

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The solar water-splitting protein complex, photosystem II (PSII), catalyzes one of the most energetically demanding reactions in Nature by using light energy to drive water oxidation. The four-electron water oxidation reaction occurs at the tetranuclear manganese-calcium-oxo (Mn₄Ca-oxo) cluster that is present in the oxygen-evolving complex of PSII. The electronic and geometric structure of the Mn₄Ca-oxo cluster, which is exquisitely tuned by smart protein matrix effects, is central to the water-oxidation chemistry of PSII. However, the mechanism of water oxidation at the Mn₄Ca-oxo cluster is not well understood because of the inability of conventional methods to directly probe the reaction intermediates. We are developing high-resolution two-dimensional (2D) hyperfine sublevel correlation spectroscopy methods that provide direct ‘snapshots’ of the photochemical water oxidation intermediates of the Mn₄Ca-oxo cluster of PSII. I will describe ongoing efforts in our laboratory to understand the mechanism of water oxidation in Photosystem II.

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Mechanisms of Solar Fuel Reactions as Probed by Advanced EPR Spectroscopy

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The oxygen evolving complex of Photosystem II splits water with visible light, producing molecular oxygen and electrons and protons (1). The electrons and protons can be recombined to make hydrogen with the appropriate catalyst. One such catalyst is the [FeFe] hydrogenase enzyme, which rapidly evolves H\textsubscript{2} at a 6-Fe catalytic site termed the H-cluster. The H-cluster consists of a traditional [4Fe-4S] cluster linked via a cysteine bridge to a dinuclear Fe subcluster [2Fe]\textsubscript{2} that possesses unusual biological ligands: two terminal CN\textsuperscript{-} ligands, two terminal CO ligands, and azadithiolate and CO bridges, all of which are thought to be synthesized and installed by a set of Fe-S proteins denoted HydE, HydF, and HydG. With the James Swartz laboratory (Stanford University) we can generate [FeFe] hydrogenase in high yield using cell free synthesis methods, allowing for specific isotope labelling of its components as needed for definitive spectroscopic studies (2).

The radical S-adenosylmethionine (SAM) enzyme HydG lyses free L-tyrosine to produce CO and CN\textsuperscript{-} for the assembly of the H-cluster. We use electron paramagnetic resonance (EPR) spectroscopy to detect and characterize HydG reaction intermediates generated with a set of \textsuperscript{2}H, \textsuperscript{13}C, and \textsuperscript{15}N nuclear spin labeled tyrosines. 5'-deoxyadenosyl cleavage of tyrosine at the C\textsubscript{alpha} - C\textsubscript{beta} bond generates a transient 4-oxidobenzyl (4OB) radical and dehydroglycine (3). The dehydroglycine is converted to Fe-bound CO and CN\textsuperscript{-}, producing an organometallic precursor in the assembly of the [2Fe] subunit of the H-cluster. We have used stopped-flow FTIR, Mössbauer, and electron-nuclear double resonance (ENDOR) spectroscopies to develop a detailed proposed mechanism for HydG's key role in assembling the H-cluster (4-7). Many open issues remain to be explored in our ongoing studies of H-cluster synthesis, including the roles of the two additional Fe-S maturation proteins HydE and HydF (8).

The radical transfer mechanism across the subunit interface of *E. coli* ribonucleotide reductase studied by advanced EPR spectroscopy

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Enzyme-mediated proton-coupled electron transfer (PCET) processes are fundamental and ubiquitous in biology. They are central to our understanding of reactions in primary metabolism including photosynthesis, respiration and synthesis of DNA building blocks. These reactions share a common catalytic feature, a tyrosyl or modified tyrosyl radical (Y•) that is involved in either concerted or stepwise PCET steps. *E. coli* class Ia ribonucleotide reductase (RNR) serves as a paradigm for diverse PCET mechanisms in enzymes. It catalyzes the reduction of ribonucleotides to deoxyribonucleotides via an unprecedented radical transfer process over 35 Å using at least four Y•s (Y122 ⇌ [W48] ⇌ Y356 in β2 to Y721 ⇌ Y730 ⇌ C439 in α2). In this work, we used multi frequency EPR (9, 34, 94 and 263 GHz), PELDOR/DEER (at 34 GHz) and 1H/2H ENDOR (at 34 and 94 GHz) to study the PCET process at the subunit interface of the *E. coli* class Ia RNR.1 We utilized protein-engineering methods to trap Y356•, the essential residue formed during PCET. High field EPR (94 and 263 GHz) reveals a g value of 2.0062, one of the lowest reported so far for a tyrosyl radical. Furthermore, these data provided evidence for communication between the two essential residues Y356 and Y731 across the subunit interface. All EPR and ENDOR spectroscopic features of Y356• are consistent with a DFT model, in which Y356• is coordinated to a cluster of two water molecules forming almost in plane moderate H bonds to Y356•. This finding has mechanistic implications regarding PCET at the interface. So far only H bonds perpendicular to the aromatic ring plane have been observed that were involved in collinear PCET in one RNR subunit.2,3 Our results provide strong support for a new PCET mechanism based on water molecules as the mediator of radical transfer at the subunit interface. It is shown that water molecules enhance electron transfer at protein-protein interfaces. However, the extension to PCET steps across the protein interfaces is much rarer. Here we report the first experimental study that suggests the role of water for a PCET mechanism over a long distance, through the subunit interface of an active protein.

Adenosine triphosphate (ATP) is a crucial part of many biochemical reactions which depends on ATP hydrolysis as an energy source. The ATP hydrolysis, occurring while the ATP is bound to the relevant protein, requires the presence of Mg$^{2+}$. The Mg$^{2+}$ is coordinated to the ATP at the nucleotide binding site of the protein. While Mg$^{2+}$ is diamagnetic, it can be replaced with the paramagnetic Mn$^{2+}$, which usually retains the activity. This allows interrogating the ATP hydrolysis through EPR hyperfine spectroscopic techniques. Despite the importance of Mg$^{2+}$-ATP (or Mn$^{2+}$-ATP) complexes for the multitude of biological reactions there seems to be no consensus of the structure of the complex, even when free in solution. The possibility of the coordination of Mn$^{2+}$ with the adenine ring and with phosphates of one ATP molecule and nitrogen of the adenine ring of the other, stacked next to the first one in the crystal [2].

To resolve the coordination sphere of Mn$^{2+}$-ATP at equimolar concentrations in solution we applied W-band ENDOR (electron-electron nuclear resonance) [3], EDNMR (electron-electron double resonance detected NMR) [4] and THYCOS (triple hyperfine correlated spectroscopy) [5]. Using $^{15}$N enriched ATP we could resolve the $^{15}$N hyperfine coupling (Fig. 1b). Moreover, using TYHCOS we showed that the Mn$^{2+}$ is coordinated simultaneously with N$_7$ of the adenine ring and with phosphorus of the phosphate chain (Fig. 1b). DFT calculations on model complexes gave $^{14}$N and $^{31}$P hyperfine couplings in reasonable agreement with the experimental values.

![Fig. 1. (a) EDNMR spectra of Mn-ATP (black), Mn$^{2+}$-ATP (red). b) Davies ENDOR (black) and THYCOS (red) of Mn$^{2+}$-ATP in solution. For the THYCOS the HTA pulse frequency is set to the high frequency component of the $^{15}$N doublet ($\Delta v _{HTA}=16.3$ GHz).](image-url)
SOLUTION NMR

Tuesday, July 25
13:45-14:45

Steffen J. Glaser
Optimal Control Sequences for Relaxation Dispersion and Cooperative Spin Echo Experiments

Gyula Batta
Disentangling the Protein Dark Matter: The Case of Antifungal Disulfide Proteins

Sven G. Hyberts
Evaluating Better Ways of Sample, Acquire, and Extending NUS NMR Spectra
OPTIMAL CONTROL SEQUENCES FOR RELAXATION DISPERSION AND COOPERATIVE SPIN ECHO EXPERIMENTS

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Analytical and numerical tools of optimal control theory (OCT) make it possible to explore the ultimate performance limits of pulse sequences. In the last decade, these tools not only provided pulse sequences of unprecedented quality and capabilities, but also new analytical and geometrical insight and a deeper understanding of pulse optimization problems [1].

In this talk, a novel approach to optimize experiments for the analysis of spin systems in the presence of chemical exchange will be introduced. Rather than designing individual pulse sequence elements, such as broadband refocusing pulses, entire relaxation dispersion sequences can now be optimized in the form of a single shaped pulse to closely approximate the performance of ideal CPMG sequences.

In addition, the extension of the concept of concurrently optimized cooperative pulses [2] to broadband spin echo sequences has been analyzed in detail. The underlying mechanism of the mutual cancellation of errors of cooperative excitation and refocusing pulses will be presented and the improved performance of broadband cooperative spin echo sequences will be discussed.

As intuitive visualizations of the dynamics of coupled spin systems are extremely useful in the design and analysis of pulse sequence, novel theoretical and experimental aspects of the powerful DROPS representation [3] (implemented in the free SpinDrops app) will also be discussed.

References

DISENTANGLING THE PROTEIN DARK MATTER: THE CASE OF ANTIFUNGAL DISULFIDE PROTEINS

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Small disulfide proteins like PAF$^{1,2}$ are generally believed to exist as rock hard entities, though they have many charged lysine residues. We have recently shown$^{3,4}$ by thermal unfolding, $^{15}$N chemical exchange saturation transfer (CEST) and ensemble molecular dynamics that this is not the case. Even under the conditions of maximum protein stability, considerable amount of NMR invisible protein states may persist due to their conformational and dynamical heterogeneity. The hidden nature of these states for everyday NMR methods might be caused by their low population and/or the fading effect due to exchange between two or more states in the intermediate time scale (ms-us) regime. Partially unfolded states can be biologically relevant, e.g. consequence or support for disulfide shuffling or chirality switching. More generally, less structured intermediate states can be useful for conformational-selection principle of molecular recognition and suggests that it is difficult to find a sharp borderer between the folded and disordered protein world (IDPs). Practical consequences have impact on the validation of MD simulations or protein concentration measurement. The present contribution is an extension of our earlier work to PAF variants and new antifungal proteins (sfPAFB and NFAP) involving chemical unfolding studies.

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References
EVALUATING BETTER WAYS OF SAMPLE, ACQUIRE, AND EXTENDING NUS NMR SPECTRA

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Non-uniform sampling (NUS) has the potential to exploit the optimal resolution of high-field NMR instruments, which is not possible when using uniform sampling in 3D and 4D NMR experiments due to the long overall measurement time. It is however not clear even for the indirect dimension in 2D how far one should sample to obtain optimal sensitivity, resolution and peak accuracy. In our most recent publication [1] we come to the conclusion of $1/2T_2^*$ and then extrapolate with hmsIST to $2T_2^*$, rather than the $5/4T_2^*$ that previously been suggested [2]. Neither it is clear which sampling strategy to apply, though our own Poisson Gap sampling [3] enjoys favor by many. We systematically test these questions with extensive simulations and evaluate the conclusions with experimental test situations.

To explore the questions about sensitivity, resolution and peak accuracy, we apply sampling schedules that require a reconstruction with a mixture of interpolation and/or extrapolation. We create a measurement $p_{\infty} = -\log_{10}(\text{max}_i |s_{r,i} - s_{0,i}|/\text{max}_i |s_{r,i}|)$, in order to evaluate 7 common sampling strategies and graph the worst outcome of 100 seed values. We also evaluate deterministic sampling schedules and find that a modified sinegap sampling [4] is a great insurance against the worst-case scenarios that happens using sample strategies relying on seed values. Finally, we extend our evaluation to 2D sampling schedules for 3D NUS NMR experiments.

Figure 1. Full spectral reconstruction plots of a singlet with line width and successive apodization by a cosine square apodization. A 1k long FID is reduced to 256 datapoints with 7 typical sampling strategies. 100 different reductions are done for each sampling strategy using a psudo-randomgenerator using 100 different seed values. For each reconstruction and processing spectrum, the $p_{\infty}$ is measured. The worst outcome is presented using the signal on and off the Nyquist grid. Each spectrum is magnified by a factor of 350.

SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Tuesday, July 25
13:45-14:45

Neeraj Sinha
Probing Role of Water in Bone Nano-Structure by Solid State NMR Spectroscopy

Daniel Stöppler
Dynamic Nuclear Polarization Provides New Insights into Chromophore Structure in Phytochrome Photoreceptors

Kristin Möbius
Automatic and Manual Solid-State NMR Resonance Assignment and Mechanistic Experiments on the Integral Membrane Protein Diacylglycerol Kinase
Probing role of water in bone nano-structure by solid state NMR spectroscopy

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The multi-scale organo-mineral hierarchical structure of composite biomaterial bone is important to understand its biological and mechanical functions. The mechanical properties of bone cannot be understood without understanding all its structural levels as well as the interactions among these levels. Among organic and inorganic components of bone extracellular matrix, water plays a very crucial role in governing bones mechanical property[1]. Solid state NMR (ssNMR) provides an attractive spectroscopic tool to probe bone structural features and interactions in native state.

The content of water in bone matrix is approximately 20% of volume along with fibrillary collagen (~ 35 – 45%) and hydroxyapatite (~ 35 – 45%) in the semi-crystalline state. The water reservoirs in bones are referred to as free, mobile, bound, or pore water. The water reservoirs and its distinguishable population in the bone can be probed by T₁ T₂ correlation experiments[2] and changes in these reservoirs when sustained to fracture and mechanical load. This talk will highlight different ssNMR experiments to probe role of water in bone nano-structure. The role of water in collagen hydroxyapatite interface is probed by $^{13}$C{$^{31}$P} REDOR experiments[3]. The collagen structural perturbations as a function of water content is probed by measuring site-specific natural abundance $^{13}$C chemical shift anisotropy (CSA) measurement by SUPER[4] and $^1$H detected DQ – SQ correlation experiments[5]. Finally water dependent backbone dynamics measurement of collagen in native state by natural abundance $^{15}$N relaxation measurements reveals structural insight into water mediated hydrogen bonding network[6].

References

Dynamic Nuclear Polarization Provides New Insights into Chromophore Structure in Phytochrome Photoreceptors

Daniel Stöppler1*, Chen Song2, Barth-Jan van Rossum1, Christina Lang3, Anil P. Jagtap4, Michel-Andreas Geiger1, Maria-Andrea Mroginski5, Jörg Matysik2, Snorri Th. Sigurdsson4, Jon Hughes3, and Hartmut Oschkinat1*

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Phytochromes are red/far-red photochromic photoreceptors acting as master regulators of development in higher plants, thereby controlling transcription of about 20% of their genes. Light-induced isomerization of the bilin chromophore leads to large rearrangements in protein structure, whereby the role of protonation dynamics and charge distribution is of particular interest. To help unravel the inherent mechanisms, we present two-dimensional dynamic nuclear polarization (DNP) enhanced solid-state magic-angle-spinning (MAS) NMR spectra of the functional sensory module of the cyanobacterial phytochrome Cph1 (Fig. 1a). Using bcTol, a new highly water-soluble biradical for efficient DNP of biomolecules, the pyrrole ring nitrogen and carbon signals were assigned unequivocally. This enabled us to locate the positive charge of the phycocyanobilin (PCB) chromophore at ring B and C. To help analyze proton exchange pathways, the proximity of PCB ring nitrogens and functionally-relevant H2O molecules was also determined (Fig. 1b).

Our results show that the positive charge must move from ring B and C to ring D during the photocycle. In the back-reaction, it returns to them, enabled through changes in the hydrogen-bonding networks. Our findings allow a more detailed view on the structural changes in phytochromes related to their signaling process. This study demonstrates the value of DNP in biological solid-state MAS NMR.
Automatic and manual solid-state NMR resonance assignment and mechanistic experiments on the integral membrane protein diacylglycerol kinase

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Abstract

*E. coli* diacylglycerol kinase (DGK) is a homotrimeric (43 kDa, 121 residues per monomer) integral membrane protein, which transfers γ-phosphate of ATP to lipid diacylglycerol (DAG) converting it into phosphatidic acid (PA). Lipid substrate as well as lipid product play important roles in different signaling pathways. Previous studies used $^{31}$P-solid-state NMR to visualize the coupled reactions at the membrane interface (Ullrich et al., 2011).

The specific aim of this project is the investigation of DGK’s functional mechanism at molecular level directly within the native-like lipid bilayer using solid-state NMR. Therefore, resonance assignment is of key importance. Although most of the residues of the thermostable triple mutant (I53C, I70L, V107D) of DGK have been assigned by solid-state NMR (Chen et al., 2014), a transfer of these assignments to the wild-type sample is not possible.

Thus, a combination of dipolar-coupling based 3D experiments (NCACX, NCOCX, CONCA) for sequential assignments of immobile domains and scalar-coupling based experiments for highly mobile regions were recorded. Since we are able to observe well-resolved NMR-spectra of high signal-to-noise ratio, we mainly performed $^{13}$C and $^{15}$N assignments using uniformly labeled samples. Residual ambiguities could be resolved by reverse labeling. The NMR time could be reduced by paramagnetic doping with Gd$^{3+}$-DOTA (Ullrich et al., 2014). Manual analysis lead to 85% resonance assignment. We also explored automatic assignment procedures. The use of ssFLYA (Schmidt et al., 2013) returned 90% correctly assigned resonances. The use of such assignment procedures for membrane protein applications is discussed.

Based on these assignments, catalytic intermediate states of DGK (nucleotide and lipid-substrate bound) were analyzed and specific alterations in chemical shifts and lineshapes were observed, which provide important molecular details about the functional mechanism of DGK.

In addition, cross-protomer interactions within the DGK trimer were probed by DNP-enhanced heteronuclear, dipolar experiments.


Kevin M. Brindle
Imaging Treatment Response and the Tumour Microenvironment with Hyperpolarized $^{13}$C MRI

Robert L. Blum
Exploring the Sparse-Sampling Limit of the Iterated Maps Approach to Reconstructing a Single Peak in a 2D NMR Experiment

Jared Rovny
Accelerated Acquisition of 2D NMR Relaxation Dispersion Experiments Using Iterated Maps
Imaging treatment response and the tumour microenvironment with hyperpolarized $^{13}$C MRI

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Molecular imaging is likely to play an increasingly important role in predicting and detecting tumor responses to treatment and thus in guiding treatment in individual patients. We have been developing imaging methods for detecting the early responses of tumours to therapy, including metabolic imaging with hyperpolarized $^{13}$C-labelled substrates, which we have used both to detect treatment response and to investigate the tumor microenvironment (reviewed in [1]). Exchange of hyperpolarized $^{13}$C label between lactate and pyruvate and net flux of label between glucose and lactate have been shown to decrease post-treatment and hyperpolarized [1,4-$^{13}$C]fumarate has been shown to detect subsequent cell necrosis. Tumour pH can be imaged using hyperpolarized $^{13}$CO$_3^-$ and redox state can be determined by monitoring the oxidation and reduction of [1-$^{13}$C]ascorbate and [1-$^{13}$C]dehydroascorbate respectively. More recently we have shown that we can follow, using hyperpolarized [1-$^{13}$C]pyruvate, the progression of pancreatic precursor lesions, in a genetically engineered mouse model of the disease, which potentially could be used clinically to guide earlier intervention. We also have used hyperpolarized [1-$^{13}$C]pyruvate to investigate glycolytic metabolism in patient derived xenograft (PDX) models of glioblastoma. These latter measurements have shown significant heterogeneity between tumors derived from different patients. We conducted our first clinical study in Cambridge with hyperpolarized [1-$^{13}$C]pyruvate in 2016 and some recent results will be presented.

References

Exploring the sparse-sampling limit of the iterated maps approach to reconstructing a single peak in a 2D NMR experiment

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Sparse sampling is a common approach to speeding up the acquisition of large multidimensional data sets in NMR. These methods generally involve omitting indirect-dimension data points (which require entire 1D scans to acquire) and using a post-processing algorithm to reconstruct the spectrum. In prior work [1] we developed one such reconstruction method, based on the Fourier transform and using iterated maps and the “difference map” algorithm [2], which was able to accurately reconstruct entire 2D NMR spectra with an undersampling fraction of roughly 60% [1]. To improve on this result, we attempted to push to lower sampling fractions by focusing our attention on the amplitudes of large spectral features.

Fig. 1. The minimum number of sampled points that was reached for the entire 2D NMR spectrum in our prior study [1] is shown (N_t1=75, black dashed line). By focusing on the 48 largest peaks, the current work pushes that N_t1 for each peak down to much lower values (blue points), very close to the theoretical ‘linear-algebra-derived’ limit (dotted boundary of grey region).

Fig. 2. 1D spectrum in the indirect dimension of “peak 7” (see arrow) of the 48-peaks list from Fig. 1. Using the mask shown and 23/128 (18%) sampled points, the peak amplitude can be reconstructed to high fidelity.

In this work, we study the interaction of quasi-evenly-spaced undersampling patterns and mask shape on the output of the difference map. We use the knowledge gained to develop a way to predict the minimum sampling fraction that will allow accurate (within 5%) reconstruction of a given peak in a spectrum. Our method is able to locate good sampling fractions very close to a theoretical information-based lower bound. We discuss potential applications of this method, as well as its limitations.

References
Accelerated Acquisition of 2D NMR Relaxation Dispersion Experiments Using Iterated Maps

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Resolving the frequency spectra of relaxation dispersion experiments using a discrete Fourier transform requires sampling the full Nyquist grid of data, making these experiments very costly in experiment time \cite{1}. Practitioners often reduce the experiment time by omitting 1D experiments in the indirectly observed dimensions (“sparse sampling”), and reconstructing the spectra using one of a variety of post-processing algorithms (Fig. 1).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{Figure1.png}
\caption{Experiments can be sped up by sparsely sampling data in the time domain. However, applying a Fourier transform to this “sparse” data results in a distorted spectrum (red). The Iterated Maps reconstruction produces a spectrum very similar to the original spectrum while using only 30 of 128 1D experiments.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{Figure2.png}
\caption{(Left) In a relaxation dispersion experiment with many 2D spectra, each spectrum may be sparsely sampled in the same “Regular” way, or the samples “Staggered”. Staggering enables reconstruction of higher quality R2 decay curves from far less data. (Right) A reconstruction from only 23.4\% of the data.}
\end{figure}

In prior work, we described a fast, Fourier-based reconstruction using an iteration scheme according to the “difference map” algorithm of Veit Elser, providing good reconstructions from \textasciitilde75/128 rows of data \cite{2,3}. Here we describe a similar and very fast reconstruction method to provide high quality results, but requiring only \textasciitilde30/128 rows of data (Fig. 2). We apply this method to reconstruct dispersion curves from a relaxation dispersion experiment, and show that our method provides useful quantitative results with fast processing. We gratefully acknowledge our colleagues Greg Manley & J. Pat Loria, who generously provided the experimental data used in this work.

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\begin{itemize}
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\end{itemize}
EPR

Tuesday, July 25
13:45-14:45

Norikazu Mizuochi
Electrical Control of Spin Coherence of NV Center in Diamond

Petr Neugebauer
First High Frequency Electron Spin Resonance Spectrometer Operating in Field and Frequency Domain

Jens Anders
Energy-Based Modeling of CMOS Voltage-Controlled Oscillators for Continuous-Wave and Rapid Scan ESR Experiments

Brian M. Hoffman
Electron-Nuclear Double Resonance (ENDOR) in Metallobiochemistry

Alex I. Smirnov
Nanopore-Confined Lipid Bilayers for Oriented Sample EPR and NMR Studies of Membrane Proteins

Peter Martin
Simulating Electron Paramagnetic Resonance Spectra of Slow-Motion Systems in the Time Domain
A single electron spin in a nitrogen-vacancy (NV) center in diamond has excellent properties such as single spin manipulation and readout at room temperature [1]. Thus NV center has potentials to realize quantum information processing, nano-scale magnetic- and electric-fields sensors. Control of coherence of the NV-electron spin is very important because the excellent performance of the applications is realized by the superior properties of coherence of the NV center. We will show recent results about electrical control of spin coherence of NV center [2].

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References
First High Frequency Electron Spin Resonance Spectrometer Operating in Field and Frequency Domain

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Electron Spin Resonance (ESR) is a powerful technique to investigate the electronic and magnetic properties of various materials. We present the first combined High Frequency Field and Frequency Domain THz Electron Spin Resonance (HFESR/FDMR) spectrometer capable to investigate for the first time the electronic and magnetic properties of molecular systems, thin films and bulk materials in the very broad frequency range of 85-1100 GHz. The combined HFESR/FDMR spectrometer operates at high magnetic fields up to 17 T and temperatures from 300 K down to 1.8 K. We use low-loss quasi-optical microwave propagation, where special care was given to eliminate standing waves in the system. We developed a variable sample holder supporting liquid, powder and single crystal measurements, including a tunable Fabry-Pérot resonator with measured Q = 1200 at 330 GHz, which allows a sensitivity of only 10^7 spins/Gauss. The outstanding performance of the combined HFESR/FDMR spectrometer will be demonstrated on molecular systems, thin films and bulk materials as well as on radicals at DNP relevant frequencies.

Fig. 1. Frequency-Field map of powdered [Mn$_2$(salpn)$_2$(H$_2$O)$_2$](ClO$_4$)$_2$ sample (inset) created by stacking fastly swept frequency spectra from 170 GHz to 390 GHz in slowly sweeping magnetic field up to 15 T. On the right side in black is ESR spectrum recorded at 320 GHz compared to the extracted one from the map at the same frequency. The resolution of the map is 10 000 points x 10 000 points and was recorded in less than 8 hours.
Energy-based modeling of CMOS voltage-controlled oscillators for continuous-wave- and rapid scan ESR experiments

Jens Anders¹, Anh Chu¹, Jonas Handwerker¹, Benedikt Schlecker¹, Maurits Ortmanns¹, Silvio Künstner², Jannik Möser², Alexander Schnegg² and Klaus Lips²

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Oscillator-based spin detection has recently gained significant attention in the ESR community due to its excellent spin sensitivity in continuous-wave ESR experiments with operating temperatures down to 4 K.¹,² Last year, our group has extended the concept of oscillator-based ESR detection by using voltage controlled oscillators (VCOs) which allow for operation in fixed $B_0$-fields by using wide frequency sweeps with constant sensitivity enabled by the operating principle of the VCO³. At last year’s Rocky Mountain Conference we have shown that the VCO-based detection concept is also ideally suited for frequency-sweep rapid scan ESR experiments with very fast equivalent sweep rates exceeding 500 MG/s. This year, we will present an improved energy-based modeling of the VCO-based ESR detection, which covers both continuous-wave and rapid-scan ESR detection. The model both allows for a prediction of the ESR performance of VCO-based detectors prior to manufacturing and for a deconvolution of the distorted rapid-scan ESR signals. The model is verified against measured data from a 14 GHz VCO prototype operating inside a 1 T field in both continuous-wave and rapid-scan experiments. The work is supported by the DFG through the priority program SPP1601 (Ulm and Berlin).

3. Handwerker et al., ISSCC 2016 Digest of Technical Papers, p. 476-478
An understanding of a metalloenzyme catalytic mechanism requires determination of the composition, structure, bonding, and reactivity of the active site for each of the catalytic intermediates that form along the reaction pathway. For paramagnetic states this information can be obtained through analysis of the electron-nuclear hyperfine and nuclear-electric quadrupole interactions of the metal-ion nuclei themselves, of nuclei that form endogenous metal ligands, as well as of enzyme-bound substrates, inhibitors, and products. These interactions are optimally determined by EPR and ENDOR spectroscopies, which most importantly can interrogate freeze-trapped intermediates not amenable to crystallization. Parallel studies of biomimetic inorganic complexes not only illuminate the studies of metalloenzymes, but also probe intrinsically interesting electronic and dynamic properties. Not least, ENDOR can reveal otherwise unknowable details of metal-ion speciation in vivo. This talk will select from among our ongoing studies, including: (i) the determination of the mechanism by which nitrogenase carries out perhaps the most challenging transformation in biology, the reduction of the N≡N triple bond; (ii) the structure and bonding in biomimetic analogs of nitrogenase intermediates; (iii) hydride tunneling in enzymes; (iv) and the mechanism of radical generation by ‘Radical-SAM’ enzymes.
NANOPORE-CONFINED LIPID BILAYERS FOR ORIENTED SAMPLE EPR AND NMR STUDIES OF MEMBRANE PROTEINS

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Fully hydrated bilayer membranes composed from either synthetic or natural lipids provide an optimal environment for folding and maintaining membrane proteins in their functional states. Previously, we have developed methods for forming self-assembled lipid nanotubular bilayers inside cylindrical nanopores composed of anodic aluminum oxide (AAO). Such hybrid nanostructures, named lipid nanotube arrays, represent a new type of substrate-supported and macroscopically-aligned lipid bilayers that have many attractive features for both biotechnology and structure-function protein studies by magnetic resonance.

Here we demonstrate a dramatic improvement in lipid nanotube technology vs. our previous studies [1] by developing AAO substrates with exceptionally uniform high-density nanopore structure. This development allowed for employing oriented sample (OS) solid state NMR for a detailed examination of lipid-induced conformational changes of Pf1 coat protein over exceptionally broad range of environmental conditions, including temperature, pH, and lipid composition. We also report on the effect of lipid saturation on the bilayer viscosity and Pf1 dynamics as evidenced by the changes in the linewidths in the Pf1 spectra, which are directly affected by the uniaxial rotational diffusion of the protein within the membrane.

We also extended the oriented sample (OS) lipid nanotube technology to spin labeling EPR including W-band (94.3 GHz) (HF EPR) spectroscopy. For the latter, a new photonic band-gap W-band resonator to accommodate planar AAO substrates has been developed and tested. Such a resonator/AAO system provides additional resolution for studies of conformational changes of model ion channels by the spin labeling EPR (Fig.1 (b)). We also provide an initial demonstration of DEER from a macroscopically aligned membrane protein.

References

We have used computational molecular and stochastic dynamics trajectories to simulate electron paramagnetic resonance (EPR) spectra of spin-labeled biomolecules. EPR spectroscopy is a powerful tool for probing local structure and dynamics in biological systems, but the corresponding spectra can be complex and difficult to interpret. To accurately model experimental data, user-friendly computational tools have been developed to simulate spectra, especially for continuous-wave (CW) EPR in the slow-motion regime (dynamical time scales of \( \approx 10-100 \) ns for nitroxides at 9-10 GHz). The standard method is to simulate spectra in the frequency domain, e.g. by numerically solving the stochastic Liouville equation [1,2]. Such programs are very fast, but are often restricted to specific models, e.g. only including one diffusion frame, restricted to electron spin-1/2, etc. When more complex models are needed, trajectory-based time domain simulation methods provide a promising alternative, but there are varying techniques in the literature [3-8]. Here we demonstrate our implementation of time domain methods in EasySpin [9], which allows us to simulate spectra using these different methods for direct comparison. As a starting point, the program can use trajectories that are calculated either internally using stochastic dynamics with an arbitrary orienting potential or externally by molecular dynamics. The latter feature will allow for detailed studies of how both spin label and protein dynamics contribute to EPR spectra, and the program will be made publicly available.

References
SOLUTION NMR / HYPERPOLARIZATION

Tuesday, July 25
15:15-16:30

Ryo Kitahara
Hydrostatic- and Gas-Pressure NMR Studies of the Cavity-Enlarged Mutant L99A of T4 Lysozyme

Thomas Theis
Hyperpolarization Chemistry for Affordable Biomolecular MRI

Alexander B. Barnes
Electron Decoupling with Frequency Agile Gyrotrons, MAS Below 15 Kelvin, Fluorescent Biradicals, and DNP in Human Cells

Mengxiao Liu
Profiling of Metalloenzyme Catalysis Using Dissolution Dynamic Nuclear Polarization
Hydrostatic- and gas-pressure NMR studies of the cavity-enlarged mutant L99A of T4 lysozyme

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NMR spectroscopy with hydrostatic pressure allows structural characterization of less-ordered conformers (i.e. high-energy conformers) [1]. We investigated the pressure-induced responses from the cavity-enlarged L99A mutant of T4 lysozyme [2,3]. Peak-intensities for side chain methyl groups in $^1\text{H}/^{13}\text{C}$ hetero-nuclear single quantum coherence (HSQC) spectra, in particular for those around the enlarged cavity (cavity 4 in Fig. 1), decreased with increasing pressure (approximately 300 MPa), without the appearance of new peaks. These residues displayed a close match with those exhibiting a large conformational change between the ground and high-energy states at atmospheric pressure. In the high-energy state, the aromatic side chain of F114 gets flipped into the enlarged cavity [4]. $^{13}\text{C}$ and $^1\text{H}$ line-shape simulations revealed that the pressure-induced loss of peak intensities may be attributed to the increase in the high-energy state population. Recent studies using high pressure circular dichroism and EPR reported that L99A retained its folded conformation up to 300 MPa [5].

We investigated the location of, and ligand accessibility to, the internal cavities of L99A using O$_2$ gas-pressure NMR spectroscopy [6]. The increase in the O$_2$ concentration to 8.9 mM (7 bar) induced longitudinal relaxation enhancements for the backbone amide and side chain methyl protons located around the two hydrophobic cavities (cavities 3 and 4 in Fig. 1), including the enlarged cavity. Our results showed the binding preference of O$_2$ to hydrophobic over hydrophilic cavities. Furthermore, 500-ns MD simulations revealed the rotational and translational motions of O$_2$ in the cavities as well as its ingress and egress. These results suggest that the conformational fluctuation within the ground-state ensemble may facilitate O$_2$ association with the hydrophobic cavities. The channel comprising helices D, E, G, H, and J is thought to be the potential gateway for ligand binding to the hydrophobic cavities.

References

Hyperpolarization Chemistry for Affordable Biomolecular MRI

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Virtually all clinical MR images are of water, and virtually all of these images are acquired with expensive superconducting high-field magnets. High fields are needed to maximize polarization and sensitivity. Yet even with the strongest magnets, it is extremely challenging to detect biomolecules at low concentrations directly reporting on biological function deep inside human organs. The presented research focuses on low-cost hyperpolarization and low-cost, low-field MRI to deliver ultrasensitive and affordable biomolecular MRI.

We develop and characterize parahydrogen based polarization transfer catalysis (SABRE-SHEATH), which we use to hyperpolarize metabolites, drugs and other small molecules directly in room temperature solutions (organic and aqueous).1-4 The hyperpolarized substrates are designed with heteronuclear (15N and 13C) spin labeling schemes for long-term retention of hyperpolarization. We achieve decay time constants of above 20 minutes enabling hour-long molecular tracking.2 We observe particularly long lifetimes at low magnetic fields of 1 T and below because of reduced relaxation from chemical shift anisotropy.5 Therefore, low magnetic fields become attractive, not only because of their affordability, but because they enable metabolic tracking on biological timescales. We discuss sensitivity scaling as a function of magnetic field, and present first principle insights as well as experimental demonstrations showing significant gains for longer waiting times as we move to lower fields.3,5 Figure 1 illustrates our nascent technology and its potential for hyperpolarized low-field molecular MRI.

References

Electron Decoupling with Frequency Agile Gyrotrons, MAS Below 15 Kelvin, Fluorescent Biradicals, and DNP in Human Cells


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Electron decoupling and time domain DNP show considerable promise to improve DNP NMR performance. Attenuation of detrimental paramagnetic relaxation effects, increased DNP enhancement at room temperature, and efficient targeted DNP within intact human cells are the primary applications of methods and technology development in our laboratory. Custom high power frequency agile gyrotrons and cryogenic MAS have already enabled the experimental demonstration of electron decoupling in MAS DNP experiments. Advancements in DNP instrumentation and methodology is being applied to study the activation of protein kinase C in membranes. Novel fluorescent DNP polarizing agents developed for in-cell NMR show both sub-cellular localization and targeted DNP enhancements in HEK293 cells.

We demonstrate electron decoupling experiments in conjunction with DNP and magic angle spinning (MAS) NMR spectroscopy. Microwave frequency sweeps through the EPR lineshape are shown as a time domain strategy to significantly improve electron decoupling. For $^{13}$C spins on biomolecules frozen in a glassy matrix, electron decoupling reduces linewidths by 11% (47 Hz) and increases intensity by 14% at 90 Kelvin. Custom frequency agile gyrotrons (Figure 1) supply >100 W of tunable power at frequencies above 200 GHz and provide the requisite control over EPR spins to implement time domain DNP transfers and also decouple electron-nuclear dipolar interactions. A custom counter flow heat exchanger reduces the liquid nitrogen consumption to 100 L per day for MAS at 82 K. Using helium gas for all spinning and cooling gases enables MAS below 15 K (Figure 2). A novel trimodal DNP polarizing agent is introduced containing a fluorescent marker, biradical, and targeting moiety. Fluorescent DNP polarizing agents enable sub-cellular targeting and optical localization of DNP enhanced NMR signals (Figure 3).

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Profiling of Metalloenzyme Catalysis using Dissolution Dynamic Nuclear Polarization

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Dissolution dynamic nuclear polarization (D-DNP) can yield sufficient NMR signal to allow determination of enzyme turnover using real-time $^{13}$C NMR. The time-dependent NMR signals are governed by both the reaction rates and spin relaxation. Here, we demonstrate that paramagnetic relaxation enhancement in the D-DNP measurement can be used to obtain long-range structural constraints of the catalyzed product in metalloenzymes. Spectra of reactions catalyzed by pseudouridine monophosphate glycosidase (ΨMPG), which couples uracil and ribose-5-phosphate with diamagnetic Mg$^{2+}$ or paramagnetic Mn$^{2+}$ in the active site, are measured. In both cases, transient signals of reaction product pseudouridine monophosphate (ΨMP) are visible upon consumption of hyperpolarized uracil (Fig. 1a). The reaction with paramagnetic enzyme further exhibits line broadening due to paramagnetic $R_2$ relaxation (Fig. 1b). Based on the dependence of $^{13}$C signals on time and on enzyme concentration, a multi-parameter model incorporating line widths as well as integrals is used to fit reaction and relaxation rates. The paramagnetic enhanced $R_1$ and $R_2$ rates can be used to determine distances between the product atoms and the metal center in the metalloenzyme (Fig. 1c). Because of the readily observed signals of hyperpolarized spins even in complex mixtures, similar methods may further be applicable to in-vivo profiling of metalloenzyme catalysis.

Fig. 1. a) Real-time $^{13}$C NMR signal from ΨMPG catalyzed reaction, in-vitro occurring left to right, coupling uracil (1) and ribose-5-phosphate (2) to form ΨMP (3). b) Line broadening in Mn$^{2+}$-enzyme reaction. In a) and b) fitted curves are plotted together with data points. c) Calculated distances from paramagnetic relaxation enhanced $R_1$ and $R_2$ rates are indicated on the crystal structure [1].

References

SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Tuesday, July 25
15:15-16:30

Daniel Huster
Dynamics and Ligand Binding of the G Protein-Coupled GHS Receptor in Bilayer Membranes Investigated by Solid-State NMR Spectroscopy

Mei Hong
$^{13}$C and $^{19}$F Solid-State NMR Determination of Cholesterol-Bound Structure of a Membrane Protein

Akira Naito
Photo-Intermediates in the Photo-Reaction Pathways of Bacteriorhodopsin as Revealed by in Situ Photo-Irradiation Solid-State NMR

Lynmarie K. Thompson
Signaling-Related Mobility Changes in Functional Chemotaxis Receptor Arrays by Solid-State NMR
The molecular dynamics of the human growth hormone secretagogue (GHS) receptor reconstituted into either DMPC or POPC membranes was studied. The receptor was expressed in E. coli, refolded, and reconstituted into bilayer membranes. The dynamics of the receptor in the absence and in the presence of its natural agonist ghrelin or an inverse agonist was studied using $^{15}$N and $^{13}$C solid-state NMR spectroscopy. Static $^{15}$N NMR spectra of the uniformly labeled receptor are indicative of axially symmetric rotational diffusion of the G protein-coupled receptor in the membrane. In addition, about 25% of the $^{15}$N sites undergo large amplitude motions giving rise to very narrow spectral components. For a more quantitative assessment of the receptor mobility, $^1$H-$^{13}$C dipolar coupling values, which are scaled by molecular motions, were determined quantitatively. From these values, order parameters, reporting the motional amplitudes of the individual receptor segments can be derived. Backbone order parameters were determined with values between 0.51 and 0.69, corresponding to motional amplitudes of 40-50° of these segments. Differences between the receptor dynamics in DMPC or POPC membranes were within experimental error. Furthermore, agonist or inverse agonist binding only insignificantly influenced the average molecular dynamics of the GHS receptor [1].

The peptide hormone ghrelin activates the GHS receptor. This 28-residue peptide is acylated at Ser3 and is the only peptide hormone in the human body that is lipid-modified by an octanoyl group. We carried out solid-state NMR studies of ghrelin in lipid vesicles, followed by computational modeling of the peptide using Rosetta. Isotropic chemical shift data of isotopically labeled ghrelin provide information about the peptide’s secondary structure. Spin diffusion experiments indicate that ghrelin binds to membranes via its lipidated Ser3. Further, Phe4, as well as electrostatics involving the peptide’s positively charged residues and lipid polar headgroups, contribute to the binding energy. Other than the lipid anchor, ghrelin is highly flexible and mobile at the membrane surface. This observation is supported by our predicted model ensemble, which is in good agreement with experimentally determined chemical shifts. In the final ensemble of models, residues 8-17 form an α-helix, while residues 21-23 and 26-27 often adopt a polyproline II helical conformation. These helices appear to assist the peptide in forming an amphipathic conformation so that it can bind to the membrane [2]. First results on the changes of the isotropic chemical shifts of ghrelin upon binding to the GHS receptor will be reported.

References
13C and 19F Solid-State NMR Determination of Cholesterol-Bound Structure of a Membrane Protein

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Cholesterol is a ubiquitous component of the phospholipid membranes of eukaryotic cells, and cholesterol interactions with membrane proteins have been reported for a number of membrane proteins. However, high-resolution structures of cholesterol-complexed membrane proteins in phospholipid bilayers are scarce, due to the difficulty of most high-resolution structural techniques to probe membrane protein structures in cholesterol-containing lipid membranes. Here we present a 13C, 19F, and 2H solid-state NMR study of the interactions and binding between cholesterol and the influenza M2 protein. While M2 is well known as a proton channel that initiates virus uncoating, it has a second function of mediating membrane scission during the last step of virus budding. This second function has been shown to require cholesterol, which is enriched in the region of the budding virus. Combining 13C, 19F and 2H-labeled cholesterol with 13C, 15N-labeled M2, we have measured M2-cholesterol interactions in lipid membranes. 13C-19F REDOR experiments and 13C-13C correlation spectra revealed cholesterol-proximal residues, while 2H spectra of cholesterol allowed the determination of the orientation of the bound cholesterol. These results present direct experimental constraints of functionally relevant cholesterol-complexed structure of a membrane protein.

![Fig. 1. Determination of the cholesterol-binding interface with the influenza M2 protein. (a) Structural model of a potential cholesterol binding pocket of M2. (b) Static 2H spectra of cholesterol deuterated at the sterol head in the absence and presence of M2. (c) Representative 13C-19F REDOR data showing fluorinated cholesterol’s contact with protein residues in the transmembrane domain of M2. (d) Simulation of the 13C-19F REDOR curve of L36 Ca.](image)

Acknowledgement: This work is supported by NIH grant GM088204.
PHOTO-INTERMEDIATES IN THE PHOTO-REACTION PATHWAYS OF BACTERIORHODOPSIN AS REVEALED BY IN SITU PHOTO-IRRADIATION SOLID-STATE NMR

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It is essential to elucidate the photoreaction cycles to understand the function of photoreceptor membrane proteins. The photointermediates that appear in the photoreaction cycle typically have short half-lives, therefore, it is difficult to detect the photointermediates using solid-state NMR. Recently, it has become possible to observe photointermediates using in situ photo-irradiation solid-state NMR\textsuperscript{[1,2]}. \textit{In situ} photoirradiation solid-state NMR is designed to irradiate light from the top part of the zirconia rotor through a glass cap, which makes it possible to irradiate the inside of the rotor, and leads to efficient photo-irradiation. This method has made it possible to observe photointermediates of bacteriorhodopsin (brR) and its Y185F mutant.

The photoreaction pathways of Y185F-bR were examined using \textit{in situ} photo-irradiation solid-state NMR \textsuperscript{[3]}. \textsuperscript{13}C CP-MAS NMR spectra were acquired at -40 \textdegree C in the dark (D1), under irradiation with 520 nm light (L1), subsequently in the dark (D2), and again under irradiation with 520 nm light (L2). Between D1 and L1, the CS (brR548) state changed to a CS* (13-cis, 15-syn)-intermediate. Simultaneously, AT (brR568) state transformed to an N-intermediate. Under the D2 condition, the N-intermediate transformed to an O-intermediate, which was observed in the first time by NMR. Consequently, the O-intermediate transformed to the N-intermediate through the AT state. In this experiment, the chemical shifts of the [20-\textsuperscript{13}C, 14-\textsuperscript{13}C]retinal provided the 13C=C and 15C=N configurations. From these data, the configurations of the AT and CS states, and the CS*, N-, and O-intermediates were determined as (13-trans, 15-anti), (13-cis, 15-syn), (13-cis, 15-syn), (13-cis, 15-anti), and (13-trans, 15-anti), respectively. In situ photoirradiation solid-state NMR spectroscopy thus revealed the photoreaction pathways and structures of the AT and CS states, and the CS*, N- and O-intermediates of the Y185F-bR mutant (Fig. 1)

References

![Fig. 1 Photo reaction pathways of Y185F-bR](image-url)
Bacteria employ remarkable ~200 nm membrane-bound nanoarrays to sense their environment and direct their swimming. Arrays consist of receptor trimers of dimers (rainbow) that extend perpendicular to the membrane to a “baseplate” where they are linked by hexagons of alternating CheA (black) and CheW (gray) proteins (Fig. 1). Binding of attractant ligands to the receptors causes a 2 Å downward piston of an alpha helix in the periplasmic and transmembrane domains (not shown). How does the signal propagate an additional ~200Å through the cytoplasmic domain to control the kinase CheA? Because changes in receptor dynamics are proposed to propagate the signal, we have used INEPT to selectively detect a mobile region within native-like arrays of U-13C, 15N receptor cytoplasmic fragment (CF), CheA, and CheW assembled on vesicles. This region is identified by chemical shifts of distinctive residues (Fig. 2). Comparison of 40 kHz MAS INEPT spectra of functional signaling states suggests increased flexibility of this region in the kinase-off state, which appears consistent with proposed changes in dynamics during signaling.
MRI METHODS, MEDICINE, MATERIALS / SOLID-STATE NMR MATERIALS/METHODS

Tuesday, July 25
15:15-16:30

Elodie Salager
NMR Spectroscopic Imaging for the Study of Electrochemical Storage Devices

Sungsool Wi
Adiabatic Sweep Cross-Polarization Magic-Angle-Spinning NMR of Spin-1/2 and its Extension to Integer and Half-Integer Quadrupolar Spins

Daphna Shimon
CO$_2$ Chemisorption/desorption Studied with $^{15}$N Solid State NMR of Surface Amine Groups for Carbon Capture

Asher Schmidt
Biomimetic CaCO$_3$ Crystallization and Stability Regulated by L-Asp: The Structure and Transformation of the Interaction Interface
NMR SPECTROSCOPIC IMAGING FOR THE STUDY OF ELECTROCHEMICAL STORAGE DEVICES

M. Tang$^{1,2,†}$, C.-E. Dutoit$^{1,2}$, G. Oukali$^{1,2}$, M. Ménétrier$^{2,3}$, J.-M. Tarascon$^{2,4}$, E. Raymundo-Piñero$^{1,2}$, V. Sarou-Kanian$^{1,2}$, M. Deschamps$^{1,2}$, E. Salager$^{1,2,*}$

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The development of renewable energies, inherently intermittent, as well as the development of electrical cars call for efficient energy storage. Electrochemical storage is performed with lithium-ion batteries or supercapacitors depending on application. A better understanding of the phenomena limiting the energy storage capacity and the rate at which it can be stored is essential to enhance the characteristics of those devices.

We combine NMR spectroscopy and imaging to study supercapacitors and batteries operando, i.e. while they are functioning. Classical spectroscopic imaging methods work well for the characterization of the components with a sharp spectrum, like electrolyte in supercapacitors$^{[1]}$, electrolyte in batteries$^{[2]}$ or metallic lithium$^{[3]}$. We are currently exploring the effect of conditioning in the case of supercapacitors. Conversely, the signal of the active material in the solid electrodes of batteries is in general not detected with standard spectroscopic imaging techniques. The transition metal centers in the electrodes are indeed paramagnetic in some stages of the charging-discharging process and result in very short lifetimes of the NMR signal. We recently developed S-ISIS, a way to retrieve this signal in a functioning 5-mm diameter Li-ion battery$^{[4]}$. It was inspired by the ISIS localized spectroscopy concept$^{[5]}$ that takes advantage of the longer longitudinal relaxation time of those materials. We could reconstruct, for the first time, the full 1D $^7$Li spectroscopic image of a battery, including the solid paramagnetic electrodes (Li$_{1-x}$CoO$_2$, 0<$x$<0.5 and Li$_{4+y}$Ti$_5$O$_{12}$, 0<$y$<3), with a resolution of 100 μm. As a result the lithiation fronts formed in those 400-μm thick electrodes can now be studied in situ for various (dis)charging conditions. We will discuss the constraints on spatial, temporal and spectral resolution in the framework of in situ or operando characterization of functioning batteries.

References

Adiabatic Sweep Cross-Polarization Magic-Angle-Spinning NMR of Spin-1/2 and its Extension to Integer and Half-Integer Quadrupolar Spins

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The use of frequency-swept radiofrequency (rf) pulses was explored in cross-polarization magic-angle spinning (BRAIN-CPMAS) experiments for obtaining broadband rare nuclei signals, such as \textsuperscript{13}C, \textsuperscript{2}H, \textsuperscript{23}Na, and \textsuperscript{11}B, by utilizing a broadband adiabatic inversion pulse. Optimal Hartmann-Hahn (HH) matching conditions, zero-quantum (ZQ) and double-quantum (DQ) modes were found by employing low rf pulse strengths for both I (abundant) and S (rare) spin channels, at a very fast ($\nu_r \approx 60$ kHz) MAS spinning rate for S=1/2,1, and 3/2 cases [1-3]. Also observed are the cases of involving a pseudo-static zero-quantum mode that is driven by a quadrupolar-modulated rf/dipolar recoupling term and a MAS-driven rotary resonance phenomenon in the presence of the chemical shift anisotropy or quadrupolar interaction. For S=1/2 case ultrawide CP profiles that span frequency ranges of nearly $6 \cdot \gamma B_1^S$ were observed (Fig. 1A). For $^2$H (S=1) case the CP lineshapes generated are very close to the ideal $^2$H MAS spectral lineshapes, facilitating the extraction of quadrupolar coupling parameters (Fig. 1B). At a easily attainable (ca. 10 kHz) S-spin rf field condition for quadrupolar S=1 and 3/2 cases, adiabatic level-crossings among different $m_S$ energy levels, which are known to complicate the CPMAS of quadrupolar nuclei, are avoided. Our experimental spectra were analyzed by extensive numerical simulations as well as by average Hamiltonian theories (AHTs). Our CP method was named as BRoadband Adiabatic INversion Cross-Polarization Magic-Angle Spinning (BRAIN-CPMAS) experiment.

Fig. 1. Offset-frequency dependent CP profiles of BRAIN- and conventional Hartmann-Hahn (HH) CPMAS spectra measured on $^{13}$C Gly at $\nu_r = 65$ kHz (A). Comparisons of BRAIN-CPMAS, HH-CPMAS, and directly acquired $^2$H spectra measured at $\nu_r = 40, 50$, and 60 kHz on Glycine-2,2-d$_2$ (B).

References

CO$_2$ chemisorption/desorption studied with $^{15}$N Solid State NMR of Surface Amine Groups for Carbon Capture

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Carbon dioxide (CO$_2$) is a well-known greenhouse gas that results in negative consequences to the environment when present at high concentrations in the atmosphere, prompting research on carbon capture and storage (CCS) worldwide. One method of minimizing CO$_2$ emissions is by reacting it with amine groups grafted to the pore-surface of mesoporous materials. The amine groups capture the CO$_2$ by chemisorption, and understanding these processes is critical to help make CCS more efficient.

In this work, we study a candidate material for CCS that consists of $^{15}$N labeled aminopropylsilane (APS) grafted on the surface of mesoporous silica SBA-15. During exposure of the sample to CO$_2$, the CO$_2$ reacts with the nitrogens to create chemisorbed species such as carbamate, carbamic acid, bicarbonate and others. The assignment of the chemisorbed species is somewhat controversial, because of the closely-spaced $^{13}$C resonances for bicarbonate, carbamate and carbamic acid. As a consequence, $^{15}$N-NMR is used to shed light on some of the assignments.

$^{15}$N{$^1$H} solid state cross-polarization magic angle spinning (CPMAS) NMR experiments were conducted to understand the nature of the grafted $^{15}$N-APS groups on the pore surface before and after CO$_2$ chemisorption. In particular, we discuss the reactions that take place during CO$_2$ adsorption and (partial) desorption (see figure 1). This work discusses intriguing and seemingly contradictory results that deal mechanistically with the chemisorbed products and the results of desorption.

![Fig. 1. $^{15}$N{$^1$H}-CPMAS NMR spectrum of 2 mmol N/g $^{15}$N-APS SBA15, measured as a function of time, t, after loading CO2 gas into the sample. The spectra were recorded at (left to right) t=0 hrs and t=102.2 hrs. A spacer with an o-ring was used to slow down the release of CO2. The peaks are assigned to aminopropylsilane (blue), ammonium propylsilane (green), di-tethered byproduct (red) and carbamate (cyan).](image-url)
Biomimetic CaCO\textsubscript{3} crystallization and stability regulated by L-Asp: The structure and transformation of the interaction interface

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CaCO\textsubscript{3} crystallization in the presence of acidic amino acids is a model system for biomineralization. Including \textsuperscript{13}N]L-aspartic acid in the precipitation solution of calcium \textsuperscript{10%-13}C]carbonate leads to precipitation of the least stable polymorph, vaterite; in its absence, only calcite forms. We find that the kinetic stability of the obtained vaterite is switchable by changing Asp concentration in the precipitation solution: high-Asp yields thermally and temporally stable vaterite while low-Asp yields metastable vaterite that spontaneously transforms to calcite at ambient conditions. Such regulation of polymorph selection and imprinted stability is often encountered in biomineralization pathways exploited by organisms. Solid-state NMR shows that molecularly dispersed amino-acids were occluded in the crystalline lattice and that the interaction shell surrounding each amino-acid consists of two chemically distinct types of disordered carbonates and water molecules, hence exemplifying the molecular details of “intracrystalline” occlusion in such lattices. We further identify the rearrangement of the interaction shell that accompanies the spontaneous solid-solid phase transformation of bulk vaterite to calcite, reflecting the readjustment to the new crystalline host matrix.

Biomolecules and/or inorganic ions in the precipitation medium of (bio)minerals, which are often subsequently entrapped, have been shown to vastly influence the resulting solids: regulation of early nucleation events, polymorph selection, imprinting of lattice characteristics and macroscopic properties such as lattice stability in this study.[1] The network of organic-inorganic interface as exposed herein, although comprising a minute fraction of the total bulk, is of critical importance for the understanding of the molecular-chemical-structural principles and mechanisms that underlie the above effects. The scarcity and disordered nature of the interaction interface of “intracrystalline” occlusions severely impedes their characterization, singling out solid-state NMR as the best-equipped technique.[2,3]

Fig. 1. Spontaneous solid-solid, vaterite-to-calcite, transformation at ambient conditions and the rearrangement of the carbonates interaction interface with occluded L-Asp, adjusting to the new host polymorph.

References
Lilian Childress  
Spin-Based Sensing with NV Diamond

Susumu Takahashi  
Nanoscale EPR Spectroscopy Using NV Centers in Diamond

Hitoshi Ohta  
Development of Multi-Extreme THz ESR: Present and Future

Dmitry Akhmetzyanov  
Nanometric Distance Determination Using Manganese Centers

Hannah S. Shafaat  
Model Nickel Metalloenzymes for Energy Conversion

Jessica A. Clayton  
High-Field CW EPR with Gd(III) Tags for Structure-Dynamics Studies of Proteorhodopsin

Valentina Lukinović  
Investigation of the Electronic Structure of B_{12} Coenzymes by TREPR: Methylcobalamin

Guillem Brandariz-de-Pedro  
Cryo-EPR of NADPH: Protochlorophyllide Oxidoreductase
Spin-based sensing with NV diamond

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The NV center in diamond is a remarkable EPR system in that its spin states can be optically prepared and detected, even under ambient conditions, and even for single spins. These properties motivate using its spin states to detect magnetic fields, with the potential for excellent sensitivity and spatial resolution. Nevertheless, the internal dynamics of the NV defect under optical excitation limit the signal to noise that can be obtained with room-temperature fluorescence detection of its electronic spin. In this talk, I will introduce NV sensing and consider in detail: (1) the limitations imposed and possibilities opened by internal dynamics – in particular, the charge state dynamics of the NV present alternate measurement modalities – and (2) application to sensing resonance of spin-torque-driven ferromagnetic devices, whose stray fields can be detected via proximal NV defects.
Magnetic resonance (MR), such as nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR), can probe the local structure and dynamic properties of various systems, making them among the most powerful and versatile analytical methods. However, their intrinsically low sensitivity precludes MR analyses of samples with very small volumes; e.g., more than $10^{10}$ electron spins are typically required to observe EPR signals at room temperature. A vast improvement in the current limits of MR will enable the imaging of structures and conformational changes of molecules in solution at the single molecule level.

A nitrogen-vacancy (NV) center in diamond is a promising candidate for applications in room temperature magnetic sensing with single spin sensitivity. In this presentation, we will discuss EPR spectroscopy using on NV centers in diamond. By employing EPR and double electron-electron resonance (DEER) techniques, we investigate impurities and coherence in diamond. We also demonstrate EPR of several electron spins using NV-based EPR spectroscopy [1,2]. Moreover, we will discuss development of a high-frequency NV-based EPR system [3]. This work is supported by NSF (CHE-1611134 and DMR-1508661) and the Searle Scholar program.

References
Development of Multi-Extreme THz ESR: Present and Future

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Development of multi-extreme THz ESR, which covers the frequency region from 0.03 to 7 THz, is in progress in Kobe. Our multi-extreme conditions correspond to the high magnetic field up to 55 T using the pulsed magnet [1], the high pressure up to 1.5 GPa using the transmission type piston cylinder pressure cell [2], and the detection of micrometer size sample using the micro-cantilever ESR [3]. Recently following new developments have been achieved on our multi-extreme THz ESR.

First we have developed the transmission type hybrid-type pressure cell, which consists of NiCrAl alloy inner cell and Cu-Be alloy outer cell, and have achieved the pressure up to 2.7 GPa [4]. Although the magnetic field is limited up to 10 T using the super conducting magnet due to the increase of pressure cell size, the observation of pressure induced phase transition in Shastry-Sutherland Model Substance SrCu$_2$(BO$_3$)$_2$ became possible.

Secondly we have succeeded in extending the micro-cantilever ESR measurements of Co-Tutton salt up to 1.1 THz using the torque method, which is applicable to the sample with the magnetic anisotropy [5]. To our knowledge, this is is the world record frequency for such mechanical detection of ESR.

Thirdly we have also developed the micro-cantilever ESR measurement system using the Faraday method [6]. In this method the field gradient is applied to the sample, and it is applicable to the isotropic sample. The application to hemin, which is the model substance of myoglobin, will be shown [7] and compared to our conventional transmission THz ESR [1], which requires 6 orders of magnitude larger amount of hemin for the measurement.

References

NANOMETRIC DISTANCE DETERMINATION USING MANGANESE CENTERS

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Dipolar EPR spectroscopy \cite{1} is a valuable technique for precise measurements of distances in the range of 1.5 to 10 nm, and is a useful tool for determining structures and conformational flexibilities of macromolecules. Distances are often measured between pairs of nitroxide radicals that have been site-specifically attached to biomolecules. Recently high-spin Gd\textsuperscript{3+} and Mn\textsuperscript{2+} centers have been introduced as alternative labels for distance measurements \cite{2,3}. Mn\textsuperscript{2+} ions are especially interesting because they are redox stable when an appropriate ligand sphere is used, and they are also endogenous to cells. Indeed numerous enzymes contain manganese ions as cofactors. Furthermore, Mn\textsuperscript{2+} can readily replace diamagnetic Mg\textsuperscript{2+}, an essential cofactor in enzymes, nucleic acids and nucleotide binding domains of membrane proteins.

We have investigated a model compound doubly labelled with Mn\textsuperscript{2+}-DOTA centers \cite{4} by dipolar EPR spectroscopy at high microwave frequencies. Pulsed electron-electron double resonance (PELDOR/DEER) \cite{1} at 94 GHz and relaxation-induced dipolar modulation enhancement \cite{5} (RIDME) experiments at 94 and 263 GHz yielded distances that are in good agreement with predictions \cite{4,6}. RIDME measurements revealed up to three times larger sensitivity compared to PELDOR. Also the RIDME experiments featured harmonics of the dipolar coupling frequency that appeared due to the high electron spin multiplicity of Mn\textsuperscript{2+} centers. Although harmonics were accounted for by extending the kernel function in Tikhonov regularization analysis, interpretation of the RIDME measurements appeared to be more complex compared to PELDOR \cite{6}. Possible applications of manganese-based distance measurements for biological systems will be discussed.

References

Model nickel metalloenzymes for energy conversion

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Nickel-containing metalloenzymes such as hydrogenase, carbon monoxide dehydrogenase, and acetyl coenzyme A synthase play important roles in regulation of global gas cycles and are highly relevant in the current energy climate. These enzymes efficiently and reversibly generate and oxidize energetic compounds such as hydrogen, carbon monoxide, and thioesters such as acetyl-CoA. However, the complexity of the multimetallic active sites in these systems along with the presence of many auxiliary cofactors obscure distinguishing spectroscopic features and render detailed analyses challenging. As a result, the molecular mechanisms of catalysis remain relatively poorly understood, thwarting efforts to build synthetic systems that are based on the native enzymes.

To better understand these valuable enzymes, we have developed structural and functional models of hydrogenase, carbon monoxide dehydrogenase, and acetyl coenzyme A synthase within small model protein scaffolds such as rubredoxin and azurin. Vibrational and magnetic resonance spectroscopic techniques have been used to probe the geometric and electronic structures of the active sites with high resolution. Density functional theory calculations used in conjunction with experiments reveal structural information across different oxidation states and substrate-bound forms. The simplicity of these scaffolds allows for comprehensive and unambiguous characterization of enzymatic intermediates, providing insight into the model catalytic mechanisms. Moreover, these observations establish a chemical precedence for key intermediates that may contribute to activity in the native enzyme systems.

References

HIGH-FIELD CW EPR WITH Gd(III) TAGS FOR STRUCTURE-DYNAMICS STUDIES OF PROTEORHODOPSIN

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EPR in combination with site-directed spin-labeling has proven to be a very powerful tool for elucidating the structure and organization of biomolecules in native-like environments. At high fields, \(S = 7/2\) Gd\textsuperscript{3+} spin labels have been shown to be particularly effective in increasing the sensitivity of distance measurements via DEER at Q- and W-band frequencies and above, and are now being developed as distance probes for use with CW EPR lineshape analysis at very high frequencies. Proof of concept experiments on random solutions of GdCl\textsubscript{3} have shown that line broadening measurements of the central \(|-1/2> \rightarrow |1/2>\) transition of Gd\textsuperscript{3+} with CW EPR at 240 GHz are sensitive to inter-spin distances up to \(\sim 3.8\) nm, and at elevated temperatures [1]. This greatly increased distance sensitivity persists in pairwise distance measurements in a series of model molecular rulers labeled with Gd-PyMTA. Dipolar broadening in these Gd-rulers follows a \(1/r^3\) dependence and is resolvable up to inter-spin distances of at least \(3.4\) nm at \(30\) K, with similar line broadening trends observed at \(215\) K and up to room temperature [2]. This extension in the flexibility of the CW EPR technique is applied to the study of proteorhodopsin (PR), a seven-alpha helical transmembrane protein that functions as a light-activated proton pump. First results applying this method to inter-protein measurement of Gd(III) tagged PR oligomers reveals distances consistent with the penta- or hexameric organization determined by crystal structure. Finally, we present progress towards development of measurement methods that will enable observation of light-induced conformational changes in the EF-loop region of PR at temperatures above the protein dynamical transition.

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References

Investigation of the electronic structure of B\textsubscript{12} coenzymes by TREPR: methylcobalamin

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Pernicious anaemia is caused by the deficiency of vitamin B\textsubscript{12} resulting in insufficient number of red blood cells. The vitamin has to be obtained from the diet and once in body, it is bound to one of the many enzymes involved in metabolism.

Methylcobalamin (MeCbl) and adenosylcobamon (AdoCbl) are forms of vitamin B\textsubscript{12} that serve as enzyme cofactors. Their structure contains a corrinoid ring with dimethylbenzylimidazole as a lower axial ligand and methyl or 5'-deoxyadenosyl as an upper axial ligand. The Co-C bond has a crucial role in enzymatic reactions of enzymes involved in the metabolism of vitamin B\textsubscript{12}. AdoCbl-dependent enzymes generate radical pairs upon substrate binding whereas MeCbl-enzymes proceed through heterolysis. However, both cofactors produce analogous radical pairs following photoexcitation. Flash photolysis time resolved EPR (TREPR) has been used to determine differences between electronic structure of two cofactors and origin of the precursor molecules.

Progress on biological time resolved EPR of B\textsubscript{12} coenzymes will be presented using a bespoke continuous-flow system with FID detection at 9 and 34 GHz and direct detection EPR at 9 GHz. Photolysis of was conducted at different excitation wavelengths and solvent conditions to study the effect on radical pair polarisation mechanisms. Apparently in contrast to published magnetic field effect studies \cite{2}, the observed polarisation is predominately from triplet-born radical pairs, concluded after observing chemically induced dynamic electron polarization (CIDEP) of the formed alkyl radicals. \cite{3,4}

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\cite{4} Hore, P.J., Joslin, C.G., McLauchlan, K.A. The role of chemically-induced dynamic polarization (CIDEP) in chemistry. Chem.Soc.Rev. 1979, 8, 29-61.
Cryo-EPR of NADPH:protochlorophyllide oxidoreductase

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Protochlorophyllide (Pchlide) is the main pigment in seedlings and dark-grown plants. It is the substrate for the key light-driven enzyme protochlorophyllide oxidoreductase (POR) in the chlorophyll biosynthesis pathway and plays an important role in plant growth and development. Pchlide has unique excited-state properties that facilitate catalysis and forms a long-lived triplet by intersystem crossing [1-3].

We have used time-resolved absorption measurements in combination with EPR to characterise the triplet excited-state of this crucial chlorophyll precursor and confirmed that its cryogenic excited-state dynamics relate to those in physiological temperature conditions, thus, opening up the possibility of using EPR to investigate the potential role of the Pchlide triplet state in this reaction and better understand how the POR active site affects the electronic properties of its substrate (Fig. 1) [4]. Moreover, the Pchlide excited-state triplet was found to react with oxygen, suggesting that highly reactive oxygen species could be formed, which may lead to photo-damage in the cell. By using EPR, we want to interrogate the potential photoprotective role of POR in preventing cell damage.

We are extending the use of these spectroscopic techniques to a range of samples consisting of standalone and protein-bound Pchlide and analogues in order to translate changes in EPR and absorption properties into answers to relevant biophysical questions (Fig. 1).

References
PLENARY SESSION 5

Wednesday, July 26
08:30-09:15

Klaus Schmidt-Rohr
Composition and Supramolecular Structure of Complex Organic Materials: New Insights from Solid-State NMR
Composition and Supramolecular Structure of Complex Organic Materials: New Insights from Solid-State NMR

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Complex organic and hybrid organic–inorganic functional materials, such as natural and synthetic polymers, biological nanocomposites, metal–organic frameworks, carbon materials, biochar, or pharmaceutical dispersions, continue to be of great practical interest. Reliable structural information is needed for the rational design of materials with improved properties, and for correct modeling of the carbon and nitrogen cycles in the environment. Due to the limited long-range order in these systems, their molecular structure is often not accessible to crystallography. Quantitative [1] and spectrally edited [2] $^{13}$C NMR is usually the best available method for comprehensively characterizing the organic components in these complex materials, resolving different sites better than XPS or XANES and providing much more quantitative intensities and more reliable peak assignments than IR or Raman spectroscopy. Furthermore, NMR can combine the compositional analysis based on chemical shifts, peak intensities, and spectral editing, with proximity measurements based on dipolar couplings [3] or spin diffusion [4]. This combination of composition and distance analysis makes it possible to localize spectrally identified organic components within the supramolecular structure on the 0.5 – 20 nm scale, below the resolution limit of spectrally resolving microscopy methods for organic materials. This enables NMR to characterize the surface of nanocrystals in bulk materials [3, 4], determine domain sizes and assign domains in nanostructured systems, as well as assess the distribution of structural defects.

For instance, the surface of 5-nm diameter nanodiamond particles, which was previously assumed to be partially covered by an aromatic layer, based mostly on modeling, has ≤ 1% aromatic C but is instead covered by C-H, C-OH, and C=O groups [3]. NMR confirms that polyethylene chains in alternating crystalline and amorphous layers end at the crystal surface and tilt in the crystallites in order to avoid amorphous layers with a density higher than in the crystallites, revising the structural model used for 50 years [4]. Based on spin diffusion and spectral editing data, the sheet-like light domains of the bio-polyester suberin in cork has to be assigned to polymethylene rotator-phase crystals instead of amorphous bent molecules. This presentation will highlight a dozen examples where NMR has overturned accepted structural models, sometimes against quite vehement objections by the experts.

References

PLENARY SESSION 6

Wednesday, July 26
09:15-10:00

Christina M. Thiele
Structure Determination of Organic Compounds Using (Anisotropic) NMR Parameters
Information about the three dimensional structure of organic or organometallic compounds can improve our understanding of their function. Thus the determination of their 3D-structure in as native an environment as possible is necessary.

Together with $^3\!J$ couplings and NOE parameters residual dipolar couplings (RDCs) can be used for this approach. RDCs belong to the class of anisotropic NMR-parameters and can yield complementary information. The prerequisites and limits for using RDCs on organic compounds (alignment media, simultaneous determination of configuration and conformation, flexibility, etc.) will be discussed.[1]

An overview of the use of RDCs for organic structure determination will be given and their complementarity with other NMR parameters will be discussed on one selected example, in which NMR allowed the understanding of enantioselective catalysis:

The peptide-based organocatalyst 1 mediates the enantioselective monoacylation of trans-cycloalkane-1,2-diols.[2] A dynamic binding-pocket within the acylated catalyst intermediate was proposed by molecular mechanics as well as by DFT computation (Fig. 1). But no experimental evidence had been found. The peptide-based catalyst 1 was studied alone using RDCs and NOEs and in a mixture with trans-cycloalkane-1,2-diol 2. Significant structural changes of the catalyst were found after the addition of the diol, which is preferentially acylated. To prove a key dispersion interaction between the cyclohexyl part of the catalyst and the diols proposed by DFT computation, we developed a new pure shift pulse sequence. We combined the EASY ROESY experiment with PSYCHE homodecoupling to observe through-space intermolecular contacts between these two cyclohexyl moieties.

References
SOLUTION NMR

Wednesday, July 26
10:30-12:00

Ana Paula Valente
Conformational Diversity in Protein Complexes: NMR Studies of Allergens Bet V 1 and Fag S 1 with Ligands and DIII of Dengue Virus E Protein with Antibodies

Rodolfo Rasia
Preformed Structural Elements within the Structural Ensemble of the Intrinsically Disordered dsRBD-1 Domain from Arabidopsis Thaliana DCL1

Kenji Sugase
High-Sensitivity Rheo-NMR Spectroscopy for Protein Studies

Steffen Merz
Diffusion NMR for Ionic Liquid Electrolyte Dynamics in Gas Diffusion Electrode Frameworks
We studied the structure and molecular dynamics of DIII, highlighting their role in the molecular mechanisms of Dengue virus infection and antibody recognition. Using a combination of nuclear magnetic resonance (NMR) experiments, we characterized, at the residue level, the motion of DIII free in solution, at different pHs, and in complex with a neutralizing antibody. We analyzed chemical shift perturbation and relaxation parameters to evaluate the importance of motions in the ps-ns and µs-ms timescales in complex formation.

We observed conformational exchange in the isolated DIII, in regions important for the packing of E protein dimers on the virus surface. Antibody binding not only removed the exchange regime in the epitope region, in a process reminiscent of conformational selection, but, somewhat surprisingly, also caused exchange in other parts of DIII through allosteric effects.

We also studied the modulation of dynamics in allergens from Bet v 1 family observed upon ligand binding and the importance for antibody-epitope complex formation. We analyzed the dynamics in the free and ligand-bound state of Bet v 1 and Fag s 1, in the presence and absence of antibodies. Our data suggest that the decrease in conformational exchange upon ligand binding and no change in the structure of epitope may modulate antibody affinity revealing that the ligand-binding cavity of the allergen and the related changes in structural dynamics, as a key structural element for allergenicity.
Preformed structural elements within the structural ensemble of the intrinsically disordered dsRBD-1 domain from Arabidopsis thaliana DCL1

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DCL1 is the ribonuclease that carries out miRNA biogenesis in plants. The two double stranded RNA binding domains (dsRBDs) located in tandem at the C-terminus are essential for the function of the protein. We have previously found that the first domain, DCL1-A, is intrinsically disordered but it binds its substrate pri-miRNA while acquiring a canonical dsRBD fold, and we described the mechanism of binding induced folding of this domain [1]. We have further shown that DCL1-A recognizes mismatched base pairs in the substrate [2].

The existence of a stable disordered form in DCL1-A enables access to the study of the unfolded state without the need of destabilizing the fold by means of denaturants or mutations, making it a good model of a binding-induced folding event. We present here a detailed structural analysis of the free form of DCL1-A. Analysis of spectroscopic data shows that the free disordered form shows tendency to adopt folded conformations, and goes through an unfolded bound state prior to the folding event. Using chemical shifts, RDCs, PREs and hydrodynamic radius measured on the protein we generated a structural ensemble that represents the conformational space sampled. The ensemble reveals preformed structural elements with restrained flexibility that could be essential to drive binding and subsequent folding of the protein on its target RNA. We discuss the importance of the residual structure present in the unfolded state in the folding pathway of the protein.

References

High-Sensitivity Rheo-NMR Spectroscopy for Protein Studies

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Shear stress can induce structural deformation of proteins, which might result in aggregate formation. Rheo-NMR spectroscopy has the potential to monitor structural changes in proteins under shear stress at the atomic level; however, existing Rheo-NMR methodologies have insufficient sensitivity to probe protein structure and dynamics. Here we present a simple and versatile approach to Rheo-NMR, which maximizes sensitivity by using a spectrometer equipped with a cryogenic probe. As a result, the sensitivity of the instrument ranks highest among the Rheo-NMR spectrometers reported so far (Fig. 1).

![Fig. 1. the Rheo-NMR instrument (left) with an enlarged view of the geometry of the NMR sample tube (right).](image)

We applied the newly established Rheo-NMR instrument to study the fibril formation of an aggregation-prone protein. Although the formation of amyloid fibrils from intrinsically disordered or chemically unfolded proteins has been well studied, the conformational conversion of folded proteins into fibrils remained elusive. Therefore, it is of vital importance to investigate changes in the natively folded structure at the atomic level. Previously, we found that M1-linked hexa-ubiquitin forms amyloid fibrils upon the application of shear stress[1]; however, the structural changes that take place during fibril formation remain unclear. Indeed, application of shear stress inside the Rheo-NMR Couette cell resulted in the formation of amyloid-like fibrils of hexa-ubiquitin. We observed changes in the chemical shift of cross-peaks in real-time ¹H-¹³C HSQC spectra. The affected cross-peaks correspond to surface-exposed residues of ubiquitin subunits in hexa-ubiquitin.

References

DIFFUSION NMR FOR IONIC LIQUID ELECTROLYTE DYNAMICS IN GAS DIFFUSION ELECTRODE FRAMEWORKS

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Understanding the dynamics of fluids in porous gas diffusion electrode (GDE) frameworks is crucial for the development and performance enhancement of batteries, fuel cells and electrolyzers since these processes constitute a major bottleneck for reaction kinetics, ion and electron transport [1]. With the advent of ionic liquids (IL) a new class of “green solvents” has been introduced with the potential to replace classical aqueous electrolytes. IL’s are liquid salts, solely composed of ions; causing complex diffusion processes in porous hosts that can be contradictory to what is commonly exhibited by fluids. Moreover, properties of IL’s are difficult to predict, since theories for property estimations usually do not compensate strong coulomb effects as observed in IL’s.

In this contribution the dynamics of pyrrolidinium and choline based ionic liquid electrolytes confined to electrospun carbon cathode frameworks were assessed by PGSTE-NMR and T₁ measurements. The results indicate different IL mobility regimes/environments inside the GDE framework with moderate exchange between the environments. Furthermore, diffraction of diffusion patterns were found for certain IL confined to the GDE framework. Therefore, the classical approach for the interpretation of NMR in porous media [2] needs to be rethought for electrospun GDE frameworks. Nevertheless, diffusive diffraction can serve as a proxy for the identification of topological features in the fibrous material.

Based on our findings, the potential and limitations of PGSTE-NMR for studying the dynamics of ionic liquid electrolytes in GDE frameworks were discussed and evaluated.

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References
IN VIVO/IN-CELL / SOLID-STATE NMR BIOLOGICAL APPLICATIONS/ METHODS

Wednesday, July 26
10:30-12:00

Isabelle Marcotte
Solid-State NMR of Intact Cells - Approaches and Challenges

Lynette Cegelski
Composition and Architecture in Intact Bacterial Cells, Cell Walls and Biofilms

Frances Separovic
Solid-State NMR Study of Live Bacteria in the Presence of Antimicrobial Agents

Lucia Banci
Advancements and Applications of In-Cell NMR
Solid-state NMR of intact cells – approaches and challenges

Alexandre A. Arnold¹, Marwa Laadhari¹, Zeineb Bouhlel¹,², Jean-Philippe Bourgouin¹, Frances Separovic³, Dror E. Warschawski¹,⁴ and Isabelle Marcotte¹*

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The high level of complexity of intact cells is a challenge to their molecular level study by solid-state NMR. Yet, NMR can provide valuable insights on the structure and dynamics of cell constituents with no alteration of cell integrity. This information is of great importance because it can contribute to better understand the biological function as well as the role of cell constituents, such as the cell membrane for example in the action of drugs and contaminants. We will present solid-state NMR approaches to study intact cells that rely on specific and unspecific isotopic labelling. We will first present the study of the interaction of antimicrobial agents with bacterial cell membranes. In this case, the specific deuteration of membrane lipids’ chains was combined to ²H solid-state NMR and magic-angle spinning to refine the action mechanisms of antimicrobial peptides caerin 1.1 and aurein 1.2 on E. coli and B. subtilis, and the microalgal pigment marennine on the marine bacteria V. splendidus. We will then present the case of non-specific labelling, where NMR strategies were introduced to selectively focus on unique constituents of fully ¹³C labelled C. reinhardtii microalgae. These approaches, which rely on differences in the excitation profiles and relaxation parameters of the different constituents, allowed distinguishing the cell wall, starch, and lipids in whole C. reinhardtii cells. A platform is thus established to study changes or perturbations of intact microalgae as a function of their environment.
Composition and Architecture in Intact Bacterial Cells, Cell Walls and Biofilms

The bacterial cell wall is essential to cell survival and is a major target of antibiotics. Beyond the cell surface, bacteria assemble complex macromolecular architectures during biofilm formation. Biofilms are implicated in serious infectious diseases and have emerged as a target for anti-infectives. Our research program is inspired by the challenge and importance of elucidating chemical structure and function in these complex biological systems and we strive to transform our discoveries into new therapeutic strategies. We have introduced uniquely enabling approaches using whole-cell and macromolecular solid-state NMR, integrated with new protocols for microscopy and biochemical analysis, in order to reveal how the physical properties and biological functions of cell walls and biofilms depend on their chemical composition and architecture.

I will report on our major discoveries including the determination of bacterial cell-wall components that must be measured in the whole-cell context by whole-cell NMR and the determination of composition of intact biofilm extracellular matrices. In our NMR analysis of the E. coli extracellular matrix, we made the unanticipated discovery that the polysaccharide portion consists of a chemically modified form of cellulose. We identified the genetic determinants for installation of the modification and established that the modification is required for community phenotypes. Thus, solid-state NMR analysis of the intact polysaccharide was able to identify this biologically important modification that is produced naturally in Nature and evaded detection by conventional approaches. This discovery has implications for understanding bacterial biofilm assembly and presents opportunities for biofilm inhibition and for the generation of new cellulosic materials.
Solid-State NMR Study of Live Bacteria in the Presence of Antimicrobial Agents

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Antimicrobial peptides (AMPs) have been extensively studied as promising alternatives to traditional antibiotics. Solid-state NMR has been used to characterise their effect on lipid bilayers, which are the primary target. Such studies are used to provide high-resolution details within a relatively controlled and homogenous system, but correlation with in vivo situation is tentative, especially in view of the complex modulations observed with slight changes in sample conditions (such as pH, temperature, lipid composition or peptide concentration). Studying AMPs in live bacteria is, therefore, appealing but challenged by the inhomogeneous and intricate cell architecture. We have used $^{31}$P solid-state NMR to study the impact of the AMP maculatin 1.1 on live E. coli and S. aureus bacteria. A combination of dynamic filtering coupled with paramagnetic reagents was used to identify signals from different molecular species such as nucleic acids, lipids or inorganic phosphate. At peptide concentrations below the minimum inhibition concentration (MIC), a significant impact on the DNA packing of E. coli and S. aureus was observed, which previously has not been reported. Interestingly, the peptide effect on S. aureus was significantly less, particularly on the phospholipid signals, despite having a lower MIC than E. coli. Furthermore, peptide treatment in E. coli induced the production of a new phosphorus signal, consistent with a phosphonate species. Overall, an extensive solid-state $^{31}$P NMR study of live bacteria and, although in an early stage of development, in-cell DNP-NMR of labelled peptides will be reported to provide new insights into bactericidal mechanisms of AMPs.
Advancements and applications of in-cell NMR

Lucia Banci – CERM and Department of Chemistry, University of Florence, Sesto Fiorentino, Italy

One of the most challenging and of higher impact applications of magnetic resonance is in-cell NMR, i.e. collection of high resolution NMR spectra of biomolecules in intact, living cells. This type of experiments allows us to obtain information on the conformational and functional properties of biomolecules at atomic resolution in conditions as closer as possible to the physiological ones.

A few examples of the striking power of this approach will be presented for a few systems and functional aspects, with particular focus on the meaningful differences in biomolecules properties in living cells with respect to the in vitro features. Methodological aspects and innovations will be also discussed, with particular focus on making this type of measurements of broader and broader applicability.


Barbieri L, Luchinat E and Banci L. Protein interaction patterns in different cellular environments are revealed by in cell NMR. Scientific Reports 5:14456: DOI: 10.1038/srep14456, 2015.


SOLID-STATE NMR MATERIALS/ METHODS

Wednesday, July 26
10:30-12:00

Hellmut Eckert
Modern Solid State NMR Spectroscopic Strategies for Structural Studies of Disordered Materials

David L. Bryce
Dynamics and Equivalence from J Splittings Associated with Pairs of Quadrupolar Nuclei in Solids

Dinu Iuga
Homo and Hetero-Nuclear Correlation Experiments Involving Quadrupolar Nuclei

Danielle Laurencin
Mechanochemistry: A Unique Opportunity for $^{17}$O NMR Spectroscopy
Disordered materials, such as defective crystals, glasses, and nanocomposites are of great importance in materials science and technology, as key functional properties are directly linked to the lack of translational symmetry. Designing the physical properties of materials to technological demands requires detailed knowledge of their structural and dynamic properties. For the investigation of the solid state, nuclear magnetic resonance (NMR) is an ideal complement to the various diffraction (x-ray, synchrotron, neutron) techniques, by its specific property of being element-selective, inherently quantitative as well as selective to the local environment. An additional strength of the solid state NMR approach lies in the opportunity of tailoring the effective Hamiltonian by manipulations in physical space (magic angle sample spinning) or spin space (multi-dimensional NMR), offering a toolbox of complementary selective averaging experiments.

A particularly powerful NMR approach towards structural elucidation is based on the site-resolved measurement and quantitative analysis of internuclear magnetic dipole-dipole interactions, which can be translated into distance information in a straightforward manner. This approach can give detailed insights into internuclear connectivities, spatial distributions, and intermolecular interactions in disordered states of matter, such as inorganic frameworks, glasses, ceramics and nanocomposites. Applications of this principle will be presented in the area of crystalline, glassy, and glass-ceramic solid electrolytes.
**Dynamics and Equivalence from $J$ Splittings Associated with Pairs of Quadrupolar Nuclei in Solids**

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We describe the implementation and application of two-dimensional double-quantum filtered $J$-resolved solid-state NMR experiments as applied to homonuclear pairs of quadrupolar nuclei. For non-equivalent spin pairs, the spectral splitting in the indirect dimension is equal to the value of the $J$ coupling constant. For magnetically equivalent spin pairs, the splitting is augmented relative to the true value of $J$ by a factor related to the principal spin quantum number [1]. Several applications of the experiment are described.

Firstly, one can distinguish between crystallographic symmetries in related compounds featuring, e.g., $^{11}\text{B}$ spin pairs, by interpreting the magnitude of the $^{1}J(^{11}\text{B},^{11}\text{B})$ spectral splitting [2]. Conversely, if the crystallographic relationship between the spins is known independently (e.g., from diffraction methods), one can interpret the values of $^{1}J(^{11}\text{B},^{11}\text{B})$ with the aid of natural localized molecular orbital theory to provide insight into the nature of the B-B bond. We describe results for the singly-bonded diboranes, doubly-bonded diborenes, and triply-bonded diborynes [3]. Insight into the elusive nature of the digallane, digallene, and purported digallyne bonds is obtained through measurements on stationary powders for which the $^{71}\text{Ga}$ quadrupolar interaction is orders of magnitude larger than $^{1}J(^{71}\text{Ga},^{71}\text{Ga})$ [4].

As a final example, we demonstrate the influence of molecular dynamics on the spectral splittings obtained for a series of synthetically important electron-precise dianionic diboranes featuring two-centre two-electron bonds [5]. In selected cases, non-equivalent boron pairs are rendered magnetically equivalent on the timescale of the $J$-resolved experiment, resulting in unexpectedly amplified spectral splittings. Careful interpretation of this phenomenon has provided a new tool for probing molecular dynamics of solids. The measured values of $^{1}J(^{11}\text{B},^{11}\text{B})$ in these compounds, in concert with DFT computations, provide new insight into their electronic structure.

In summary, if one has independent knowledge of the symmetry relationship between two quadrupolar spins, the observed effect can be used to detect the presence or absence of dynamics in the system. Or, if one has independent knowledge of the presence or absence of dynamics, the effect can be used to provide information on the crystallographic equivalence or non-equivalence of the spin pair.

**References**


Homo- and heteronuclear correlation NMR spectra are an abundant source of information in the quest for structural constraints, hence their diversity and popularity. However, for quadrupolar nuclei these experiments are challenging due to the presence of the quadrupolar interaction. Despite difficulties, sufficient progress has been recently reported that homo and heteronuclear correlations involving quadrupolar nuclei are more often used for structural characterization of complex materials. Such sequences have also been combined with two dimensional isotropic sequences (like MQMAS) in tri-dimensional experiments such that isotropic homonuclear correlation can be obtained. Numerical simulations of two coupled quadrupolar nuclei reveal several peculiarities, such as compensation of the first order quadrupolar interaction between the two single quantum transitions involved in a double quantum coherence. However, numerical simulation can be performed on each quadrupolar orientation independently and this gives the possibility to group the orientations in a statistical way such that the overall result can be better analysed and optimized. Boosted recently by the availability of higher magnetic fields and faster spinning speeds, heteronuclear sequences have been demonstrated. In this presentation the statistical approach used to investigate DQ homonuclear correlation experiments and 13C-35Cl REDOR distance measurements is extended for the study of 1H-35Cl HMQC experiment. The effect of quadrupolar interaction (with and without WURST saturation of the satellite transitions) on the 1H recoupling scheme (SR4, R3 and others) can be quantified such that the inverse detected lineshape can be simulated.

Fig 1. Top: Dependence of 1H-35Cl SR4 recoupling on Quadrupolar orientation. Bottom: Example of second order quadrupolar lineshape of 35Cl.

References:
5. We thank Dr. Aaron Rossini for sharing with us his work on Proton Detection of MAS Solid-State NMR Spectra of Half-Integer Quadrupolar Nuclei
6. The 1H-35Cl work is performed in collaboration with Ms. Emily Corlett and Professor Steven Brown
Mechanochemistry: a unique opportunity for $^{17}$O NMR spectroscopy

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Although $^{17}$O NMR has long been the object of much attention, it intrinsically suffers from a very poor sensitivity, the natural abundance of $^{17}$O being only 0.04% [1]. In most situations, $^{17}$O-labelling of the molecules or materials of interest is thus necessary. This is a major issue, because the protocols which are the most frequently used for $^{17}$O-enrichment purposes are time-consuming (lasting several hours or days), costly (using excessive amounts of expensive $^{17}$O-labelled precursors) and/or constraining (involving hazardous reagents or harsh experimental conditions).

In this presentation, we will show that mechanosynthesis is uniquely suited for the $^{17}$O-labelling of a wide variety of organic and inorganic compounds of interest [2]. The protocols we have developed are fast, cost-efficient and user-friendly, and also very well adapted to $^{17}$O NMR applications (Fig 1).

Fig. 1. Illustration of the advantages of ball-milling for $^{17}$O-labelling purposes.

References
UNUSUAL

Wednesday, July 26
10:30-12:00

Marcel Utz
Integration of NMR Spectroscopy with Microfluidic Devices for the Study of Live Systems

Sami Jannin
Transportable Hyperpolarized Metabolites

Herve Desvaux
Introducing Nuclear Spin-Noise Spectroscopy

John W. Blanchard
NMR Meets Dark Matter: The Cosmic Axion Spin Precession Experiment (CASPEr)
Integration of NMR Spectroscopy with Microfluidic Devices For The Study Of Live Systems

Marcel Utz*

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NMR is arguably the most versatile tool available to study live systems. It is non-invasive and provides a very high degree of chemical resolution. NMR spectroscopy and imaging are widely used for metabolic profiling and in-situ studies of life processes.

On the other hand, microfluidic lab-on-a-chip technology has made great strides to provide artificial environments that support life systems such as cells, cell spherulites, tissue samples, and small organisms under highly controlled experimental conditions.

Combining the rapidly emerging LoC technology with NMR observation is an attractive development goal for the NMR community, but one that holds considerable challenges. While NMR is inherently less sensitive than other spectroscopic techniques, its mass sensitivity (Signal/Noise ratio per spin) scales favourably with smaller detector sizes [1]. As other authors and our own work have shown, this can be exploited to design miniaturised NMR detectors that provide sufficient sensitivity to study metabolic processes at sample volumes around 1 µl.

We have designed a transmission line NMR detector, which consists of two planar conductors separated by a gap of 1 mm. Microfluidic LoC devices can be inserted into this gap [2]. We have shown recently that this device can be used to follow the metabolism of a culture of about 2000 human cancer cells (MCF-7) over periods of 48h.

In this presentation, general design principles for integrated NMR/LoC systems will be discussed, along with specific solutions and applications to the culture of cells and tissue slices.

References

Transportable Hyperpolarized Metabolites

Xiao Ji\textsuperscript{1}, Aurélien Bornet\textsuperscript{2}, Basile Vuichoud\textsuperscript{2}, Jonas Milani\textsuperscript{2} David Gajan\textsuperscript{2}, Aaron J. Rossini\textsuperscript{1}, Lyndon Emsley\textsuperscript{1}, Geoffrey Bodenhausen\textsuperscript{1,3,4,5} and Sami Jannin\textsuperscript{*}\textsuperscript{2}

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Nuclear spin hyperpolarization by dissolution dynamic nuclear polarization (d-DNP)\textsuperscript{[1]} can be used to enhance the signals of \textsuperscript{13}C-labeled metabolites by several orders of magnitude. Given the short lifetimes of hyperpolarized magnetization (typically, \(T_1^{(13}C) < 50\) s), d-DNP must be carried out close to the NMR or MRI apparatus. Removing solid hyperpolarized samples from the polarizer normally results in complete loss of hyperpolarization, since \(T_1^{(13}C)\) becomes prohibitively short at low magnetic fields and high temperatures because of the proximity of the \textsuperscript{13}C spins to the statistically dispersed polarizing agents.

Here, we introduce a concept to dramatically extend the lifetimes \(T_1^{(13}C)\) from seconds to hours or even days. The hyperpolarized sample can now be removed from the polarizer, stored and transported to remote MRI or NMR sites. This is achieved by suitable microparticulate sample architecture. Hyperpolarization is relayed to the protons of the metabolites by proton-proton spin diffusion and is transferred from \textsuperscript{1}H to \textsuperscript{13}C by cross polarization (CP).\textsuperscript{[2]} Since the \textsuperscript{13}C spins are effectively isolated from the polarizing agents they remain hyperpolarized for prolonged periods, typically hours or even days.

As a proof of concept we show that \textsuperscript{13}C hyperpolarization in alanine and glycine can endure storage and transport for at least 16 hours, resulting in a final enhancement up to three orders of magnitude after ‘remote’ dissolution. This is to our knowledge the first demonstration of remote hyperpolarization of small molecules by d-DNP.\textsuperscript{[3]}


This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement HP4all n° 714519)

\textbf{Figure 1. Remote DNP concept.}
NMR spectra are usually obtained by exciting, through a rf field, the nuclear magnetization and then by monitoring the induction, it creates. An alternative approach, named spin noise, exists: it consists in searching for correlations in the noise signal at the probe detection output, a concept up to now used for only a single spin species [1]. Here, we report its extension for looking to small signals in the presence of a major one and show that this technique allows sensitivity enhancement for their detection, in particular when the temperature of detection coil is lower than that of the sample. Signals resulting from small species appear as bumps, superimposed on the dip which results from the main component contribution.

For the description and the processing of the experimental spectra, a new analytical equation is introduced. Its derivation is based on fluctuating rf fields due to the preamplifier and coil resistances and magnetization fluctuations and coherent rf field due to the feedback field (radiation damping). Its generality allows analytical explanation of the difference of tuning conditions [2,3] and the treatment of static magnetic field inhomogeneity, which has an enhanced spectral signature in spin-noise spectra and provides a chemical shift reference.

We have combined all these aspects and proved the enhanced detection capability of the nuclear spin-noise approach for characterizing secondary isotopic effect [4], proving the opening of a new "spin-noise" spectroscopy. Perspectives of this work in terms of classical NMR in the presence of radiation damping but also in terms of unconventional detection scheme will be discussed.

Fig. 1. Numerical simulation of a pulsed (A) and spin-noise spectra (B) for a spin system including three species: a main one and two minority components shifted by ± 5 Hz with a relative intensity of 0.5%. (A) The main line is broadened and shifted by feedback field and secondary contributions are essentially undetectable. (B) the nuclear spin-noise spectrum unambiguously shows fine and well resolved peaks related to minority components.

References
NMR Meets Dark Matter: The Cosmic Axion Spin Precession Experiment (CASPEr)

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The nature of dark matter is one of the most important open problems in modern physics. Axions (originally introduced to resolve the strong CP problem, related to the imbalance between matter and antimatter in the universe), or axion-like particles are strongly motivated dark matter candidates, but are difficult to detect experimentally. The Cosmic Axion Spin Precession Experiment (CASPEr) [1] uses NMR techniques to detect spin precession induced by background axion dark matter.

CASPEr is naturally divided into two main efforts, based on the two relevant couplings between axions and nuclear spins [2]: CASPEr-Wind searches for the “axion wind” effect – the direct coupling of nuclear spins to the relative velocity of the axion field, and CASPEr-Electric searches for the oscillating nuclear electric dipole moment caused by the QCD axion. A general picture of the CASPEr concept is shown in Fig. 1. Under appropriate experimental conditions, both axion couplings behave analogously to RF magnetic fields, in that they induce measurable spin precession if the frequency of oscillation of the axion field (corresponding to the axion mass) is equal to the nuclear Larmor frequency. As such, CASPEr is essentially a CW-NMR experiment where the field is swept from 0—14.1 T in order to search for transverse nuclear magnetization produced by the axion pseudo-RF field.

In this presentation, we will discuss the experiment and technical developments from an NMR perspective. In particular, we will consider methods for maximizing experimental sensitivity via sample hyperpolarization and the implementation of sensitive detection techniques. We will also address applications of the experimental design to conventional (“non-exotic”) NMR.

Fig. 1. Cartoon of the CASPEr concept, and projected sensitivity of CASPEr-Wind, reaching unexplored parameter space beyond current laboratory and astronomical limits.

References

EPR

Wednesday, July 26
10:30-12:00

P. Chris Hammel
Magnetic Resonance Studies of Spin Transport and Dynamics of Collective Spin Excitations

Peter C. Maurer
Nanoscale Control of Biological Systems by Quantum Sensing

Uwe Gerstmann
Radio Frequency STM-Spectroscopy for Single-Molecule Spin Resonance - Experiment & Theory

Jason W. Sidabras
Resonator Developments for Studying Protein Single Crystals of Limited Dimensions at X-Band
We report studies of angular momentum transport and magnetic dynamics in diverse insulating materials. We have shown that spin transport is exponentially suppressed by insulating diamagnetic barriers, but we find that collective spin excitations in various materials can enable robust spin transport in insulators. We present studies that reveal efficient spin transport in Yttrium Iron Garnet (YIG) even in the presence of magnetic-field defined barriers that require inter-conversion between magnons of dissimilar energy. Optical detection of Ferromagnetic Resonance (FMR) in YIG by means of nitrogen-vacancy (NV) defect centers in diamond reveals the role of spin waves in this dipole-mediated spin transfer process and presents a powerful approach to broadband, spatially resolved FMR detection for these and related studies. We find that fluctuating antiferromagnetic (AF) spin correlations also enable efficient spin transport having decay lengths approaching 10 nm in insulating antiferromagnets. While the spin decay length increases with the strength of the AF correlations, AF magnon spin transport is robust against the absence of long-range order. This research performed in collaboration with F.Y. Yang, V.P. Bhallamudi, C.H. Du, R. Adur, H.L. Wang, C.S. Wolfe, A.J. Berger and S.A. Manuilov, and is supported by the U.S. DOE through Grant DE-FG02-03ER46054, by the NSF MRSEC program through Grant~1420451 and by the Army Research Office through Grant W911NF-16-1-0547.
Nanoscale control of biological systems by quantum sensing

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Quantum control of nanoscale systems under ambient conditions has wide-ranging applications from quantum computation and communication to precision measurements and sensing. In this talk I will introduce a novel metrological technique that allows for the investigation of biological questions at an unprecedented level of accuracy, by controlling individual electron spins that are associated with Nitrogen Vacancy (NV) color centers in diamond.

I will start by discussing how the toolbox of quantum optics and coherent control has realized a NV-sensor capable of precise temperature measurements with tens of nanometers spatial resolution. Combining such a nano-thermometer with laser-induced heating of plasmonic nanoparticles further allows for an external control of a temperature profile at a nanoscale. To demonstrate a biological application of our technique, we injected nanoparticles into living cells and showed that a temperature gradient can be maintained with sub-cellular resolution, resulting in alteration of basic cellular functions. I will discuss additional bio-applications of our technique, which enable us to study a variety of biological processes including embryogenesis in a developing \textit{C. elegans} worm.

Finally, I will give an outlook on how crystallographic defects in nanoparticles can not only be utilized for sensing but also offer new possibilities for fluorescent imaging. Specifically, I will introduce a multicolor electron microscopy modality that can visualize the absolute location of proteins within the context of cellular ultrastructures with up to ten nanometer resolution and five different colors. Finally, the biophysics concepts developed for nanoparticle passivation, functionalization and labeling can possibly be transferred to other fields, including NV-based nanoscale magnetometry.

Radio frequency STM-spectroscopy for single-molecule spin resonance – experiment & theory

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Recently, new techniques of radio-frequency (rf) modulated scanning tunneling microscopy have been introduced by Müllegger et al. [1] and Baumann et al. [2] allowing subnanometer spatial resolution combined with single-spin sensitivity. By utilizing resonant rf currents, spin transitions can be induced in single magnetic atoms adsorbed on isolating films [2] as well as in single molecules directly adsorbed on metallic substrates [1]. As a prototype example for such a molecular spin quantum dot, the archetypal terbium double-decker phthalocyanine molecule (TbPc₂) directly attached to a Au(111) substrate has been used.

At readily accessible magnetic fields (few mT) and temperatures (5 K) efficient spin transitions beyond the electromagnetic dipole selection rules (up to ΔJz=±12 and ΔIz=±3) can be probed. With the help of density functional theory (DFT) calculations, we show that electron tunneling via magnetic molecular orbitals is crucial for the rf-STS-based spin excitation [3], whereby subsequent charge transfer to the metallic substrate plays also an important role. Most of the observed high-spin excitations are far away from avoided level crossings, ruling out a dominant role of quantum tunneling of magnetization. The intriguing evidence for efficient higher spin excitations in rf-STS points towards an involvement of mechanical degrees of freedom for fulfilling the fundamental angular momentum conservation [4].

Fig. 1. (left, [1]:) Scheme of molecular high-spin excitation: from the magnetic tip, electrons are tunneling into molecular orbitals of the TbPc₂ molecule followed by charge transfer to the metallic Au(111) substrate. During the rf-induced recharging, the TbPc₂ molecular magnet is strongly deformed (right, [3]).

References
We present the development of micro-resonators for X-band (9.5 GHz) Electron Paramagnetic Resonance (EPR) for volume-limited samples [1]. Such samples have nanoliter volumes or limited crystal geometries that minimize their effective size. In order to increase the signal-to-noise ratio (SNR), one must design new probes that concentrate the magnetic field over a finite sample space. This work has immediate applications in the study of protein single crystals of hydrogenases. Single crystal EPR experiments of these enzymes are highly informative and may be used to resolve g- and hyperfine tensors of the active site and relate the electronic structure to the protein geometry [2].

Two challenges exist in micro-resonator design: i) magnetic field inhomogeneity over the sample volume and ii) degraded Q₀-value due to large surface currents and dielectric losses. We propose two geometries: A sapphire substrate Planar Micro Resonator (PMR) with a 0.5 mm ID omega-loop shaped geometry and a self-resonant micro-helix design with a 0.4 mm ID are the focus of this work. The magnetic field profile of the 0.5 mm PMR and 0.4 mm micro-Helix are shown in Fig. 1A and 1B, respectively. Micro-Helix geometry is encouraging with a larger Q-value, resonator efficiency, and a more homogenous magnetic field compared to the PMR.

Modeling, design, and simulations were performed using the Ansys High Frequency Structure Simulator. Experimental concentration sensitivity analysis is presented as a comparison with commercial EPR probes. Finally, spectra of the tyrosine radical YD in Photosystem II from a 0.3 x 0.3 x 0.4 mm³ single crystal are presented as a benchmark for future studies.

References
PLENARY SESSION 7

Thursday, July 27
08:30-09:15

Tatyana Polenova
Structure and Dynamics of HIV-1 Capsid Assemblies: Insights from an Integrated Approach
Structure and Dynamics of HIV-1 Capsid Assemblies: Insights from an Integrated Approach

Tatyana Polenova
University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716

HIV-1 capsids, assembled from ~1,500 copies of the capsid (CA) protein, are an integral part of mature virions [1, 2]. Conical in shape, capsids enclose the viral genetic material (two copies of RNA) together with several proteins that are essential for viral replication. In the assembled state, capsids are remarkably dynamic, with the CA residue motions occurring over a range of timescales from nano- to milliseconds [3-6]. These motions are functionally important for capsid's assembly, viral maturation, and interactions with host factors [3, 6]. In this talk, an integrated MAS NMR, DNP, MD, and DFT approach will be presented to probe the functionally important motions in assemblies of CA and their complexes with host factors Cyclophilin A (CypA) and TRIM5α, as well as assemblies of CA-SP1 maturation intermediates. The role of dynamic allosteric regulation in capsid’s assembly, maturation, and escape from the CypA dependence will be discussed. It will be demonstrated that the integration of experimental NMR and DNP methods and theory, at classical and quantum mechanical levels, yields quantitative, atomic-level insights into the dynamic processes that govern the capsid’s function.

References:
PLENARY SESSION 8

Thursday, July 27
09:15-10:00

Jiangfeng Du
Spin Magnetic Resonance Spectroscopy from Billions of Molecules to Single Molecule
Spin Magnetic Resonance Spectroscopy
from Billions of Molecules to Singe Molecule

Jiangfeng Du

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Magnetic resonance (MR) is one of the most important techniques on both of science and applications. Conversional magnetic resonance methods need billions of uniform molecules on centimeter-scale to accumulate large enough signal-to-noise ratio. Single-molecule magnetic resonance spectroscopy and imaging is one of the ultimate goals in magnetic resonance and will has great applications in a broad range of scientific areas, from life science to physics and chemistry.

A sensor to accomplish single molecule detection is the recently developed atomic sized magnetic field sensor based on the nitrogen-vacancy (NV) defect center in diamond. By combining the quantum controls and long coherence time of NV, single molecule electron/nuclear spin magnetic resonance spectroscopy have been accomplished. Along this line, we and co-workers have successfully obtained the first single-protein spin resonance spectroscopy under ambient conditions [1], realized atomic-scale structure analysis of single nuclear-spin clusters in diamond [3], detected nuclear magnetic resonance spectroscopy with single spin sensitivity [4], and succeeded in detection of (5nm)³ hydrogen nuclear spin magnetic resonance spectroscopy [5].

Recently, we not only detect the nuclear magnetic resonance spectrum of nanoscale-ice located on diamond surface, but also resolve the intra-water-molecule magnetic dipole interaction on ~20kHz. The spectrum resolution is 5 kHz by using correlation methods. The structure have been resolved by the analysis of spectrum. Furthermore, the methods developed on diamond-based MR can also be extended to conversional electron spin resonance. We first prolonged the coherence time of a molecular qubit beyond a milliseconds (~1.4 ms) by dynamical decoupling. Then we utilized this molecule qubit as a quantum probe to sense its nearby environmental noise. Nuclear spins, such as ¹H
and $^{31}$P, have been resolved.

References:


IES GOLD MEDAL

Thursday, July 27
10:00-10:30

Daniella Goldfarb
Combining High Field EPR with Metal Ions Spin Labeling - A Gold Mine?
Combining high field EPR with metal ions spin labeling - a gold mine?

Daniella Goldfarb

Department of Chemical Physics, Weizmann Institute of Science, Israel

The IES Gold Medal Award for 2017 is awarded to Prof. Daniella Goldfarb for her contributions to development of high-field pulsed EPR techniques and their applications to materials and biological research. Daniella Goldfarb received her PhD in 1984 from The Weizmann Institute of Science under the Supervision of Prof. Z. Luz. Her EPR career began with a postdoctoral fellowship in laboratory of Prof. L. Kevan in the University of Houston, Texas.

In 1987, Daniella began her independent scientific career at the Weizmann Institute, where she was quickly promoted through the ranks to become a full professor in 1998. Daniella’s research resulted in important understandings and advances in high-field pulsed EPR and ENDOR that included high-sensitivity and in-cell nanometer distance measurements. The developed methodology was successfully applied to unravel structure and mechanisms of various biomolecular systems and to obtain crucial insight into formation mechanism of porous materials.

Daniella is the author of more than 200 scientific publications.
SOLUTION NMR

Thursday, July 27
11:00-12:00

Christian Griesinger
Label-Free NMR-Based Binding Kinetics Determination

Roberta Pierattelli
Just Flexible Linkers?
Label-Free NMR-Based Binding Kinetics Determination

P. Trigo-Mourino,¹ D. Lee,¹,² C. Griesinger.¹,*

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Protein functions can be accurately modified with small-molecule ligands. In addition to the equilibrium binding affinity of such ligands to biomacromolecules the kinetics and lifetime of the complex formation are of interest. Indeed, there is now growing experimental evidence linking ligand efficiency with ligand-receptor complex lifetime (τ), raising the interest in the determination of dissociation rate constants (k_{off} = 1/τ).[1]

Recently, the High Power Relaxation Dispersion (RD) technique has been developed in our group, allowing the detection of single-digit-microsecond chemical exchange events.[2] Here we present the use of a 1D $^1$H $R_1\rho$ experiment on unmodified ligands, from which binding dissociation can be easily extracted.[3] This method has advantages over previously proposed CPMG RD techniques.[4] First, $^1$H spectra does not require expensive, and unpractical, isotope-labeled samples; second, the experimental time is significantly shorter; third, the $^1$H nucleus is differently sensitive from $^{13}$C to the conformational state, e.g. rotameric states, reducing the weight of bound ligand slow dynamics (if any) that might obscure the binding event; and fourth, the use of $R_1\rho$ experiment instead of CPMG makes possible the use of any multiplet from the ligand due to the absence of $J$ coupling modulation during the spin-lock period. Additionally, the use of the High Power RD approach improves the detection limit from residence times of 500 µs to the single digit µs.

Fig. 1. Relaxation Dispersion profiles of Epothilone A in the absence (red, flat line) and in presence (blue) of tubulin heterodimers. The solid lines indicate the individual fit of each resonance. Globally, both resonances fitted to a k_{off} = 6.88 ± 0.47 10^3 s⁻¹, which translates into a half-life τ = 14.52 ± 0.93 µs.

We show the applicability of our method with different small-molecule binding to two different target systems: the soluble bovine serum albumin and stabilized microtubules. The ligands we chose show no dispersion when free in solution. In both cases, upon the addition of the receptor, significant dispersion profiles were detected. Importantly, distant protons within the ligands could be fit to the same kinetic parameters.

References
The importance of local flexibility in determining the function of proteins has been recognized long ago and also widely scrutinized. If the extent of local flexibility is taken to its extreme conditions it leads to completely random coil behaviour of a polypeptide chain, indicated as intrinsic disorder, through a wide variety of intermediate cases both in terms of extent of mobility or in terms of protein stretches involved.

In recent years many examples of intrinsically disordered proteins (IDPs) appeared in the literature showing how their structural plasticity and intrinsic flexibility can be key features to enable them to interact with a variety of different partners and to adapt to different conditions. These properties provide functional advantages to IDPs enabling them to play key roles in many regulatory processes and their function has also been related to several diseases.

The general properties of IDPs cannot be captured in ordered crystals, preventing them to be suitable targets for crystallographic studies. Thus, nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in their investigation, being the only method that allows a high resolution description of their structural and dynamic features in solution. The high flexibility has several consequences on the NMR spectroscopic parameters that, if properly handled, can give precious information.

Large proteins are often composed of several folded domains separated in the primary sequence by flexible linkers. Although structure and function of globular domains are generally well characterized, only little information is often available for highly flexible and disordered protein segments. We will present recent results demonstrating that more complex functions than expected can be ascribed to the long disordered chains connecting well-structured protein domains.

References
IN VIVO/IN-CELL

Thursday, July 27
11:00-12:00

Dror E. Warschawski
Probing Cell Membrane Surfaces by *In Vivo* 31P Solid-State NMR

Gulin Öz
NMR of the Human and Animal Brain at High Fields: Applications in Neurodegenerative Diseases
PROBING CELL MEMBRANE SURFACES BY IN VIVO 31P SOLID-STATE NMR

D. E. Warschawski$^{1,2,*}$, A. A. Arnold$^2$, I. Marcotte$^2$

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Solid-state NMR is at a stage where it can tackle whole cells and even small living organisms [1]. Focusing on membrane lipids, saccharides, cell walls and extracellular matrix, we have been able to study various bacteria and algae by 13C, 1H and 2H NMR, allowing to identify various molecules, to measure lipid order, to quantify and locate the effect of antimicrobial peptides or nanoparticles, and more generally to assess the “health” of the studied micro-organism, all by in vivo solid-state NMR [2-4].

While we are learning about the heart of the cell membrane and wall, where many processes occur, we need to develop complementary tools to study the interface between the membrane and the aqueous environment. Since 31P NMR allows to probe the state of lipid headgroups, we want to add this tool to in vivo solid-state NMR, and thereby identify any process that would involve the surface rather than the core of the membrane.

The diversity in lipid headgroups is both puzzling and an asset for 31P NMR, allowing to differentiate various lipids and probe their properties separately. We are currently adapting pulse sequences developed for measuring chemical shift anisotropies (CSA) in carbonyl carbons [5] to the measurement of 31P CSA, in model and biological membrane lipids. This allows us to assess lipid phases in lipid mixtures and in biomembranes, and will help us identify which lipid headgroup is affected, and how, when external parameters are changed, such as temperature, salinity, or the interaction with peptides or pollutants.

References

NMR of the Human and Animal Brain at High Fields: Applications in Neurodegenerative Diseases

G. Öz1,*

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Neurochemical profiles obtained by in vivo NMR are increasingly used to monitor diseases of the central nervous system [1]. The SNR and resolution advantages at high and ultra-high fields (3T and above) allows reliable quantification of neurochemical profiles that consist of up to 18 metabolites, including neuronal and glial markers, neurotransmitters and antioxidants, in brain regions that are involved in various neurological disorders. This presentation will outline the methodology we utilize to obtain high spectral quality in the human and animal brain. Given robust methodology, excellent spectral quality can be reproducibly obtained (Fig. 1), resulting in high reproducibility of neurochemical quantification, with test-retest coefficients of variance (CVs) within 5% for the major metabolites at 3T and 7T [2]. Finally, the presentation will share examples of utilizing NMR in clinical populations with neurodegenerative diseases.

Fig. 1. Reproducibility of spectral quality at 3T and 7T. Spectra were obtained using semi-LASER (TE = 28ms at 3T and 26ms at 7T, TR = 5s, 64 transients) from one healthy volunteer, who was scanned 4 times on each scanner. Each panel contains 4 overlaid spectra obtained in weekly intervals. The voxel locations are shown on the T1-weighted images acquired at 3T. Spectra were apodized with linebroadening (1 Hz) and Gaussian multiplication (σ = 0.12 s) for display purposes. PCC: posterior cingulate cortex, CBM: cerebellar vermis. Figure from [2].

References
SOLID-STATE NMR MATERIALS/
METHODS

Thursday, July 27
11:00-12:00

Patrick C. A. Van der Wel
Amyloidogenic Self-Assembly in Peptide-Based Materials and Biology:
ssNMR Methods and Applications
Amyloidogenic self-assembly in peptide-based materials and biology: ssNMR methods and applications.

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Magic-angle-spinning (MAS) solid-state NMR was used to probe the structure of human-made amyloid-based nano-assemblies formed by a gold-binding peptide/acyl conjugate [1]. The peptide-acyl conjugates form a fibrillar framework that facilitates the assembly of metal-decorated nano-structures that are of interest because of their strong chiroptical activity. By combining ssNMR data with known principles of “biological” amyloidogenicity, a surprising mode of assembly of these particular fibrillar structures is revealed. Key in this analysis is a comparison to prior lessons learned from peptide-based amyloid-like structures, studied by ssNMR and X-ray methods[2].

I will discuss how both biological principles and common ssNMR methods are useful for understanding amyloid formation processes that may then be leveraged in the rational design of human-made functionalized nanomaterials and hydrogels. I will discuss how we deploy similar approaches for ssNMR sample preparation[3] and ssNMR characterization of hydrated biological and “peptide-materials” samples. This will include a brief discussion of the potential for using ssNMR dihedral angle measurements as valuable constraints that complement more commonly used structural measurements when probing the internal structure of amyloid-like architectures (e.g. [4]).

References
EPR

Thursday, July 27
11:00-12:00

Howard J. Halpern
*In Vivo* EPR Imaging for Redox Balance, Cancer Aggressiveness and Enhance Cancer Cure

Murali Krishna Cherukuri
EPR/MRI Imaging Biomarkers to Guide Treatment in Tumor Bearing Mice

Fraser MacMillan
Integral Membrane Transport Proteins: The Role of Magnetic Resonance is Determining Substrate Binding Affinity and Conformational Dynamics

Matthew J. Lawless
DPA-DNA: A Nucleotide Independent Site-Specific Motif for Copper(II)-Based Distance Measurements in DNA by ESR Spectroscopy

Benesh Joseph
Dipolar EPR Spectroscopy of a Membrane Transporter in Native Environments
In Vivo EPR imaging for redox balance, cancer aggressiveness and enhance cancer cure

Howard J. Halpern, Matthew C. Maggio, Martyna Krzykawska-Serda, Gage H. Redler, Richard C. Miller, Eugene D. Barth, Ralph R. Weichselbaum, Boris Epel, 1Marsha R. Rosner, 2Victor M. Tormyshev

University of Chicago, Department of Radiation and Cellular Oncology USA, 1Ben May Inst. Cancer Research & Support: NIH grants P41 EB002034 and Ro1 CA098575

2Novosibirsk State University, RU

In vivo EPR imaging at 250 MHz frequencies has proven to provide new insight and capabilities for non-invasive physiologic imaging. Very low frequencies allows minimally distorted penetration into lossy living tissues. Physiology is provided by injectable reporter molecules sensitive to crucial aspects of tissue physiology. A particularly important sensitivity is that to local molecular oxygen partial pressure in the aqueous compartment of tissues. Using spin-lattice relaxation pulse EPR imaging enabled by inverse (microsecond) relaxation rate triarylmethyl radicals with extremely low toxicity, we have imaged pO2 concentrations in <1 mm voxel size images with ~ 1 torr absolute resolution. This has allowed us to image at these resolution hypoxic tumor regions defined as those with pO2 less than 10 torr. Rapidly fabricated 3D printed tungsten loaded plastic radiation blocks and an XRAD225Cx gantry mounted x-ray source has allowed treating hypoxic subvolumes of 1/3 ml tumors. This showed that hypoxic boosts trebled the cure in tumors that had been exposed to whole tumor doses sufficient to cure ~1/6 of the tumors. Similar images of triple negative breast malignancy (MDA-MB-231 cancer cells grown in nude mice has shown that invasion and metastasis promoting BACH1 also promotes tumor hypoxia. This is reversed using siRNA silencing treatment. Other aspects of tumor oxygenation has been observed using EPR imaging. A novel rapid scan technique has been used to measure the redox state of tumors as well as local pH. Other quantitative measures using EPR imaging will be discussed.
EPR/MRI Imaging Biomarkers to Guide Treatment in Tumor Bearing Mice

Murali Krishna Cherukuri
Radiation Biology Branch, NCI, NIH, Bethesda, MD, USA

The tumor microenvironment in solid tumors is characterized by regions of poor perfusion, hypoxia and low pH. Biochemically, tumor cells, both in vitro and in vivo display the aerobic glycolysis phenotype. Imaging techniques which provide biomarkers reporting on these features will be useful in: a) providing diagnostic/prognostic information; and b) developing appropriate treatments based on a priori information of the tumor microenvironment.

In this presentation, Electron Paramagnetic Resonance Imaging (EPRI) which provides non-invasively quantitative pO2 maps and metabolic MRI using hyperpolarized 13C labeled pyruvate which provides biochemical profiles of tumors will be used to probe the microenvironments of three tumor xenografts in mice to characterize their metabolic and physiologic status. These tumor bearing mice will be treated with radiation therapy or with hypoxia activated pro-drugs to evaluate the potential of the imaging biomarkers to predict treatment response.

Data will be presented to support the capability of EPRI as well as the lactate/pyruvate from 13C MRI can predict difference in the benefit from oxygen-dependent anti-tumor treatments in individual pancreatic tumor cell lines that may help properly choose the best treatment in patients with pancreatic cancer.
Integral membrane transport proteins: The role of magnetic resonance is determining substrate binding affinity and conformational dynamics

Jenny Hall, Anna Mullen and Fraser MacMillan

*Henry Wellcome Unit for Biological EPR, School of Chemistry, Norwich Research Park, University of East Anglia, Norwich, UK*

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The Henry Wellcome Unit for Biological EPR focuses on biomacromolecular protein complexes studied using a broad range of EPR techniques. This presentation will focus on the fast-moving area of membrane transporters, which are studied in collaboration with leading groups worldwide and have arisen from the EU COST Action which we lead. I will present several examples of using both NMR and EPR (especially DEER/PELDOR) to determine substrate binding affinities as well as looking at the conformational dynamics present in several systems.

1. GltPh is an homotrimeric aspartate transporter from the thermophile *Pyrococcus horikoshii*. This archaean homolog of mammalian glutamate transporters belongs to the Solute Carrier 1 (SLC1) transporter family, which also includes the human Excitatory Amino Acid Transporters (EAATs). The EAATs have been implicated in various neurological diseases including epilepsy and Alzheimer’s disease. GltPh was the first member of the SLC1 family of membrane transporters that was crystallised and has since been used to investigate the structure-function relationship of the SLC1 family of transporters. Here I will demonstrate a novel approach for the determination of $K_D$ values by Saturation Transfer Difference (STD) NMR spectroscopy to demonstrate that this STD-NMR-based method provides a fast and accurate way of determining the substrate affinity of ligands in detergent-solubilised membrane proteins.

2. Many crystal structures of membrane transporters suggest large scale conformational changes to facilitate substrate binding and transport. Often EPR reports heterogeneous mixtures of distances and therefore remains inconclusive with respect to conformational changes associated with substrate transport. Here I will demonstrate our work on several such systems, including variants with mutations introduced that are designed to lock the transporter in specific conformations, using a combination of both cw-EPR and DEER/PELDOR to deconvolute such complex mixtures of conformations.

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1. Coauthors of this presentation include: Jesus Angulo (UEA, UK), Thomas Stockner (Vienna, Austria), Thorben Cordes (Groningen, Netherlands), Christopher McDevitt (Adelaide, Australia)

2. “Understanding Movement and Mechanism in Molecular Machines” (CM1306) www.molecularmachinery.eu

DPA-DNA: a Nucleotide Independent Site-Specific Motif for Copper(II)-Based Distance Measurements in DNA by ESR Spectroscopy

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Nanometer-range distance measurements using electron spin resonance (ESR) techniques have helped to provide valuable insight into the conformation and dynamics of DNA and RNA as well as with protein-DNA interactions. In this talk I will introduce a new method of site specifically introducing a paramagnetic Cu\textsuperscript{2+} as a probe for DNA/RNA distance measurements by ESR spectroscopy \cite{1}. We have done so by utilizing a 2,2’-dipicolylamine (DPA) as an inorganic chelating nucleic acid substitute, shown in Figure 1. The DPA phosphormadite is easily incorporated into any DNA oligonucleotide in a site specific manner during initial DNA synthesis and only requires the post-synthetic addition of Cu\textsuperscript{2+}. Hence, this motif is completely structure independent which allows for distance determinations regardless of DNA/RNA primary or secondary structure. Furthermore, the DPA containing DNA (DPA-DNA) positions the spin probe within the interior of the helix. Distance measurements, shown in Figure 1, performed on the DPA-DNA resulted in a most probable distance within 1 Å of the expected helical backbone-backbone distance as analyzed by MD simulations of a corresponding natural DNA sequence. For the first time ever, ESR is able to directly report the DNA backbone distance without further analysis. This new method will be useful to understand the role of DNA structure in mediating protein-DNA interactions by direct measurements.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Left: DPA-DNA duplex. The inset shows the chemical structure of the Cu\textsuperscript{2+} binding site. Bottom right: The experimental DEER distance distribution (solid, blue) compared to distance distribution from MD simulations (dotted, black) showing agreement in most probable distance. The inset shows the background subtracted time domain DEER data (solid blue) along with fit via Tikhonov regularization (transparent black).}
\end{figure}

\textbf{References}

Dipolar EPR Spectroscopy of a Membrane Transporter in Native Environments

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³Department of Chemistry and Center for Membrane Biology, University of Virginia, USA

Determining biomolecular structures and their conformational changes at high resolution has primarily been achieved after isolating the target molecule from its native environment. This approach masks the effect of the cellular conditions, which may significantly modulate biomolecular structure and dynamics. DEER/PELDOR [1,2] is a complimentary tool for structural biology with a great potential for in-cell investigations [3]. We discuss an approach to perform distance measurements using DEER/PELDOR on membrane proteins in E. coli and native membranes. The cysteine-free nature of the outer membrane proteins and the intrinsic reduction of spin labels those cross the outer membranes in E. coli enable selective spin labeling and DEER/PELDOR spectroscopy for outer membrane proteins in whole cells and isolated outer membranes [4]. Using the cobalamin transporter BtuB, we demonstrate the application of the in-cell EPR to observe structure and conformational changes [4,5] in E. coli and to follow protein-ligand interaction using orthogonal labels in native outer membranes [6]. Also, we discuss the suitability of different spin labels for labeling of outer membrane propteins in E. coli.

Fig. 1. MTSSL labeling of BtuB in live E. coli cells. A) Spin-labeled positions (shown in space-filling model) and the loops carrying them highlighted in green using PDB 1NQH. The core domain inside the barrel is shown in red. B) X-band RT CW EPR spectra for MTSSL- labeled E. coli expressing WT or 188C BtuB. C) Left column, background corrected Q-band PELDOR data at 50K for BtuB 188R1−399R1 mutant in E. coli cells (red) or outer membrane (blue); right column, corresponding area-normalized distance distributions. D) Q-band PELDOR at 50K in trityl (TAM)-labeled native outer membranes containing BtuB 188C and TEMPO-CNCbl. E) The corresponding distance distribution obtained from Tikhonov regularization.

References:
SOLUTION NMR

Thursday, July 27
13:45-14:45

Xun-Cheng Su
Stable Paramagnetic Tags in Analysis of Structures and Dynamics of Proteins in Vitro and in Cells Using Paramagnetic NMR Spectroscopy

Jacob Anglister
Detection of Intermolecular NOEs in Small and Large Protein/Peptide Complexes

Paul R. Gooley
Characterising the Binding Mode of a Low Affinity Peptide to the Neurotensin Receptor 1
Stable paramagnetic tags in analysis of structures and dynamics of proteins in vitro and in cells using paramagnetic NMR spectroscopy

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Site-specific installation of paramagnetic lanthanide ion in proteins has been shown a powerful method in delineating the structures, dynamics and interactions of proteins by NMR and EPR. Since most proteins don’t have a paramagnetic center, efforts towards site-specific labeling of proteins with paramagnetic ions have thus been made via thiol chemistry, click chemistry, and molecular biology. The formation of disulfide bond between a protein and the paramagnetic tag is mostly applied, whereas the disulfide bond tether succumbs to low stability in reducing conditions or high pH of disulfide. We have been focusing on development of paramagnetic tagging proteins in formation of a stable thioether bond for analysis of proteins in vitro and in cells using NMR and EPR.

We show that reaction of phenylsulfonated pyridine derivatives with solvent exposed thiol groups of proteins is an efficient way of generation of stable thioether bond between protein and paramagnetic tag. A number of stable paramagnetic tags have been designed and the performance of the respective protein conjugates has been evaluated in vitro and in crowding media by high resolution NMR spectroscopy. Using these high-performance paramagnetic tags, we have determined the 3D structure of unstable and short-lived thioester intermediate of Sortase A using pseudocontact shifts (PCSs) as structural restraints. Powered by PCS-Rosetta, we show that 3D structures of proteins in living cells can be determined via site-specific labeling of proteins with stable paramagnetic tags.
Weak protein-protein and protein-ligand interactions play important roles in biological recognition. Detection of pairwise interactions in such complexes is a major challenge for NMR. Here we demonstrate that transferred nuclear Overhauser effect, TRNOE, in combination with asymmetric deuteration of a protein and a peptide ligand can be used to detect intermolecular interactions in large protein complexes with molecular weights up to ~100 kDa. Assignment of the interactions of the gp120 HIV-1 envelope glycoprotein to the corresponding protons was accomplished by site directed mutagenesis of the protein and by the resonance assignment of the interacting peptide which corresponds to the N-terminal segment of the CCR5 receptor. In addition, we used the $^{13}$C-edited/$^{13}$C-filtered TRNOE experiment to study the interactions of small and medium sized monomeric and dimeric RANTES with a 27-residue peptide representing the N-terminal segment of the CCR5 receptor (Nt-CCR5(1-27)). The TRNOE phenomenon led to more than doubling in the signal to noise ratios (SNR) for the intermolecular NOEs observed in the $^{13}$C-edited/$^{13}$C-filtered experiment for the 11.5 kDa monomeric RANTES/Nt-CCR5(1-27) complex. An even better improvement in the SNR was achieved with dimeric Nt-CCR5(1-27)/RANTES (23 kDa). Assignment of these interactions was accomplished using the resonance assignment for the protein and the peptide.
Characterising the binding mode of a low affinity peptide to the Neurotensin Receptor 1

P. R. Gooley¹,²*, F. Bumbak,¹,²,³, T. Vaid¹,², T. Thomas⁴, B. Noonan⁴, X. Tan⁵, M.A. Johnson⁵, D.K. Chalmers⁴, R.A.D Bathgate¹,³, D.J. Scott¹,³*

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G Protein-Coupled Receptors (GPCR) are targets for around 30% of all FDA approved drugs. Conversely, over 85% (>310 receptors) of the family remain undrugged, often despite clear linkage to disease. Important reasons for this are that high-throughput screening has failed to deliver optimal drug leads and a lack of structural knowledge about how ligands engage GPCRs has hindered compound optimisation. The neurotensin receptor 1 (NTS1) is a GPCR for the tridecapeptide neurotensin (NT). Activation of NTS1 by NT has been implicated in schizophrenia, Parkinson’s disease and hypothermia, as well as progression of certain cancers and drug abuse. Crystal structures of NTS1 bound to fragments of NT, as well as mutagenesis data, have defined intermediate states of NTS1 along with the high affinity binding site for NT. It is desirable to complement these achievements by probing the binding mode of NT in solution. Furthermore, little is known about transient interactions that underlie NT binding. Cellular high-throughput encapsulation, solubilisation and screening (CHESS)-based directed evolution, yielded stable and signalling competent mutants of NTS1 suitable for solution NMR studies. Saturation transfer difference (STD) NMR, a technique commonly used for fragment screening against soluble proteins was applied to map the binding epitope of the low affinity NT peptide NT10-13 to samples of NTS1 mutants expressed to the E. coli inner membrane and reconstituted in n-dodecyl β-D-maltopyranoside (DDM) micelles. Analysis of STD spectra enabled epitope mapping of the NT10-13/NTS1 interface. The epitope maps generated are in good agreement with molecular dynamics (MD) simulations of NT10-13 bound to our NTS1 variant. Our microsecond timescale MD simulations indicate that in solution, the Y11 of NT10-13 may adopt two main sidechain conformations of which only one was evident from previously published crystal structures. This work marks an important step towards elucidating the binding pathways of peptide GPCR ligands.
IN VIVO/IN-CELL / MRI METHODS, MEDICINE, MATERIALS / SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Thursday, July 27
13:45-14:45

Michal Neeman
MRI of Implantation to Placentation in the Pregnant Mouse

Kim Potvin-Fournier
Membrane Interaction and Calcium Myristoyl Switch of Recoverin

Philip W. Kuchel
NMR of Stretched Cells
Mammalian reproduction is critically dependent on the tight communication between the mother and the fetus. The maternal circulation provides during pregnancy, for all the needs of the growing fetus, while maintaining strict isolation and integrity of the offspring. The transport and barrier functions of the maternal vasculature that interface with the embryo are essential for proper development, and inadequate maternal vascular remodeling can result in implantation failure, intrauterine growth restriction, preeclampsia and long term implications for the health of the offspring. Recent studies showed trans generational impact associated with effects of placental malfunction on the development and quality of the gametes. Over the last years we developed MRI tools that now allow up to probe the molecular mechanisms that lie at the heart of the intimate vascular barrier between the mother and the embryo [1-6].

During implantation, the maternal-embryo barrier is maintained by remodeling of the composition of the extracellular matrix (ECM). Alterations of the ECM can result in breach of the barrier and implantation failure. Remarkably, MRI is the only tool that can provide in vivo visualization of the implantation site at this early stage, through the enhanced permeability of the angiogenic vessels to macromolecular contrast media. We showed that deposition of the ECM could be altered by lentiviral infection of embryos at the blastocyst stage resulting in specific expression of the transgene in the outer trophectoderm layer of the embryo, which will eventually give rise to the placenta.

After placentation, the labyrinth layer of the placenta in which fetal capillaries exchange oxygen and nutrients with the maternal circulation accommodates the barrier functions. MRI can be used at that stage for monitoring hemodynamics and molecular transport of water, oxygen and nutrients using endogenous, exogenous and targeted contrast media.

References
MEMBRANE INTERACTION AND CALCIUM MYRISTOYL SWITCH OF RECOVERIN

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Recoverin is a peripheral protein present in retinal photoreceptors as a neuronal calcium sensor protein for phototransduction [1]. The conformation of that protein varies as a function of calcium concentration. At low Ca²⁺ concentration, recoverin is in a cytosolic form with its N-termini myristoyl group sequestered into a hydrophobic cavity whereas the binding of 1 to 2 Ca²⁺ to its functional EF-Hand motifs results in the extrusion of the myristoyl group. This is the so-called "calcium myristoyl switch" [1]. The study of the structure and membrane binding of recoverin in the absence and presence of calcium is therefore of great interest. On the other hand, the membrane of rod photoreceptors contains more than 60% polyunsatured lipids [2]. These parameters could therefore modulate the membrane interaction and binding of recoverin.

We have thus investigated the interaction of recoverin with membrane phospholipids using a combination of ²H, ¹⁹F and ³¹P solid-state NMR spectroscopy and FTIR spectroscopy. Different lipid polar headgroups and/or acyl chain lengths were used. ²H solid-state NMR results with lipid bilayers oriented between glass plates clearly demonstrate a calcium myristoyl switch for recoverin in the presence of specific lipids in given physical states in the presence of Ca²⁺. Also, recoverin is only thermally stable in the presence of Ca²⁺ both in the presence of lipids or pure in solution [3-4]. Protons at different positions on the myristoyl moiety of recoverin were replaced by ¹⁹F and ¹⁹F T₁ measurements provide information on the environment and mobility of the myristoyl moiety inside and outside the recoverin hydrophobic pocket and in membranes. Finally, ³¹P CODEX was used to understand the impact of recoverin, myristoyl moiety and calcium on lipid lateral diffusion. Overall, the results indicate that the presence of Ca²⁺, membrane fluidity and charge of the lipid polar headgroup are important for the modulation of the membrane interaction of recoverin.

References
NMR OF STRETCHED CELLS

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How do the senses of touch, pain and hearing work from a biochemical perspective? It is known that macroscopic distortions of tissues ultimately change molecular shapes of proteins in sensory nerve endings, which leads to transmission of signals to the brain. But what about mechanical sensation by any cell-type that is known to occur from the most primitive archaea to mammalian cells? The simplest human cell, the erythrocyte (red blood cell), has been the subject of our latest work in this general area of enquiry.

Imagine taking these microscopic bags of enzymes, distorting their shapes and observing that the rates of reactions inside them increase dramatically; and the transport of ions across their membranes is also substantially enhanced! This is exactly what we recently discovered while monitoring chemical reactions in cells by using NMR spectroscopy. Novel molecular and spectroscopic tools to address this field, and novel explanations for shape and volume control by cells, have emerged from this work.

Distortion of the cells is achieved in a variably-stretchable-gel apparatus [1-3]; and metabolism is followed by using $^{13}$C-labelled glucose and its conversion to $^{13}$C-labelled lactate. Concurrently, the cation transport is measured using $^{133}$Cs$^+$, a quadrupolar nucleus that fortuitously gives separate NMR signals from inside and outside erythrocytes [4]. This makes measuring membrane transport very easy and straightforward. I will present the NMR-based evidence for cell distortion, and the methods used for the metabolic and membrane-transport analyses.

How does physical distortion of cells, like stretching or compressing them, lead to a biochemical response? Our working hypothesis on the mechanism of the cell-shape-induced metabolic and membrane-transport responses will be presented, along with an outline of future directions for this project.

References


SOLID-STATE NMR MATERIALS/ METHODS

Thursday, July 27
13:45-14:45

Yan-Yan Hu
Determination of Ion Transport Pathways with Isotope-Replacement NMR

Kristopher Harris
$^7$Li MATPASS NMR Spectroscopy Combined with Monte Carlo Simulations for Structure Solution

Kevin J. Sanders
Low-Power Broadband NMR of Paramagnetic Materials at 111 kHz MAS
Determination of Ion Transport Pathways with Isotope-Replacement NMR

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Fast ion conductors, also known as solid electrolytes, are widely used in energy storage technologies, including fuel cells, batteries, and sensors. Ion transport pathways, along with charge carrier concentration and ion dynamics, determine ionic conductivities of fast ion conductors. So far, the determination of Li-ion transport pathways has been mostly carried out with computational effort. Experimental studies are limited by the challenges to follow Li ion diffusional movement in complex systems. A solid-state NMR approach is devised on the basis of ⁶Li/⁷Li isotope replacement induced by a biased electric potential to determine Li ion transport pathways [1]. The mechanism of this isotope replacement approach is illustrated in Fig. 1a. Solid electrolytes are sandwiched by two pieces of ⁶Li-enriched metal foil. An electric potential (Fig 1b) is applied across this assembly to induce Li ion transport from one ⁶Li metal electrode, across the solid electrolyte, to the other ⁶Li metal electrode. During this process, naturally-abundant ⁷Li isotope in the solid electrolyte will be replaced by ⁶Li originated from ⁶Li metal. By quantifying the changes in the amount of ⁶Li from different Li local environments in the solid electrolyte before and after the ⁶Li -> ⁷Li replacement, Li-ion transport pathways can be inferred. Fig1. c and d show an example of employing this approach to identify Li-ion pathways in a polymer-ceramic composite electrolyte. The comparison of ⁶Li NMR spectra of the pristine electrolyte and the electrolyte after electrochemically induced ⁶Li -> ⁷Li replacement showed significant increase in the ⁶Li amount in the ceramic phase, with minimal changes observed at the polymer-ceramic interface and the polymer phase. Therefore, Li ions are determined to diffuse through the ceramic phase in this composite electrolyte. Examples of Li ion transport via different crystal structural sites in ceramic fast ion conductors and various composite electrolytes will be discussed.

Figure 1. a. Schematic of possible Li ion transport pathways in a polymer-ceramic composite electrolyte. b. applied electric current and voltage profiles across the electrolyte. c. d. ⁶Li NMR and quantified results of the solid electrolyte before and after the electrochemically incuded ⁶Li -> ⁷Li replacement.

Reference
The most common commercial EV batteries contain Li[Ni\textsubscript{1/3}Mn\textsubscript{1/3}Co\textsubscript{1/3}]O\textsubscript{2} as positive electrode material; furthermore, varying the ratios of transition metals (TMs), Li atoms, and vacancies can provide capacity increases of up to 40%. The empirical formulae of this large class of materials are simple, yet their exact structures and breakdown pathways are unknown.

Here, we propose a Monte Carlo structure solution method, using a Hamiltonian that is based on local electroneutrality. The close-packed 2D TM sheet is partitioned according to valence-bonding principles and a state of local charge balance—for the TM atoms with respect to the neighboring (fixed) 2D oxygen sheet—is sought. This simple Hamiltonian allows rapid, yet realistic, sampling of the configuration space of the large sheets (up to 10,000 TM atoms) necessary to properly capture the (often) complex arrangements.

The unpaired electrons of the TM atoms generate large paramagnetic chemical shifts in the neighboring Li atoms [1]. The isotropic portion of these shifts is sensitive to the identity of the 12 TM atoms neighboring each Li: 6 in the TM sheet above, 6 in the sheet below [1]. Because the interfering anisotropic portion of the interaction is too large to remove via MAS, its effects are separated using \textsuperscript{7}Li MATPASS NMR spectroscopy under 60 kHz MAS [2].

A series of samples with compositions Li[Ni\textsubscript{x}Mn\textsubscript{x}Co\textsubscript{1-2x}]O\textsubscript{2} are investigated, where x = 2%, 10%, and 33%. In each case, structures generated by the Monte Carlo calculations are verified through an extremely accurate matching between predicted and experimental \textsuperscript{7}Li NMR spectra. Additionally, accurate 3D simulations of electrochemically charged versions of these samples, where transition-metal oxidation and delithiation occur, are presented.

Fig. 1: Schematic of the structure solution algorithm, using Monte Carlo structure prediction guided by experimental \textsuperscript{7}Li MATPASS NMR data.

References
Low-power broadband NMR of paramagnetic materials at 111 kHz MAS

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Solid-state NMR studies of samples containing abundant paramagnetic centers suffer from poor resolution due to the coupling of NMR-active nuclei and unpaired electrons, typically resulting in substantial isotropic shift dispersions and shift anisotropies often exceeding 1 MHz, as well as a significant relaxation enhancement. These effects frequently result in conventional NMR techniques under moderate magic-angle spinning (MAS) failing to produce any observable signal, often because of the insufficient bandwidth offered by conventional radiofrequency (RF) pulses. The short high-powered adiabatic pulse (SHAP) [1] has been a successful tool for inverting and refocusing broad signals but demands very high RF fields under ultrafast (>60 kHz) MAS that greatly exceed the spinning rate [2]. The single-sideband-selective adiabatic pulse (S³AP) [3] is a promising alternative to the SHAP for experiments using ultrafast MAS conditions, and has been shown in silico to have decreasing RF requirements for increasing MAS rates [2].

We present here a result that, for the first time, demonstrates better performance of a low-power (ω₁ < ω_R) S³AP pulse relative compared to a high-power (ω₁ > ω_R) SHAP pulse at an MAS rate of 111 kHz (Fig. 1a) and that these pulses can be implemented in a straight-forward way without extensive optimization (Fig. 1b). We show that for nuclei with low gyromagnetic ratios, such as ^6Li, ^15N, or ^29Si, the SHAP is comparatively inefficient due to the inability to reach sufficiently high RF fields, whereas the S³AP performs well as it requires lower powers.

For systems with large anisotropies relative to the MAS frequency, we extend this class of pulses to irradiate multiple sidebands simultaneously. Using spin dynamics simulations and experimental optimization, we show that sweeping over multiple sidebands simultaneously can improve not only the pulse efficiency but also the accuracy of the observed spinning sideband manifold.

Fig. 1. (a) 1D shifted-echo ^6Li spectra of LiFe₀.₂₅Mn₀.₇₅PO₄ with 270 μs S³AP refocusing pulses (black) and 18 μs SHAP refocusing pulses (red). (b) Normalized integrals of the 1D ^6Li spectra as a function of the RF field strength used for each pulse, showing that the S³AP pulse (black) efficiency is robust against RF field strength misset and overall more sensitive than the SHAP (red). All experiments utilized an external magnetic field strength of 11.7 T and a rotation rate of 111.111 kHz.

References
UNUSUAL / HYPERPOLARIZATION

Thursday, July 27
13:45-14:45

Dieter Suter
Coupled Dynamics of Electronic, Nuclear and Photonic Spins

Ilia Kaminker
Impact of Electron Spin Depolarization on DNP at 7T

Edward P. Saliba
Electron Decoupling with MAS DNP Using a Frequency Tunable Gyrotron
Magnetic resonance studies the dynamics of spin angular momenta that interact with external magnetic fields as well as with other spins. Depending on the system, the interaction between the different angular momentum reservoirs can present a small perturbation or it can dominate the dynamics of the system. This contribution will highlight a number of such cases, where the most interesting dynamics are due to the interaction between electronic and nuclear spins, sometimes with additional coupling to photons. Examples include free atoms [1], atomic ions in solids, defect centers in solids like the nitrogen-vacancy (NV)-center in diamond [2], or nuclear spins in semiconductor materials interacting with the quasi-free electrons of the conduction band [3]. From the perspective of the nuclear spin, the interaction with a localized electron spin can be orders of magnitude stronger than the Zeeman interaction with the static magnetic field. In addition, the hyperfine interaction can enhance the coupling between the nuclear spin and static or alternating magnetic fields – an effect known as enhanced nuclear magnetism. From the perspective of the electronic spin, the interaction with a single nuclear spin is often relatively weak. However, in systems with (near-)degenerate electronic spin states, the hyperfine interaction can strongly modify the dynamics of the electron spin, such that the resulting dynamics can no longer be described in the context of the rotating wave approximation [4]. Furthermore, if it interacts with a large nuclear spin ensemble, the combined effect can become strong and dominate even the Zeeman interaction. If the system also couples to photons, optical pumping effects can be observed, which polarize the electronic and nuclear spins on timescales that can vary from nanoseconds to minutes. Apart from the fundamental interest in these types of dynamics, such systems have a number of applications ranging from solid-state physics through sensing and quantum information.

Fig. 1. a) $^{13}$C Rabi oscillations on sub-$\mu$s timescales. b) Hyperfine tensor of NV center. c) Irregular dynamics of an electron spin under strong driving conditions.

References
Impact of electron spin depolarization on DNP at 7T

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NMR signal enhancement in dynamic nuclear polarization (DNP) experiments critically depends on an electron polarization profile obtained after prolonged microwave irradiation typical in DNP experiments. The shape and breadth of the electron polarization profile depend on the applied mw power intensity (B₁), electron relaxation rates (T₁, and T₂), and electron-electron spectral diffusion rate (λ), which in turn depend on radical type, radical concentration, and temperature. It was shown that an optimum depolarization exists and that both narrower and broader depolarization profiles lead to reduced DNP enhancement. It has only been very recently that the electron depolarization profiles were measured experimentally in the context of DNP via electron-electron double resonance (ELDOR) experiments.

Our development of a dual 7T DNP/EPR spectrometer operating at 200 GHz / 300MHz allows collection of both DNP and the corresponding EPR and ELDOR data under identical conditions. We show that the depolarization profiles relate to the extent of DNP enhancement. Previously it was shown that for a given sample composition and experimental conditions DNP enhancement can be improved if monochromatic irradiation is substituted by a frequency modulated one. Here, we demonstrate that this effect is even more pronounced at 7T compared to 3.3T and that improvement up to a factor of 5 can be achieved if chirp pulses are substituted for monochromatic irradiation. We present experimentally measured ELDOR spectra taken under chirp irradiation conditions and demonstrate that in this case, the improvement in DNP enhancement is again related to the broadening of the electron depolarization profile beyond what is achieved by spectral diffusion alone.

Figure 1. (left) ELDOR electron depolarization profiles for mono-, bi-, tri-, and spin-labelled dendrimer at 4 K. (right) Electron depolarization profiles obtained for 10 mM 4-amino TEMPO in a water/glycerol glass at 10 K with cw- and chirped excitation. The DNP signal enhancements is given in parenthesis in the figure legend.
Electron Decoupling with MAS DNP Using a Frequency Tunable Gyrotron


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The small interaction energies inherent in NMR lead to a small Boltzmann polarization, resulting in poor signal to noise. This problem can be remedied with a technique known as dynamic nuclear polarization (DNP) in which the polarization of radical electrons is transferred to the nucleus of interest [1]. DNP, however, is a double edged sword. The same electron-nuclear dipolar couplings that lead to the excellent sensitivity obtained also drastically shorten the nuclear relaxation times. This shortening of the nuclear relaxation times can lead to a decrease in resolution, inhibiting the effectiveness of experiments.

Fig. 1. (a) Urea spectra with and without electron decoupling. (b) The electron decoupling pulse sequence. (c) Effectiveness of electron decoupling with sweep width. A narrowing is observed even with continuous wave decoupling.

Data is presented demonstrating a narrowing of a $^{13}$C resonance under microwave irradiation (Figure a). The microwaves begin on the DNP matching condition during a polarization time and then are switched on resonance with the radical electron of the Trityl-OX063 radical (Figure b) [2,3].

Narrowing is observed under continuous wave irradiation of the sample, but experiment demonstrates that a considerably more effective technique is to sweep the frequency during the decoupling time using an arbitrary waveform generator (Figure c). The arbitrary waveform generator control is integrated directly into the spectrometer and allows for the gyrotron to be controlled directly from the spectrometer user interface [2,3].

References


EPR

Thursday, July 27
13:45-14:45

Gunnar Jeschke
A Dual-Band Ultrawideband EPR Spectrometer and its Application

Jack H. Freed
Four Reasons Why I Should Not Have Been Made a Fellow of the IES at This Time
A DUAL-BAND ULTRAWIDEBAND EPR SPECTROMETER AND ITS APPLICATION

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Typical EPR spectral widths of organic radicals, transition metal, and rare earth metal ion centers range from about 100 MHz to 5 GHz, thus precluding uniform excitation by rectangular monochromatic pulses. Microwave components that can cover such bandwidths are available and in 2011, the first commercial arbitrary waveform generators (AWGs) were introduced that could provide signals at 5 GHz analog bandwidth and 6 GHz Nyquist frequency. At that point, we decided to design and construct a pulsed EPR spectrometer for routine use that combines ultrawideband excitation and detection, high purity of the excitation waveforms for precise spin control, and a sensitivity that matched the one of current commercial spectrometers when used with monochromatic rectangular pulse excitation. In this contribution I discuss the basic considerations on spectrometer architecture, our application-oriented design approach, and the performance that could be achieved.

The spectrometer was designed for operation in X band, where bandwidth requirements are more benign for metal ions with large g anisotropy, and in Q band with high power (150-200 W), where concentration sensitivity is optimal for many pulsed EPR experiments. The choice of a high-end AWG with built-in sequencer provides for a spectrometer architecture with low complexity that in turn allows for performing complex experiments at the repetition rate permitted by the studied system without reprogramming overhead. We decided for an architecture with microwave resonators that ensures high sensitivity and good microwave power utilization at the expense of limit detection bandwidth to about 800 MHz. Resonator compensation during excitation provides much larger excitation than detection bandwidth.

Performance of the spectrometer will be demonstrated by experimental examples on model systems and on a few biological systems.

I thank Dr. Andrin Doll for the detailed design, the building and careful testing of the spectrometer and for many deep and fruitful discussions on spin dynamics and Dr. Yevhen Polyhach, Stephan Pribitzer, Frauke Breitgoff, and Janne Soetbeer for further helpful discussions. Funding by ETH grant ETH-23 11-2 and by DFG SPP 1601 (JE 246/5-1) is gratefully acknowledged.

References
FOUR REASONS WHY I SHOULD NOT HAVE BEEN MADE A FELLOW OF THE IES AT THIS TIME

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An IES Fellow should have made significant contributions to ESR and be completing his/her career. In fact, as these four projects illustrate, the latter requirement is not yet fulfilled. But I am still happy to be so honored.

1) Over the years we have developed the theory of slow motional ESR, and we have provided many experimental studies based on it. In recent years we have applied it for a new methodology to study the initial step in viral attack of a cell for many types of viruses. In a current study we have shown that the same transmembrane protein (HAP2) mediates the fusion of sperm and egg cells, and does the same for viral attacks of cells for virus types such as Dengue and Zika.[1] This raises the question: What came first—the virus or the egg?

2) The method of Pulse Dipolar Spectroscopy (PDS-ESR) that ACERT helped to pioneer has gained widespread use. We have been studying the complex mechanism of bacterial chemotaxis signaling; it involves a sensor that senses food or poison in its path, and its signal is then sent a distance of over 200 Å by receptor proteins to the active protein units which then signal the flagellum motor, telling it whether to move forward or away. Our extensive PDS studies[2] have elucidated key details of all these steps in the signaling chain.

3) We have pioneered Fourier transform Two-Dimensional Electron-Electron Double Resonance to study room-temperature dynamics in fluids and proteins. In recent work at 95 GHz with 1.2 kW pulses[3], we have studied the exchange of a spin probe between aqueous and lipid membrane phases. We see 2D cross-peaks emerge between the signals from the two phases. The rate of exchange was quantitatively measured in the microsecond range, which is a range not readily available by other techniques. But this is the rate of protein conformational changes, and we are adapting the method to this objective.

4) Finally, I address how to proceed when one’s experiments, after much struggle, do not provide adequate SNR. We have developed a new denoising method, based on wavelet transforms, that enables one to improve the SNR by about two orders of magnitude while preserving the fidelity of the signal. Examples of retrieving signals from noisy ESR spectra will be shown.[4]

References
SOLUTION NMR

Thursday, July 27
15:15-16:30

Malene R. Jensen
Exploring the Role of Protein Intrinsic Disorder in the MAPK Cell Signaling Pathways

Philipp Neudecker
The Nedd4-1 WW Domain Recognizes the PY Motif Peptide through Coupled Folding & Binding Equilibria

David N. Langelaan
Characterization of Oncogenic Protein-Protein Interactions Involving the Microphthalmia-Associated Transcription Factor

Yunyu Shi
Structural and Functional Insights into the Cold Shock DEAD-Box Helicase CsdA
Intrinsically disordered proteins (IDPs) are highly abundant in the human proteome and play key regulatory roles in biology. NMR spectroscopy is uniquely suited to characterize IDPs at atomic resolution, and ensemble descriptions have emerged as the preferred tool for capturing both the structure and dynamics of IDPs [1]. Characterization of IDP complexes is an entirely different challenge as exchange between free and bound conformation(s) often occurs on the micro- to millisecond time scale leading to extensive line broadening of the NMR signals. Here, methods will be presented for characterizing the structure, dynamics and kinetics of IDP complexes using a combination of nuclear relaxation rates, relaxation dispersion and chemical exchange saturation transfer experiments. The approaches are applied to IDPs within the mitogen-activated protein kinase (MAPK) cell signalling pathways that assemble specific kinases into highly dynamic signalling complexes. Our results reveal how IDPs in the MAPK pathways rely on linear motifs for governing signalling specificity, and how different IDP binding modes may induce different functional states of the folded, binding partners [2].

References
THE NEDD4–1 WW DOMAIN RECOGNIZES THE PY MOTIF PEPTIDE THROUGH COUPLED FOLDING & BINDING EQUILIBRIA

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The four WW domains of human Nedd4–1 (neuronal precursor cell expressed developmentally downregulated gene 4–1) interact with the PPxY (PY) motifs of the human epithelial Na⁺ channel (hENaC) subunits, with the third WW domain (WW3*) showing the highest affinity. The α-hENaC PY motif binding interface of WW3* undergoes conformational exchange on the millisecond time scale [1], indicating that conformational sampling plays a role in peptide recognition. The nuclear Overhauser effect-derived structure of apo-WW3* resembles the domain in complex with the α-hENaC peptide [2], although particular side chain conformations change upon peptide binding, which was further investigated by molecular dynamics simulations. Model-free analysis of the 15N nuclear magnetic resonance spin relaxation data showed that the apo and peptide-bound states of WW3* have similar backbone picosecond to nanosecond time scale dynamics. However, apo-WW3* exhibits pronounced chemical exchange on the millisecond time scale that is quenched upon peptide binding. 1HN and 15N Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion experiments at various temperatures revealed that apo-WW3* exists in an equilibrium between the natively folded peptide binding-competent state and a random coil-like denatured state. The thermodynamics of the folding equilibrium was determined by fitting a thermal denaturation profile monitored by circular dichroism (CD) spectroscopy in combination with the CPMG data, leading to the conclusion that the unfolded state is populated to ~20% at 37°C. The kinetics of binding was determined by NMR lineshape analysis [3]. These results show that the binding of the hNedd4–1 WW3* domain to α-hENaC is coupled to the folding equilibrium (Fig. 1). Our ongoing studies reveal that these coupled folding and binding equilibria are retained in the context of neighboring hNedd4-1 WW domains and likely also exist for the neighboring WW domains themselves.

References

Fig. 1. Nedd4-1 WW3* folding equilibrium (unfolded state, U, natively folded state, F) determined by CPMG coupled with the binding equilibrium of the PY motif ligand, L, determined by NMR lineshape analysis at 5°C.
Characterization of oncogenic protein-protein interactions involving the microphthalmia-associated transcription factor

Makenzie Branch and David N. Langelaan
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Transcription factors control gene expression and often coordinate fundamental processes such as cell growth and differentiation. The microphthalmia-associated transcription factor (MITF) is a melanocyte-specific protein essential for melanocyte development. MITF has also been identified as a lineage-specific oncoprotein in melanoma, and knockdown of MITF function results in cell senescence. MITF carries out its function by recruiting transcriptional co-regulators to gene promoters to modify gene transcription, including the homologous histone acetyltransferases CBP/p300. MITF contains N-terminal and C-terminal activation domains as well as a central basic helix-loop-helix DNA binding motif. Both the N-terminal and C-terminal activation domains have been shown to interact with CBP/p300. Using a combination of pull-down experiments, NMR spectroscopy, biophysical studies, and functional assays we have determined that the N-terminal activation domain of MITF is intrinsically disordered in solution and interacts with the TAZ2 domain of CBP/p300 with high affinity (Fig. 1). NMR-based titrations indicate that MITF also interacts with the KIX and TAZ1 domains of CBP/p300. These results support a model in which MITF may interact with multiple domains of CBP/p300 to activate transcription of MITF-target genes and provides insight as to how MITF may control gene expression in melanoma.

Fig. 1. $^1$H-$^{15}$N HSQC spectrum of MITF$_{81-204}$ in the absence (black) and presence (red) of the TAZ2 domain of CBP/p300.
Structural and functional insights into the cold shock DEAD-box helicase CsdA

Ling Xu¹, Lijun Wang¹, Junhui Peng¹, Fudong Li¹, Lijie Wu², Jiahai Zhang¹, Beibei Zhang¹, Rongsheng Ma¹, Mengqi Lv¹, Qingguo Gong¹, Rongguang Zhang², Zhiyong Zhang¹, Jihui Wu¹, Yajun Tang¹,*, Yunyu Shi¹,*

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The cold shock DEAD-box helicase CsdA plays an essential role in *Escherichia coli* at cold shock situation. Here we showed that CsdA contains two RecA-like domains at the core region, similar to other RNA helicases, as well as previous uncharacterized auxiliary domains, a dimerization domain (DD) and an RNA binding domain (RBD). We solved all of the domain structures by X-ray crystallography or NMR. Using small angle X-ray scattering experiments (SAXS) and ¹⁹F based paramagnetic relaxation enhancement experiments (¹⁹F-PREs), an integral structural model for this multi-domain protein was established. This approach is a valuable attempt to track the conformational rearrangement of CsdA in the presence of different substrates. *In vitro* and *in vivo* assays revealed that DD plays an indispensable role in stabilizing the structure of CsdA protein. We demonstrated for the first time that CsdA functions as a stable dimer in solution at low temperature and the C-terminal regions are main determinant for RNA binding. Moreover, the C-terminal region could bind to the RNA that contains the regions comprising of the stem of H92 of 23S rRNA with high affinity. Since CsdA has been reported to be involved in the biogenesis of ribosome 50S subunit, this kind of interaction may have biological implications.
MRI METHODS, MEDICINE, MATERIALS / IN VIVO/IN-CELL

Thursday, July 27
15:15-16:30

Saifeng Liu
Practical Micro-Imaging of Tissue/Vessels at 3T and 7T

Roland Hergenröder
Planar Waveguide NMR Detectors for Nanoliter Biological Sample Volume

Gil Farkash
Accurate Slice Selection near Metal by Utilizing Frequency-Swept and Offset-Corrected Pulses

Saeed Bakhshmand
Multimodal Exploration Platform for MRI-Driven Brain Networks (MultiXplore)
A key challenge in imaging the vascular system of the brain is to see small arteries. The cerebrovascular system, in particular the micro-arterial system, plays a key role in delivering oxygen and glucose to the brain to meet the high metabolic demand. Microvascular or small vessel abnormalities have been increasingly identified as the basis of many neurovascular and neurodegenerative disorders. Although MRI has matured significantly in imaging the brain and can do so with an in-plane resolution of 500µm at 3T and 200µm at 7T, it has not yet been used to study microvascular details in humans in vivo with an in-plane resolution of 50-100µm. Recent advances in Susceptibility Weighted Imaging (SWI) have dramatically improved the visibility of small veins such as the medullary veins and venules. However, in vivo detection of tiny arterioles, where vasculogenic neuropathology often begins, remains challenging and uninvestigated. The primary reason is that there is no susceptibility difference between the arteries and the surrounding tissue. Therefore, to make the arteries visible in the same way we see veins, we propose to introduce a susceptibility shift in the vasculature by using ultra-small superparamagnetic iron oxide (USPIO) contrast agent, Ferumoxytol. We have found that Ferumoxytol has saturated by 1.5T and that the product of susceptibility times field strength is roughly 90 ppm*T for unit concentration (mg/ml) of Ferumoxytol. Using simulations, we have shown with TE=10ms and a voxel aspect ratio=1:1, that arteries with a diameter less than half a pixel (approximately 45 µm in the simulation) can be detected using tSWI, with 4mg/kg Ferumoxytol. We have since shown that vessels of this size can indeed been seen in human in vivo imaging at both 3T and 7T (Fig. 1).

**Figure 1.** a. Maximum intensity projection (MIP) of pre-contrast magnitude images. b. MIP of pre-contrast QSM. c. MIP of post-1mg/kg QSM. d. MIP of post-3mg/kg QSM. e. MIP of the post-3mg/kg ΔR2* images. f. minimum intensity projection (mIP) of pre-contrast SWI. g. mIP of post-1mg/kg SWI. h. mIP of post-3mg/kg SWI. The arteries and veins are indicated by the red and blue arrows, respectively. Effective slice thickness is 3.2mm.
Planar waveguide NMR detectors for nanoliter biological sample volume

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The optimum experimental setup for NMR on mass-limited biological samples is to dissolve the sample in the minimum volume of solvent to get the highest possible concentration, and to construct the smallest radio frequency coil that will enclose the sample. The first microcoils that have been used for high-resolution \(^1\)H NMR of nanoliter-volume samples were solenoidal coils[1]. Recently, two new types of rf microcoils were introduced, which are based on planar, electromagnetic waveguides. Microstrip transmission line resonators were first used for surface coils in MRI and later as NMR detector [2, 3]. A slightly different approach was taken by Maguire and co-workers who used a small microslot in a microstrip waveguide that creates a pure series inductance as the rf coil [4]. Based on this design we built a microslot probe by means of femtosecond laser ablation, which is capable of all basic NMR measurements for metabolomics studies on in-vitro 3D Cell culture models [5, 6]. NMR microprobes based on radio frequency planar waveguide technology such as stripline and microslot are suitable for investigations of volume-limited samples in the low nanoliter range. Spectra of sample quantities in the 100 pmol range can be obtained with this probe in a few seconds. The planar geometry of the probe is easily adaptable to the size, geometry and physiological requirements of the samples such as microfluidic chips for studying metabolomics of cells. The concentrations and dynamics of the metabolites not only give direct evidence of the cells’ physiological condition. In order to study interfaces, a B1 gradient NMR detector technique delivers spatially resolved NMR information which can be used to study cellular spheroids patho-physiological gradients. It can be combined easily with microfluidic elements to conduct in-vitro experiments on living 3 dimensional cell culture models.

Fig. 1. Different designs of NMR-microslot detectors.

References
Accurate Slice Selection Near Metal by Utilizing Frequency-Swept and Offset-Corrected Pulses

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Orthopedic metal implants, such as total hip or knee replacements, are increasingly used in hospitals worldwide. Adjacent tissues should be monitored by MRI for associated pathologies, however, these regions are associated with severe $B_0$ inhomogeneities. Currently used techniques include one readout and two phase encoding dimensions, thereby encoding and reconstructing a volume, from which the non-distorted slices are extracted [1-2]. None of these excite a slice that is uniformly thick and perpendicular to the X-Y plane. Here we present two sequences, based on SPEN [3], that can accurately select such a slice near metal. The first one uses four adiabatic pulses and selects a diamond in the chemical shift ($\Omega$) and spatial (Z) dimensions (Fig. 1A). The second, derived from LASER [4], applies two adiabatic pulses, which selects a parallelogram in $\Omega$-Z (Fig. 1B). In both sequences, transmission and reception offsets are stepped, which tiles the $\Omega$-Z diamonds or parallelograms along $\Omega$ (Fig. 1D-E). As the transmission offsets decrease, the slice thickness becomes more uniform. In addition, the SNR, given by the integral of the area along Z, is constant across $\Omega$. Results were compared to SEMS and LASER using a 7 Tesla magnet (Fig. 1F-G,I-J).

Fig. 1. Coronal spin-echo images of a cucumber with an inserted titanium screw (depicted in H,K) at 7 T (A) OC-SPEN SL sequence, (B) OC-LASER sequence, (C) SEMS sequence, (D) selectivity of OC-LASER in $\Omega$-Z, and (E) selectivity of OC-SPEN SL in $\Omega$-Z. MRI images: (F) OC-SPEN SL, (G) OC-LASER, (I) SEMS, and (J) LASER.

References
Multimodal Exploration Platform for MRI-driven Brain Networks
(MultiXplore)

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Multimodal Imaging has been traditionally used to measure brain activity in accordance to its structural features. While functional and structural MRI modalities (fMRI and DTI) have been used now for decades, being able to analyze their intricate nature is of growing interest especially when looking at brain networks [1]. Interactive exploration of functional and structural brain networks is crucial not only in cognitive neuroscience but also clinically in studying neurological disorders [2]. Here, we present an implementation of an open-source software platform which supplements conventional displays of graph theoretical brain networks (e.g. matrices and node-edge 3D brain graphs) with novel interaction functionalities such as an input matrix (implemented in Qt) that holds user-selected regions of the brain and represents anatomical counterparts of dual brain networks (functional and anatomical connectivity) in an interactive 3D view (implemented using VTK) (Fig.1a) upon selection of relevant cells. It also allows for annotation of deterministic tractography as can be seen in Fig.1b. We have made MultiXplore [3] available through NITRC and designed it as an investigational tool to visually represent both resting-state and structural networks to mitigate intrinsic distance between user level representation of brain imaging MRI modalities.

References
SOLID-STATE NMR MATERIALS/METHODS

Thursday, July 27
15:15-16:30

Thomas Vosegaard
EasyNMR: A New Platform for NMR Simulations

Sean T. Holmes
Refining Crystal Structures with Quadrupolar NMR and Dispersion-Including DFT Calculations

Giulia Mollica
DNP NMR for Structural and Morphological Investigation of Polymorphic Powders at Natural Isotopic Abundance

Cory M. Widdifield
Assessing the Potential of NMR Crystallography for Structure Distinctions and Verifications of Organics
This presentation summarises our efforts to establish a new platform for NMR simulations. The simulation platform is web-based to ensure full cross-platform compatibility including mobile devices and to allow easy sharing and access to NMR data through public available data-storage platforms like Dropbox and Google Drive.

The EasyNMR platform provides simple and intuitive graphical interfaces for spectrum visualisation (1), basic spectrum processing, routines for fitting experimental spectra, and interface for data and file management. We have been very careful to design a highly modular setup, which provides simple ways to implement own models and tools, or for using existing programs like SIMPSON (2–4), which are available through the web interface. Thus, when fitting an experimental spectrum, the user can freely choose between SIMPSON models or other implemented models – or create his/her own models.

As an example, simulating the characteristic lineshape from exchange in a two-site jump given by the equation $S(\omega) = k(\omega_A - \omega_B)^2 / ((\omega - \omega_A)^2 (\omega - \omega_B)^2 + k^2 (4\omega - \omega_A - \omega_B)^2)$ can be implemented with just a few lines of code, since EasyNMR handles all spectrum import/export, user interface, etc. Thus, as a user you only need to concentrate on the science in your model, as everything else is handled by the program.

Examples that exploit the flexible and customizable interface of EasyNMR are given in Fig. 1 and include analysis of single-crystal NMR spectra (5) and the spectroscopy game iSpec.

References

Solid-state nuclear magnetic resonance (NMR) crystallography has emerged as a robust collection of techniques for refining or even predicting atomic coordinates in crystals. Often, the interpretation of NMR results is aided through computation of NMR tensor parameters by periodic density-functional theory (DFT) [1]. This work examines several computational strategies for refining the coordinates of atoms in molecular or ionic solids, with an emphasis on the structures of active pharmaceutical ingredients formulated as hydrochloride salts (HCl APIs) [2]. In particular, the role of dispersion forces on DFT-based structural refinements of crystals is assessed, especially as they relate to the prediction of electric field gradient (EFG) tensors. It is illustrated that quadrupolar parameters obtained from experimental NMR studies can be used to reparameterize common dispersion force fields [3], such that the resulting structures yield accurate and reliable predictions of EFG tensors. This analysis is based on $^{35}\text{Cl}$ EFG tensors from a wide variety of organic solids. Although changes in bond lengths between non-hydrogen atoms are generally under 0.02 Å, these small differences are strongly reflected in the computed EFG tensors. The correlation between calculated and experimental principal components of $^{35}\text{Cl}$ EFG tensors is shown in Fig. 1. Refinement of crystal structures using the reparameterized dispersion force field typically leads to a reduction of mean-absolute errors, relative to experimental values, of over 50% relative to the results obtained from calculations on structures derived from X-ray diffraction or structures refined through DFT without the dispersion force field. Similar results are found for the prediction of $^{14}\text{N}$ and $^{17}\text{O}$ EFG tensors.

**References**


DNP NMR for structural and morphological investigation of polymorphic powders at natural isotopic abundance

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Diffraction techniques currently remain unparalleled in structural elucidation of materials for which single crystals of sufficient dimensions and quality can be grown. However, despite recent technological advances in microdiffraction, there still is a large number of systems that cannot provide suitably sized single crystals, either because of the preparation procedure of the substance, or because such crystals cannot simply be grown. In such cases, structure can hardly be retrieved using currently available methodologies. This hampers our understanding of the physico-chemical behavior of numerous samples, such as functional organic powders, hence precluding the design of new materials with tailored properties and limiting polymorph control.

Solid-state NMR has the potential to be the key to access the crystal structure of powders because it can provide atomic-level information without requiring any long-range order of the sample. Among the different NMR observables available, the dipolar coupling is naturally the one with the highest structural content, since it depends on the inverse cube of the distance between the coupled nuclei. Notwithstanding its potential for structural elucidation, dipolar coupling has been mainly used to extract qualitative information about nuclear spatial proximity, but, differently from chemical shift [1], it has rarely been considered as a systematical tool for crystal structure elucidation of solids, notably on samples at natural isotopic abundance. The main limitation to access such information lies in the inherently low sensitivity of NMR to spin-spin couplings, especially when dilute spins such as ¹³C or ¹⁵N, ubiquitous in organic samples, are investigated.

Here, we show how we can capitalize on the tremendous NMR sensitivity enhancement provided by Dynamic Nuclear Polarization (DNP) to obtain unique insights into the structure and morphology of sub-micrometric organic powders at natural isotopic abundance. First, we demonstrate how a new dipolar-based NMR crystallography approach sensitive to both molecular conformation and crystal packing allowed the correct crystal structure of powdered anhydrous theophylline to be rapidly and effectively identified among a set of both existing and predicted crystal structures [2][3]. Second, we illustrate the potential of DNP solid-state NMR in the investigation of the morphology of challenging powdered samples that could not be characterized using X-ray or synchrotron diffraction experiments.

References
Assessing the Potential of NMR Crystallography for Structure Distinctions and Verifications of Organics

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Numerous studies have established the complementary nature of X-ray/neutron diffraction, nuclear magnetic resonance (NMR) and computational modelling. When these techniques are used for crystal structure refinements, determinations, etc., they may be classified as applications of “NMR crystallography”. Although the diffraction community has internal structure verification protocols, as solid-state NMR (SSNMR) is a sensitive probe of local structure and dynamics, it should complement existing verification protocols. Although ad hoc examples exist [1], the potential of NMR crystallography to validate diffraction crystal structures has not been quantitatively established. Likewise, a systematic understanding of the scope of systems which may be distinguished using NMR crystallography remains undeveloped. Structure databases, such as the Cambridge Structural Database (CSD) and the Inorganic Crystal Structure Database (ICSD), are important repositories of (primarily) diffraction structures. With the goal of developing crystal structure verification tools that may be generally applied to organic powders, we present our analysis of repeat crystal structures in the CSD. We surveyed over 200 000 organic crystal structures, and find ca. 4200 repeat structure pairs corresponding to the same polymorphic form under similar measurement conditions. Using gauge-including projector augmented-wave density functional theory (GIPAW DFT), we determine which structure pairs do not relax to the same energy minimum structure (as per the method of van de Streek and Neumann [2]). Magnetic shielding calculations are performed to establish the $^1$H and $^{13}$C NMR spectral signatures which are characteristic of the various types of local differences, and building on previously established guidelines [3], we postulate whether given pairs could be distinguished using SSNMR. The local differences in these repeat structures are found to typically involve H positions; hence, methods such as powder X-ray diffraction would rarely be expected to offer conclusive distinctions. Particular functionalities where NMR would be most useful at distinguishing between structures are identified. As an example, we consider two repeat structure determinations of furosemide, which differ only in the placement of one H atom [4]. Not only are solid-state NMR methods (when paired with GIPAW DFT calculations) able to distinguish between these two crystal structures, but the crystal structure of one form can be verified, while substantial evidence that the other CSD structure is not valid is presented.

References


UNUSUAL

Thursday, July 27
15:15-16:30

Ronald Walsworth
Nano and Micron-Scale Magnetic Resonance Using NV-Diamond

Victor M. Acosta
Solution NMR and EPR on a Diamond Chip

Ashok Ajoy
Quantum Interpolation for High Resolution Nanoscale NMR

Carl A. Michal
Sculpted Excitation and Solvent Suppression with Noise Excitation
I will describe the use of nitrogen vacancy (NV) color centers in diamond for nano and micron-scale NMR and EPR via optically detected magnetic resonance (ODMR). In recent years, NV-diamond has allowed NMR and EPR detection of small ensembles of statistically-polarized spins under ambient conditions, and even demonstrations of single proton and single protein NMR for samples on the surface of the diamond. Current challenges include improving the spectral resolution to perform useful NMR and EPR spectroscopy, co-localizing the sample and NV sensor without significantly perturbing the sample, and developing techniques for single molecule and quantum defect structure determination. I will outline progress to meet these challenges, including a straightforward technique that enhances NV spectral resolution by several orders of magnitude to about 1 millihertz, allowing high-resolution NMR spectroscopy on picoliter volume samples.
Solution NMR and EPR on a diamond chip
Victor M. Acosta, University of New Mexico, Dept of Physics and Center for High Technology Materials.

The power of NMR spectroscopy as a tool for determining the composition, structure, and function of complex molecules is somewhat offset by the the cost and bulk of commercially available spectrometers. A technique for NMR based on nitrogen-vacancy (NV) color centers in diamond foregoes the need for strong magnetic fields and cryogenic temperatures by relying on the statistical rather than thermal polarization of the analyte spins. NV NMR was recently demonstrated [1] at room temperature and low magnetic field (≈20mT), but the long integration times (hours to days) hindered practical applications.

We have developed a platform for sample-limited NMR spectroscopy with improved sensitivity using nanostructured diamond chips [2]. The NMR sensitivity depends on the contact surface between NV centers and external analyte. We fabricated high aspect ratio diamond nanogratings, effectively increasing the contact area by ≳15X. The end result is that more than 10 million NV centers can be used to measure NMR spectra of less than 1 picoliter of analyte that lie within their adjacent grooves. Using CsF:glycerol solutions, we determine that 4 ± 2 x 10^{12} spins in a 1 pL volume can be detected with a signal-to-noise ratio of 3 in 1 second integration time. This represents a two orders of magnitude improvement over previous reported results on picoliter and NV NMR. To improve spectral resolution and sensitivity further, we are pursuing hyperpolarization strategies. Concurrently, we are extending this platform to perform solution EPR.

Fig. 1. Picoliter NMR. (a) Overview of ambient temperature NMR techniques for small volumes [2]. Points represent minimum detectable nuclear-spin concentration in 1 s with SNR = 3. (b) Epifluorescence diamond NMR setup. The sensor region consists of dense, high aspect-ratio diamond nanogratings fabricated via interferometric lithography and doped with NV centers. The analyte's precessing nuclear statistical polarization produces an oscillating magnetic field which is sensed by adjacent NV centers. (c) Scanning electron micrograph of 400-nm pitch diamond nanogratings. (d) Photo of first benchtop optical NMR setup. (e) NMR spectrum of 19F nuclei in ~1 pL of Fomblin® oil. (f) Measured 1H and 19F gyromagnetic ratios. Dashed lines are literature values.

Quantum Interpolation for High Resolution Nanoscale NMR

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In the past few years, the development of "quantum sensors" based on the Nitrogen Vacancy (NV) center in diamond has shown tremendous promise for nanoscale Magnetic Resonance Imaging (MRI). Highlights have included the nanoscale sensing of few-to-single nuclear spins and single proteins [1-2]. The promise of these sensors for a new generation of single molecule structural determination tools is very compelling [3].

However, an outstanding challenge remains boosting the resolution of the obtained nanoscale NMR spectra, in order to measure individual J-couplings or chemical shifts. Indeed, the conventional technique for sensing of external nuclear spins involves monitoring the effects of the target nuclear spins on the NV center coherence under dynamical decoupling (the CPMG/XY8 pulse sequences). However, the nuclear spin affects the NV coherence only at precise free evolution times -- and finite timing resolution set by hardware often severely limits the sensitivity and resolution of the spectra.

In this work, we overcome this timing resolution barrier by developing a technique to perform a "quantum interpolation" of the spin system dynamics. This allows for the high resolution sensing of spins and AC magnetic fields via the NV center (see Fig 1). Experimentally we demonstrate how quantum interpolation provides resolution boosts by over a factor of 100, although ultimate limits of the method could approach resolution gains by $10^4$. The method is shown to be robust, versatile to sensing normal and spurious signal harmonics, and ultimately limited in resolution only by the number of pulses that can be applied to the system (Fig 2).

References:
We present new strategies for sculpting the excitation spectra for NMR using pseudo-random noise excitation. We describe and demonstrate the collection of solution-state NMR spectra with broadband noise sequences that exclude the excitation of specific chosen frequency windows with the aim of suppressing solvent signals in high-resolution spectra.

The collection of NMR spectra with noise spectroscopy, also known as stochastic excitation, has a number of advantages: it requires no high-power rf pulses, simplifying rf circuit design and lowering power requirements. The excitation bandwidth can be very broad and uniform, even with low power excitation. Ring-down times are reduced due to the low power of the applied pulses.

A strategy for selectively exciting specific spectral regions was introduced by Tomlinson and Hill in 1973 [1], and reintroduced later by Yang et al. [2].

In this work, we present two distinct strategies for sculpting the excitation spectrum, the first of which expands upon this earlier work, but utilizes a Zadoff-Chu sequence [3] rather than random phases to generate the sequence. This modification makes the amplitudes of the excitation pulses much more uniform than the random case enabling more efficient excitation. This technique does allow multiple suppressed windows to be included to allow suppression of solvents with multiple peaks.

The second strategy utilizes a modified maximal length binary sequence to create a hole in the excitation spectrum. This strategy has the advantage that the noise excitation sequence retains the ideal two-level auto-correlation properties of the unmodified sequence, allowing perfect reconstruction. We will discuss the performance, advantages, and disadvantages of both strategies.

**References**


EPR

Thursday, July 27
15:15-16:30

Betty J. Gaffney
An EPR Take on Enzymology
AN EPR TAKE ON ENZYMOLOGY
Betty J. Gaffney1,2*

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The cytosolic protein lipoxygenase (LOX) is a large (100kD) football-shaped enzyme with a non-heme iron buried centrally. The iron, as ferric, oxidizes polyunsaturated lipids forming lipid hydroperoxides. As ferrous, it turns over the same hydroperoxides to form other products. Opportunities abound to study quantities of free radicals, iron oxidation state, and protein structures by EPR. Two themes have dominated years of attempts to pin down the mechanism. One involves the conformation of a fatty acid within its binding cavity: where are the polar and non-polar ends of the substrate and could they be reversed? The other theme addresses the nature of an impossibly large deuterium isotope effect when a fatty acid C-D bond is the site of the first step in the oxidation reaction. In summary, EPR experiments involving metal ions, anaerobic enzyme, and spin labeling have brought me, and others, closer to understanding classic questions about the lipoxygenase mechanism. Lipoxygenases exist in many organisms and they have a similar core structure but interesting variations in detail, such as manganese instead of iron. Formulating a unified mechanism and raison d'être for all of these is the goal, but the approaches are broadly applicable to other paramagnetic enzymes and proteins. Elucidating the enzymology of lipoxygenase by EPR includes quantitative simulation of S=5/2 metals (iron and manganese), determining the nature of spin label fatty acid binding, locating the entrance to the buried active site by DEER, and probing protein motions involved in opening that entrance. These points will be illustrated.

Figures. A Illustrates the distribution of S=5/2 iron spectra that contribute to the observed EPR spectra (X-band) of a bacterial lipoxygenase [1]. B is an interpretation of motion revealed in EPR spectra from a series of spin label fatty acids bound to lipoxygenase [2]. C Illustrates design of the DEER experiment to locate added spin labeled lipid [3].

References
SOLUTION NMR / MRI METHODS, MEDICINE, MATERIALS / SOLID-STATE NMR BIOLOGICAL APPLICATIONS/METHODS

Friday, July 28
08:30-09:45

Conggang Li
NMR Spectroscopy of Proteins and Nucleic Acids in Living Cells

Galina E. Pavlovskaya
Mechanically Induced $^{23}$Na MQF (Multiple Quantum Filtered) Responses in Biofluids as Potential Disease Markers

Bingwen Hu
New SSNMR Methods for $^{14}$N Direct/Indirect Acquisition
NMR SPECTROSCOPY OF PROTEINS AND NUCLEIC ACIDS IN LIVING CELLS

Conggang Li*, Yansheng Ye, Wenwen Zheng, Jiajing Zhao, Guohua Xu, Qiong Wu, Xiaoli Liu, Maili Liu

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In-cell NMR technique has attracted more and more interest because it can provide protein biophysics and biochemistry properties at atomic resolution. In the past decade, tremendous progress have been made in cellular NMR spectroscopy, including tailored isotope labeling and paramagnetic tag attachment, protein structure determination, protein posttranslational modifications, protein interactions, protein stability and so on.

In this talk, I will show that NMR spectroscopy, especially $^{19}$F NMR is a useful tool to assess biomacromolecular structure and function in living cells, which is difficult for other biophysical methods if not possible. Two examples, phosphorylation dependent protein degradation and G-quadruplex in the nucleus of living cells will be given to demonstrate the unique advantage of in-cell NMR.

References

Mechanically induced $^{23}\text{Na}$ MQF (multiple quantum filtered) responses in biofluids as potential disease markers.

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The discovery of markers specific for maladies is crucial as they provide timely assessment for diagnosis, prognosis and theranostics. While diagnosis and prognosis are well established, stratified medicine requires additional markers to timely monitor body response to treatment. Conventional markers can be either biochemical markers, imaging markers or a combination of both. We probe in this work potentially novel type of disease markers that appear only when molecules in body fluids and tissues are sheared or compressed.

Many body fluids are complex in nature, driven by body physiology. Their $\textit{in situ}$ rheology is determined by their molecular composition and also on how molecules adapt themselves in a deformation field as determined by fluids function in a body, for example how synovial fluid in load bearing joints performs as a lubricant and a shock absorber, how bile is passed through the duct during digestion, or how mechanics of red blood cells becomes impaired after dialysis. We demonstrate that $^{23}\text{Na}$ MQF methods are very efficient in detecting the formation of special molecular responses induced by shear field in a wide range of model biofluids, for example solutions of xanthan gum (Fig.1), kappa-carrageenan and bile salts. We also report the observation of shear-induced $^{23}\text{Na}$ MQF signals in solutions of hyaluronic acid and in the drug $\textit{Hylaritin V}$ that are currently used in osteoarthritis (OA) treatment. We have measured both $^{23}\text{Na}$ TQF and DQF MA signals in these fluids under physiological sodium concentration and strong $^{23}\text{Na}$ DQF MA responses in both fluids upon shear were found. Remarkably, the signals were absent in the absence of shear. Furthermore, no $^{23}\text{Na}$ TQF signals were detected with or without shear in some biofluids. To the best of our knowledge this intriguing effect has never been reported before.

We demonstrate that this effect is in line with theoretical predictions for a given combination of residual quadrupolar coupling constant values and MQF build up rates (Fig. 2).

The work is of great importance in designing the strategies for in vivo $^{23}\text{Na}$ MQF MRI as this technique is currently undergoing through a revolution. Physiologically body fluids and many body tissues are constantly sheared and are never at rest, therefore $^{23}\text{Na}$ DQF MA signals must always be probed even in the absence of $^{23}\text{Na}$ TQF response.


Fig. 1. Shear-induced molecular alignment detected by $^{23}\text{Na}$ DQF MA at 0.1M Na concentration in the solution. Molecular alignment is detected only in the shear changing zones [1].

Fig. 2. Theoretical simulation demonstrating time evolution of shear-induced MQF responses in 1% hyaluronic acid at 0.1M Na concentration in the solution.
New SSNMR methods for $^{14}$N direct/indirect acquisition

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To acquire the $^{14}$N wideline spectra directly, WURST-CPMG is usually employed[1]. However, strong baseline distortions may be observed, which make the extraction of the tensor information difficult. We propose a slight modification of the sequence, WURST-CPMG$_M$, in which we skip the first $M$ echoes and we only acquire the following ones. This simple treatment mostly eliminates the strong background signal and the ring down effects, leading to a flat baseline.[2]

To acquire $^{14}$N spectra indirectly, D-HMQC in overtone (OT) framework is often used. We recently modified the 90° composite pulses (e.g. COM-I$_m$ and COM-II$_m$), which are originally designed for spin 1/2 nuclei, for the $^{1}$H-$^{14}$N$_{OT}$ D-HMQC experiments, and showed that composite pulses enable symmetric excitations of $^{14}$N sites with large shift differences (Fig 2).[3] However, 90° composite pulses only excites symmetric horn bands (Fig. 1).

Furthermore, we modified the 180° composite pulses (e.g. COM-R5$_m$ and COM-R6$_m$), which are originally designed for spin 1/2 nuclei, for the $^{1}$H-$^{14}$N$_{OT}$ D-HMQC experiments. These 180° composite pulses could excite horn bands plus one center band (Fig. 1). Single pulse (SP) only excites center band. We used SPINACH to simulate D-HMQC experiments.

References:
UNUSUAL / MRI METHODS, MEDICINE, MATERIALS

Friday, July 28
08:30-09:45

Jens Michael Boss
Nanoscale NMR Using a Diamond Spin Sensor

Jan G. Korvink
Miniaturized Microfluidic Solutions for Chip-Scale Hyperpolarization

Melanie M. Britton
MRI of Electrochemical Systems: Batteries, Corrosion and Electroplating
Abstract

Nanoscale NMR using a diamond spin sensor

¹Department of Physics, ETH Zurich, Otto Stern Weg 1, 8093 Zurich, Switzerland.
(Dated: March 31, 2017)

Diamond has emerged as a unique platform for a variety of applications, both because it is very robust and because it can host defects with interesting properties. One of these defects, the nitrogen-vacancy (NV) center, has a single electron spin associated with it that shows quantum behavior up to room temperature. Our group is harnessing the properties of single NV centers for nanoscale NMR applications.

In this talk, I will present our efforts in the field of nano-NMR/MRI using the NV center as sensor. I will first introduce the basics of NV centers, and explain how it is used to sense ac magnetic fields. As an application, I will discuss the detection of NMR signals from nuclear spins in nanometer proximity to the NV center. I will show examples of Fourier NMR spectroscopy on single 13C nuclei within diamond, and small ensembles (~1e2-1e4) of proton spins on the diamond chip. I will conclude by discussing 3D mapping of single nuclear spins inside the diamond host with Angstrom precision, which is a first step towards atomic-resolution MRI.
Miniaturized microfluidic solutions for chip-scale hyperpolarization


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A consistent challenge to be overcome in magnetic resonance is detection sensitivity. Hyperpolarization techniques [1,2] aim to overcome Boltzmann population statistics and have been shown to improve sensitivity by orders of magnitude, enabling experiments that would have been otherwise impossible. One regime which stands to benefit from such signal improvement is the small sample regime (here taken to be sample volumes < 5 µL). In this regime there is an additional benefit to be gained by decreasing the detector size to more closely conform to the sample. Such micro detectors often have dimensions less than 1 mm, and present novel manufacturing challenges targeting high precision and mass fabrication. In this presentation, we will discuss a selection of unique opportunities to be explored using micro fabrication technologies aiming to co-integrate MR micro detectors and hyperpolarization techniques. We will focus on micro fabrication processes towards para-hydrogen and dynamic nuclear polarization hyperpolarization implementation within a microfluidic environment featuring integrated MR detectors. The challenges associated with sample handling, gas-fluid contact, and MW/RF sample co-access at the microscale within the context of MR will be discussed.

Fig. 1.: A) Combined microfluidic sample holder and EPR resonator. B) Measured S-parameters. C) Self-filling sample using capillary forces. D) PDMS membrane micro-contactor for parahydrogen delivery. E) PDMS contactor inside a 1.5mm Helmholtz microcoil for 500 MHz NMR.

References


MRI of electrochemical systems: batteries, corrosion and electroplating
Melanie M. Britton
School of Chemistry, University of Birmingham, Birmingham, UK.

The design and development of improved energy storage devices, and other electrochemical technologies, requires detailed understanding of the electrochemical reactions, ion transport and concentration gradients within these systems. However, there are few methods that are able to visualise and quantify these non-invasively, spatially, in situ and in real time. Magnetic resonance imaging (MRI) has proved to be an excellent tool for non-invasively studying complex, spatially heterogeneous chemical systems in materials, engineering and chemical research\(^1\). While, MRI has enormous potential for in situ investigation of the spatial distribution, speciation, and mobility of molecules and ions in electrochemical devices, there are currently very few examples of MRI being used to probe such systems. This is largely due to the experimental challenges associated with setting up an electrochemical cell inside a strong magnetic field and the imaging artefacts caused by the presence of metals that lead to undesirable variations in the radiofrequency (RF) and magnetic fields across the sample\(^2\). However, it has been found recently that such technical issues can be overcome and that it is possible to collect viable, quantitative, in situ data\(^3\)-\(^6\). This paper will discuss the challenges of studying electrochemical systems by MRI and demonstrate how they can be overcome to enable the collection of unique, viable, quantitative, in situ data during the operation of batteries, as well as other electrochemical systems, including corrosion\(^3\),\(^7\) and metal electroplating in ionic liquids.

(a) Model Zn-air cell (b) MRI of Zn dissolution in Zn-air cell\(^5\) (c) Cu corrosion cell (d) Cu\(^{2+}\) concentration map\(^7\) during electrodissolution of Cu\(^{2+}\).

SOLID-STATE NMR MATERIALS/ METHODS

Thursday, July 27
08:30-09:45

Robert W. Schurko

Philip J. Grandinetti
NMR Non-Crystallography: Measuring Bond Angle Distributions from $^2J$ Couplings in Silicate Glasses

Luís Mafra
Unprecedented Details on the Structure of CO$_2$ Species in Mesoporous Materials Using SSNMR and Modeling

Austin W. Lindquist\(^1\), Lucas D.D. Foster\(^1\), Luke A. O’Dell\(^2\) and Robert W. Schurko\(^1\,*\)

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High-power, rectangular pulses are often insufficient for the uniform excitation of ultra-wideline (UW) NMR patterns, which range in breadth from 250 kHz to upwards of 10 MHz, due to large anisotropic NMR interactions.\(^1,2\) For many years, UW powder patterns were commonly acquired with the application of frequency-stepped techniques, in which sub-spectra were acquired at even transmitter increments across the entire breadth of the powder pattern.\(^2\) Our research group suggested the use of wideband uniform-rate smooth-truncation (WURST) pulses\(^3\) for broadband excitation and refocusing of magnetization associated with anisotropically broadened patterns, in the form of the WURST-CPMG pulse sequence (for direct excitation, DE) and the broadband adiabatic inversion cross polarization (BRAIN-CP) pulse sequence (for indirect excitation, via CP).\(^4,5\) Both sequences are easy to use, require very low rf powers, and function under a variety of experimental conditions for both spin-1/2 and quadrupolar nuclides.

![OCTOBER pulses](image)

Our research group is currently trying to discover new pulse shapes that may provide comparable or superior excitation/refocusing characteristics to those of WURST pulses. Recently, we have been exploring optimal control theory (OCT),\(^6\) as implemented in the SIMPSON software package,\(^7\) as a means of identifying such pulses. In our work, OCT is used to find optimum pathways between spin states during pulse and evolution periods, for purposes of optimizing population transfer, efficient selection of specific coherences, and increasing signal-to-noise. The new pulses that emerge from our OCT simulations can be implemented in real-time experimental tests, aimed at producing high-quality spectra which are uniformly excited.

In this presentation, I will discuss a new class of pulses known as Optimal Control Theory Optimized Broadband Excitation and Refocusing (OCTOBER) pulses, for use in UW NMR experiments on stationary samples. First, the use of OCTOBER pulses for the DE of anisotropically broadened patterns of spin-1/2 and quadrupolar nuclides will be discussed. Second, the application of OCTOBER pulses in a Bloch-decay type experiment, which is capable of providing uniformly excited UW NMR spectra without the need for echo collection, will be presented. Third, preliminary studies of the use of OCTOBER pulses in BRAIN-CP (static) and \(^14\)N overtone (MAS) experiments will be shown. Finally, experimental examples involving \(^14\)N (\(I = 1\)), \(^35\)Cl (\(I = 3/2\)), \(^71\)Ga (\(I = 3/2\)), \(^59\)Co (\(I = 7/2\)), \(^119\)Sn (\(I = 1/2\)), and \(^195\)Pt (\(I = 1/2\)) will be given.

References
NMR Non-Crystallography: Measuring Bond Angle Distributions from $^2J$ Couplings in Silicate Glasses

Deepansh Srivastava, Philip J. Grandinetti*, Ohio State University, Columbus, OH, USA,
Jay Baltisberger, Berea College, Berea, KY, USA,
Pierre Florian and Franck Fayon, CEMHTI-CNRS, Orléans, France.

A great advantage of solid-state NMR spectroscopy is its ability to reveal and quantify atomic-level structure in materials where diffraction techniques fail. This is generally true in non-crystalline solids, where diffraction methods rarely reveal structural details beyond the first-coordination sphere except in the simplest of compositions. While it also true that the spectra of non-crystalline solids in many spectroscopies are often broad and featureless, magnetic resonance spectroscopy has a unique advantage in that the different NMR frequency contributions leading to these broadenings can be separated and correlated in multi-dimensional experiments. In this talk we describe recent efforts by our laboratory to:

1. develop a method called shifted-echo Phase Incremented Echo Train Acquisition (PIETA) for rapidly obtaining the 2D $^2J$ $^{29}$Si spectra at natural abundance in silicate glasses. By working at $^{29}$Si natural abundance levels (4.67%) we obtain the $J$ splittings as simpler overlapping doublet patterns, arising from isolated $^{29}$Si-O-$^{29}$Si linkages, instead of the overlapping multiplet patterns in $^{29}$Si enriched samples. Another advantage of natural abundance is that the homonuclear dipolar coupling between $^{29}$Si is easily removed with MAS due to its inhomogeneous nature. Because $^{29}$Si at natural abundance is a low sensitive nucleus and possesses exceptionally long relaxation times the use of PIETA is critical in obtaining a fast and accurate measure of the 2D $J$ spectrum.

2. identify relationships between $^2J_{\text{Si-O-Si}}$ and local structure. We show that the $^2J_{\text{Si-O-Si}}$ coupling correlates strongly with the inner Si-O-Si linkage angle between two SiO$_4$ tetrahedra and also exhibits a moderate dependence on the outer Si-O-Si linkage angles of the two tetrahedra. Combined with the dependence of the $^{29}$Si chemical shift on the mean Si-O-Si linkage angle we obtain a mapping of the 2D $J$ resolved resonances to the $^{29}$Si-$^{29}$Si inner linkage angle and mean linkage angle of the two tetrahedra.

3. measure and analyze the 2D $J$ resolved spectrum of silica glass in terms of a quantitative distribution of Si-O-Si bond angles present in the glass.

We determine a Si-O-Si bond angle distribution in silica glass with a mean/mode at $147^\circ \pm 1^\circ$ and a FWHM of $21^\circ \pm 3^\circ$. These results and their implications for various network structural models of silicate glasses will be described.
UNPRECEDENTED DETAILS ON THE STRUCTURE OF CO₂ SPECIES IN MESOPOROUS MATERIALS USING SSNMR AND MODELING

Luís Mafra¹*, Tomaž Čendak¹, Sarah Schneider¹, Paul Wiper¹, José R. B. Gomes¹, Moisés L. Pinto²

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Amine-functionalized porous sorbents are among the best materials for selective adsorption/capture of CO₂.[1,2] In these materials, the mechanism of CO₂ capture by the amines is assumed to be essentially the same as in aqueous amine solutions. In this presentation, we will show that this assumption is not correct and that the nature of the grafted amines and the silica substrate strongly influences the nature of the CO₂ species present. Studies focusing on the atomic-level characterization of the CO₂ species formed within the pore cavities are still scarce, with most reports based primarily on IR spectroscopy, often leading to controversial assignments.

The present work comprises NMR and computational studies of various functionalized SBA-15 silicas with primary, secondary, tertiary and mixed primary-secondary amines loaded with ¹³C-labeled CO₂, under controlled partial pressure, to characterize the chemisorbed CO₂ species.[1] NMR identified CO₂-adducts, which explain the high selectivity (up to 15000) towards CO₂ in CH₄/CO₂ mixtures. The combination of ¹H-²⁹Si/¹³C HETCOR and ¹³C{¹⁴N} recoupling experiments in as-prepared and H/D exchanged SBA-15 showed that the CO₂ species are engaged in very complex hydrogen bonding networks involving amine···amine and amine···silanol interactions, allowing to distinguish between carbamate, carbamic acid, alkylammonium carbamate species as well as extremely water-sensitive CO₂ species.[1]

We also show how to distinguishing by NMR between CO₂ species based on carbamic acid (neutral) and alkylammonium ion pairs (charged species) generated at the pore surface, which has been a longstanding problem. This work elucidates the structure of CO₂ species formed with surface amines and readily released by pressure swing.

References

EPR

Friday, July 28
08:30-09:45

Peter Z. Qin
Site-Directed Spin Labeling Studies of DNA Recognition by CRISPR-Cas9

Stefan Stoll
New Insights from AWG-Based Pulse EPR

Thorsten Bahrenberg
Improved Sensitivity for W-Band Gd(III)-Gd(III) and Nitroxide DEER Measurements with Shaped Pulses

Dane R. McCamey
The Role of Spin in Singlet Fission

Felix Kraffert
Spin-Correlated Polaron Pairs in Organic Solar Cells

Hermann Kraus
Visualizing the Radical-Pair Mechanism of Molecular Magnetic-Field Effects by Dual Fluorescence and Phosphorescence from an OLED

Timothee Chauviré
Elucidation of Photoinduced Electron Transfer Mechanism by Electron Spin Resonance in a Pyrylium-Based Photopolymerization Reaction
Site-Directed Spin Labeling Studies of DNA Recognition by CRISPR-Cas9

Peter Z. Qin*

Department of Chemistry and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089, USA
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In a type II Clustered-Regularly-Interspersed-Short-Palindromic-Repeats (CRISPR) system, RNAs derived from the CRISPR locus complex with the CRISPR-associated (Cas) protein Cas9 to form an RNA-guided nuclease that cleaves double-stranded DNAs at specific sites. In recent years, the CRISPR-Cas9 system has been successfully adapted for genome engineering in a wide range of organisms, leading to a revolution in genome engineering that is still rapidly unfolding. Studies have indicated that a series of conformational changes in Cas9, coordinated by the RNA and the target DNA, direct the protein into its active conformation, yet, details on these conformational changes, as well as their roles in the mechanism of function of Cas9, remain to be elucidated. Building on our recent work demonstrating the use of site-directed spin labeling to monitor nucleic-acid dependent conformational changes in Streptococcus pyogenes Cas9 (1), we reported here direct detection of Cas9-mediated DNA unwinding, which is a crucial step in Cas9 target recognition, by a combination of site-directed spin labeling and Molecular Dynamics simulations (2). Our results support a model in which the unwound non-target strand is stabilized by a positively-charged patch located between the two nuclease domains of Cas9, and reveal uneven increases in flexibility along the unwound non-target strand upon scissions of the DNA backbone. This study establishes the synergistic combination of spin-labeling and Molecular Dynamics to directly monitor Cas9-mediated DNA conformational changes, and yields information on the target DNA in different stages of Cas9 function, thus advancing mechanistic understanding of CRISPR-Cas9 and aiding future technological development.

References

New Insights from AWG-based Pulse EPR

Claudia E. Tait, Stefan Stoll

Department of Chemistry, University of Washington, Seattle WA, USA

The availability of sufficiently fast arbitrary waveform generators (AWGs) for the generation of pulse shapes in pulse EPR has recently led to a host of new methods involving shaped pulses, mainly for the purpose of wideband excitation. We present results from our recent methodological work using AWG-based pulse EPR. We discuss the consequences of utilizing a fully coherent EPR spectrometer for DEER spectroscopy [1], and we demonstrate that narrowband shaped pulses can be used to improve the sensitivity of ENDOR spectroscopy [2]. These results show that AWG-based EPR opens up new experimental opportunities, but also provides new insights into the spin physics of existing methods.

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Improved sensitivity for W-band Gd(III)-Gd(III) and nitroxide DEER measurements with shaped pulses

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Chirp and shaped pulses have been recently shown to be highly advantageous for improving sensitivity in DEER (double electron-electron resonance) measurements due to their large excitation bandwidth.[1-2] The implementation of such pulses for pulse EPR has become feasible due to the availability of arbitrary waveform generators (AWG) with high sampling rates to support pulse shaping for pulses with tens of nanoseconds duration. Here we present a setup for obtaining chirp pulses on our home-built W-band (95 GHz) spectrometer and demonstrate its performance on Gd(III)-Gd(III) and nitroxide-nitroxide DEER measurements. We carried out an extensive optimization procedure on two model systems, Gd(III)-PyMTA-spacer-Gd(III)-PyMTA (Gd(III)-ruler; zero-field splitting parameter (ZFS) D_{ZFS} ~ 1150 MHz)[3] as well as nitroxide-spacer-nitroxide (nitroxide-ruler) to evaluate the feasibility for these two important classes of spin labels used in modern DEER/EPR experiments. Consequently, we applied our findings to ubiquitin, doubly labeled with Gd-DOTA-monoamide (D_{ZFS} ~ 550 MHz) as a biological model system. Our experiments were focused on the questions (1) what are the best conditions for positioning of the detection frequency, (2) which pump pulse parameters (bandwidth, positioning in the spectrum, length) yield the best signal-to-noise ratio (SNR) improvements when compared to classical DEER, and (3) how does the sample’s spectral parameters influence the experiment. For the nitroxide-ruler, we report an improvement of around 1.8 in total SNR, while for the Gd(III)-ruler, the improvement was 3.1-3.3. Here, we also investigated two different approaches, pump on maximum and observe on maximum and compared the results. We find that the choice of the best set of parameters depends on the D_{ZFS} parameter of the sample. This work indicates that the implementation and use of an AWG is highly beneficial also at W-band and it gives rise to a significant improvement in experiment sensitivity and/or run-time. Finally, we demonstrate that shaped pulses can successfully be applied to a Gd(III) labeled biological sample. Here, improvement is highly needed due to limitations such as low concentration and fast relaxation, especially when focusing on in-cell applications. To our knowledge, this is the first comprehensive demonstration of shaped pulses for DEER experiments at high field.

References
THE ROLE OF SPIN IN SINGLET FISSION

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¹ARC Centre of Excellence in Exciton Science, School of Physics, UNSW Sydney, NSW 2052 Australia

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Singlet exciton fission is a process in which an optically prepared singlet state splits into two triplet excitons with (anti-)correlated spins. If harnessed efficiently, this process can be exploited to enhance the photocurrent of solar cells, raising the limiting power conversion efficiency from 33.7% to 45.9% under 1 Sun [1]. Fission is usually studied via optical spectroscopic techniques, such as pump-probe transient absorption, which drive electronic transitions and give details about the rate and yield of fission. However experimental insight into the nature of the triplet-pair state generated upon fission is lacking, as such approaches do not have the resolution required to identify the small spin couplings which influence these processes.

In this talk, I will discuss the application of transient EPR following optical excitation in molecular systems which undergo singlet fission. The spectral resolution this provides allows us to unambiguously determine the nature and spin dynamics of the triplet-pair states on the fission reaction coordinate.

By applying this approach to a range of novel molecules comprising pentacene dimers with engineered coupling [2], we show that singlet fission proceeds via a strongly coupled Quintet (S=2) state, before dissociating to two uncoupled Triplet (S=1) states [3]. The assignment of spin states is confirmed by the relative ratios of the coherent nutation frequency of the resolved spectral features. Alongside its importance in elucidating the nature of the fission process, this work also provides the first experimental identification of a quintet state in a closed-shell organic molecule.

I acknowledge the significant contributions of M. Tayebjee, S. Sanders, E. Kumarasamy, A. Asadpoordarvish, M. Y. Sfeir and L. Campos to this work.

References


Spin-Correlated Polaron Pairs in Organic Solar Cells

Felix Kraffert1, Robert Steyrleuthner1, Jan Behrends1

1 Berlin Joint EPR Lab, Fachbereich Physik, Freie Universität Berlin, Berlin, Germany

Spins of charge carriers play an important role for loss mechanisms and electrical transport in materials used in organic solar cells. They are also involved in processes such as singlet fission that provide the possibility of building solar cells with quantum efficiencies exceeding 100 %, i.e., photovoltaic devices delivering more than one electron and one hole per absorbed photon [1]. Techniques based on EPR spectroscopy can provide valuable insight into the microscopic details behind both efficiency-enhancing and efficiency-decreasing mechanisms.

We study intermediate paramagnetic states generated upon fission of one singlet exciton into two separated triplet excitons. Particular emphasis will be given to strongly coupled triplet pairs in organic molecules that form quintet states.

Furthermore, we report on the simultaneous detection of transient EPR (trEPR) as well as transient electrically detected magnetic resonance (trEDMR) signals from fully-processed and encapsulated solar cells. Our results obtained from solar cells based on poly(3-hexylthiophene) and the fullerene-based electron acceptor PCBM show that the resonant signals observed in low-temperature ($T = 80$ K) trEDMR spectra can be attributed to positive polarons in the polymer as well as negative polarons in the fullerene phase, indicating that both centers are involved in spin-dependent processes that directly influence the photocurrent [2]. Differences and similarities between the trEDMR and trEPR signals of spin-correlated polaron pairs, which mediate the conversion between photoinduced excitons and separated charge carriers, will be discussed.

We performed multi-frequency trEPR measurements and compared the results to semi-analytical polaron pair simulations in order to estimate the influence of dipolar and exchange couplings on the polaron-pair spectra (see Figure) and to determine inter-polaron distances [3].

Visualizing the radical-pair mechanism of molecular magnetic-field effects by dual fluorescence and phosphorescence from an OLED

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Large magnetoresistance effects which arise, for example, due to spin-dependent recombination rates are well-known for OLEDs in variable static magnetic fields[1], although models are still under debate given that they remain hard to verify from a measurement of integrated current and luminance[2]. Spin resonance of paramagnetic species enables direct manipulation of charge carrier and excitonic precursor spins, providing a wealth of new insight into dynamic spin properties.

Previous work on electrical or optical detection of spin manipulation[3,4] misses out on the opportunity to directly observe the presence of triplet exciton species that are at the heart of spin-dependent recombination models. We probe the interconversion of spin permutation symmetry of weakly bound electron-hole carrier pairs in an OLED between singlet and triplet by monitoring the yield of recombinant species, singlet or triplet excitons, through fluorescence and phosphorescence. Spin mixing occurs by spin precession in local hyperfine fields and is suppressed by an external magnetic field, leading to an anticorrelation of the change in fluorescence and phosphorescence yield which follows the same functionality as magnetoresistance. A resonant radio-frequency field reverses this effect, enhancing spin mixing to raise the phosphorescence and lower the fluorescence. The experiment offers the first direct probe of both interconverting spin states in the radical-pair mechanism, which features prominently in models of natural magnetoreception.

Fig. 1. a) Electroluminescence (EL) spectrum of an OLED made of phenylene-substituted ladder-type poly(para-phenylene) (PhLPPP) b) Spectrally resolved steady-state magnetoelectroluminescence and magnetoresistance under magnetic resonance driving.

References
TITLE: Elucidation of Photoinduced Electron Transfer Mechanism by Electron Spin Resonance in a Pyrylium-Based Photopolymerization Reaction

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Light-induced polymerization reactions are used in many industrial applications [1]. However, controlling the polymerization rate and chain growth is a crucial concern in polymer research to enhance the design of polymer architecture. The concomitant use of a photocatalyst to initiate the reaction, and a chain-transfer agent (CTA) to control the reaction rate and the polymer shape, have been recently optimized to achieve the cationic photopolymerization of isobutylvinylether (IBVE) [2].

In this publication, a metal-free photocatalyst p-methoxytriphenylpyrylium and different dithiocarbamate-derived chain-transfer agents were employed. The postulated mechanism of the photo-controlled polymerization of vinyl ethers is presented in Fig.1. Until now, the exact mechanism of the photopolymerization has remained unknown and multiple questions have to be solved: 1) Does the photoinduced charge transfer take place preferentially by one-electron oxidation of the IBVE or of the CTA? 2) What is the rate of this electron transfer? and 3) How do the chemical modifications of the photocatalyst and the CTA impact the photo-induced electron efficiency and the reaction rate?

To address these issues, we used several different spectroscopic techniques, including cw-ESR and fluorescence analysis. Cw-ESR spectroscopy enables us to detect and quantify the creation of pyrylium-derived radicals. An apparent quantum yield of the photoinduced electron transfer was thus deduced and compared against the different photocatalysts and CTA tested. Furthermore, minute time-scale kinetic ESR experiments allowed us to deduce the creation of new radicals and their evolution as a function of the irradiation time.

Fig. 1. Postulated catalytic cycle of the photo-controlled polymerization of vinyl ethers. (Chart reproduced from Kottisch et al. [1]). M = Isobutylvinylether monomer, Pn = polymer, ZCS2 = dithiocarbamate chain-transfer agent).

References


PLENARY SESSION 9

Friday, July 28
10:15-11:00

Songi Han
Dynamic Nuclear Polarization at 200 GHz by Microwave Pulse, Hardware and Sample Design
Dynamic nuclear polarization at 200 GHz by microwave pulse, hardware and sample design

Songi Han\textsuperscript{1,2,*}, Ilia Kaminker\textsuperscript{1}, Sheetal Jain\textsuperscript{1}, Alisa Leavesley\textsuperscript{1}, Alicia Smith Lund\textsuperscript{1}, Asif Equibal\textsuperscript{1}

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We present an integrated dynamic nuclear polarization (DNP) instrumentation operating at a magnetic field of 7 T and an electron paramagnetic resonance (EPR) frequency of 200 GHz that is designed to concurrently carry out DNP-enhanced NMR, continuous wave (CW) and pulsed EPR and pump-probe electron-electron double resonance (ELDOR) experiments at temperatures ranging from liquid helium to room temperature. These capabilities are added as modular add-ons to a commercial 7 T NMR system to enable dual DNP-EPR experiments under static and magic angle spinning (MAS) operation, and that in frequency, time-domain, CW and pulsed mode. We showcase electron spin relaxation and dual-frequency pump-probe ELDOR measurements to precisely track the fate of electron spin polarization under DNP conditions. We present systematic ELDOR and EPR data illustrating the effect of temperature, local electron spin concentration and radical geometry on DNP-inducing saturation of the EPR spectrum under static DNP conditions. Instrumentation development to achieve equivalent capabilities under MAS is underway. Furthermore, recent advances in arbitrary microwave form generation (AWG) at 200 GHz will be presented with shaped adiabatic pulse trains achieving significantly enhanced DNP performance compared to CW irradiation, bringing us a step closer towards time-domain DNP and EPR. Rationale design of sample conditions will be discussed, including the choice at of radical type, concentration and solvents, as well as the choice of complex fluids with superior solubility, coating and vitrification properties. These new technical capabilities present powerful opportunities for basic studies of DNP mechanisms towards a rational design of DNP experiments, as well as enhanced materials characterization opportunities from the study of catalyst surfaces, Li-ion battery electrolyte systems to semiconductors, enabled by DNP, EPR and NMR at a broad temperature range from room temperature down to $< 4$ Kelvin.
PLENARY SESSION 10

Friday, July 28
11:00-11:45

Robert Tycko
Solid State NMR of Protein Assemblies
This talk will present recent results from two projects: (1) Development of a detailed molecular structural model for fibrils formed by a low-complexity (LC) protein domain, namely the low-complexity domain of the "fused in sarcoma" protein (FUS-LC); (2) Characterization of local structural features within the HIV-1 capsid protein lattice, in both immature and mature states of HIV-1. Both projects depend on pulse sequence methods and assignment methods developed in our lab, and both projects benefit from segmental isotopic labeling.

**Fig. 1.** Transmission electron microscope image and structural model for FUS-LC fibrils

**Fig. 2.** Transmission electron microscope image of tubular HIV-1 capsid protein (CA) assemblies and 2D solid state 15N-13C NMR spectra of segmentally-labeled samples, labeled either in the 148-residue N-terminal domain (NTD) or the 83-residue C-terminal domain (CTD)
POSTER SESSION 1

Monday, July 24
16:30-18:30

Sharon Ruthstein
Janet E. Lovett
Veronika Szalai
Likai Song
John McCracken
Matthew J. Lawless
Art van der Est
Molly M. Lockart
Markus Teucher
Kurt Warncke
Boora Srinivas
Bingwen Hu
Jabor Rabeah
Takuya Akita
Mikhail Agrachev
Aiko Shimada
Colin G. McKay
Matthew D. Krzyaniak
Ichiro Yamaguchi
Alina Motygullina
Ashok Ajoy
C. Blake Wilson
Laura A. Buchanan
Joseph McPeak
Jordan N. Nelson
Hoang Long Nguyen
Lukas B. Woodcock
Sushil K. Misra
Daniel Lee
Chandrasekhar Ramanathan
Warren S. Warren
Alisa Leavesley
Lorenzo Bordonali
Jihyun Kim
Sebastian Z. Kiss
Alexandre A. Arnold

Bing Wu
Bernhard Blümich
Gigi Galiana
Fatemah Azadi Chegeni
Martin D. Gelenter
Simone Kosol
Rui Li
Shenlin Wang
Maarten Schledorn
Justine Dionne
Matthew Fritz
Hajime Tamaki
Peng Xiao
Benjamin Martial
Andreas Brinkmann
You-lee Hong
Xiaolong Liu
Adam R. MacIntosh
Christopher A. Klug
Eric Breynaert
Sungsool Wi
Yasuto Noda
Annica I. Freytag
Richard Bounds
Bernhard Blümich
Fazhan Shi
Renny Mathew
Yijue XuEdy Abou-Hamad
Mohamad Niknam
Shenggen Yao
Mariana Manzano Rendeiro
Julie Géan
Marius Wanko
Christian Damblon
Chin Yu
Jyoti Singh Tomar

Jingyu Zhan
Braden M. Roth
Georg Künze
Jaimie Van Pelt
Mylène Campredon
Bernhard Blümich
Jessica Villegas-Moreno
Yunhuang Yang
Ping Xu
Lech Kozerski
Johannes Kremser
M. Sameer Al-Abdul-Wahid
Ēriks Kupče
Calem Kenward
Sarah Roy
A. Samantha Lagaida
Xiaohong Cui
Vibhuti Wadhwa
Xu Zhang
Ēriks Kupče
Ahmad Saad
O. David Redwine
Keith C. Brown
Amandeep Singh
Jonathan Zopes
Chris W. Kirby
Chandrasekhar Ramanathan
Gaining molecular level information on the cellular copper cycle by CW and pulsed EPR spectroscopy

Sharon Ruthstein
The Chemistry Department, Faculty of Exact Sciences, Bar Ilan University, Israel.

In the last couple of years, my lab has been exploring the cellular copper cycle in eukaryotic and prokaryotic systems using Electron Paramagnetic Resonance (EPR) spectroscopy. While most of the proteins involved in the copper cycle are believed to be known, as well as some of the crystal structures, there is still lack of information on the kinetic and the transfer mechanism of the copper in the cellular environment. Since dysfunction of the copper regulation system can lead to neurological diseases and to the cell death, it is essential to understand every little detail in the copper cycle to be able to control it according to specific needs.

EPR has become a powerful tool for studying complex dynamic biological systems since it is not limited to the protein size and does not require crystallization. Hence, the biological system can be studied in solution, lipids, and even the cellular environment.

While CW-EPR experiments are mostly sensitive to the close environment of the paramagnetic center (up to 1.8 nm), pulsed EPR spectroscopy can provide nanoscale type of information by measuring the dipolar coupling between paramagnetic centers in the range of 2.0-8.0 nm. With the progress of the pulsed EPR methods, several computational programs have been developed to correlate between the distance distribution functions derived from the EPR and the structure of the studied system.

In our group, we are applying various EPR measurements together with computations, biochemistry experiments, CD and NMR to identify the copper binding sites, as well as to understand how one protein in the cycle coordinated to another protein to transfer the metal ion. We target the conformational changes that occur in each protein, and we aim to gain also kinetic data on the transfer mechanism.

In this talk, I will present our results on the copper transfer mechanism in the human cell. From the blood carrier protein, human serum albumin (HSA), through the Ctr1 copper transporter, and to the metallochaperone Atox1. Utilizing EPR spectroscopy together with CD and NMR, we succeeded to target several Cu(II)/Cu(I) binding sites in Ctr1, as well as to get structural information on the interaction between HSA and Ctr1 and between Ctr1 and Atox1.

References
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Exploring Gd(III) and Cu(II) binding in a family of coiled-coil peptides with EPR distance measurements

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Peptides of form Ac-G(IAAIEQK)ₓ-G-NH₂ have a tendency to form coiled-coils due to the helical repeat of varying hydrophilicities and interhelical bridges of the side chains in the heptad repeating unit. This sequence has been modified to contain a hard oxygen binding site by replacing the Ile in one repeat with an Asn and Asp pair and a Trp replacing the Gln adjacent to this metal binding site. The peptide with 𝑥 = 5 and replacement of Ile 4 and 5 has been previously shown to bind to Ln(III) ions such as Tb(III) and Gd(III) selectively over Ca(II) ions and to display interesting luminescent and MRI contrast agent capabilities respectively [1]. Later work demonstrated that only the Asp is necessary for binding if the Ln(III) is placed in the first heptad repeat domain and that water molecules are now also directly coordinated [2].

Here we will demonstrate that the coiled coil peptides can be designed to hold two Gd(III) ions by measuring distances between pairs using DEER and comparing to the expected distances (Fig. 1). We then show that the same distances can be measured if Cu(II) ions are present rather than Gd(III). Hyperfine spectroscopy was used to probe whether the Cu(II) are directly bound to nitrogen atoms and negative results further strengthen the case that the Cu(II) is being bound in a wholly hard oxygen peptide environment. To the best of our knowledge, this is the first example of this coordination for Cu(II) in peptides/proteins.

Fig. 1. Four of the five coiled-coils bound showing distance estimates between the metal binding sites.

References

Integration of a new bridge concept in a 34 GHz pulsed/CW EPR spectrometer

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We present a 34 GHz electron paramagnetic resonance (EPR) spectrometer using a novel microwave bridge design that employs two stages of up/down conversion. As a result, a variety of excitation and detection methods have been implemented and compared. This spectrometer operates in both pulsed and continuous wave (CW) modes. Our versatile EPR spectrometer system builds on literature precedent and is functionally comparable to commercially-available systems built using a different approach. For operation in pulsed mode, the single shot signal-to-noise [1] was determined using a Böhlen-Bodenhausen refocusing scheme [2] employing sech/tanh adiabatic pulses (Figure 1) to demonstrate the ability to directly input arbitrary waveform pulses into the bridge. Four-pulse DEER on a series of doubly-spin labeled alpha-helical peptides produced DEER traces of excellent signal-to-noise in 10 min. Overall, we have successfully built a combined pulsed/continuous wave EPR spectrometer for routine use in the CNST user facility that integrates pulse shaping and operates at 34 GHz, allowing a wide variety of conventional and more advanced pulsed EPR measurements to be performed.

Fig. 1. Room temperature broadband echo from a 3 mm diameter fused quartz sample irradiated to 1 kGy. Shown is the absolute value of the quadrature detected echo generated with a single shot. The inset depicts the Böhlen-Bodenhausen refocusing scheme, where the pulses have simulated bandwidths of 120 MHz. The durations of the π/2 and π pulses are 200 ns and 100 ns, respectively. Following the approach taken in [1], the SNR is the echo amplitude divided by the standard deviation of the noise and is estimated to be 67.3 using an overcoupled commercial resonator, with Q₀ ≈ 2000.

References
HIV enters human T cells through the fusion of viral and host-cell membranes. This fusion process is mediated by a surface protein, gp41, and the platform provided by the cholesterol-rich viral membrane. The membrane proximal ectodomain region (MPER) and transmembrane domain (TM) of gp41 play critical roles in this fusion process and is a major target of anti-gp41 antibodies and vaccine design. Here, EPR and spin-labeling techniques were used to define MPER/TM conformation on the membrane, MPER/TM–lipid interaction, and how anti-gp41 antibodies recognize their membrane-immersed epitopes. The analyses of several HIV-1 clade B and clade C MPERs revealed a structurally conserved pair of helices immersed in the viral membrane separated by a flexible hinge. A few charged residues and the residues around the hinge are crucial to MPER/TM structure and orientation relative to the membrane, as demonstrated by EPR assays with mutations of these residues. Furthermore, neutralizing anti-gp41 antibodies disrupt the MPER hinge function by perturbing the MPER hinge orientation, and/or extracting part of the MPER from the membrane. The interaction can be a stepwise rearrangement through an apparent scoop-like movement of the antibodies’ long and unique CDRH3 segments. Mutations of the CDRH3 segments reduced the ability of the antibodies to extract MPER residues from the membrane. In addition, MPER/TM–membrane interaction and antibody binding are modulated by lipid composition and cholesterol content. These findings have revealed important features of protein–lipid and gp41–antibody interactions at the HIV membrane interface.
Aromatic Amino Acid Hydroxylases: Exploring the Details of a Catalytic Cycle with EPR Spectroscopy

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The aromatic amino acid hydroxylases catalyze the hydroxylation of the side-chains of the aromatic amino acids, phenylalanine, tyrosine and tryptophan, and are essential for proper function of the liver and central nervous system. This chemistry requires the binding of the amino acid substrate, a tetrahydrobiopterin cofactor, and molecular oxygen to an Fe(II) active site. In the enzyme’s resting state, the metal ion features an octahedral coordination geometry with one coordination face, consisting of the side chains of two histidines and a glutamic acid, provided by the protein, and the remaining three coordination sites occupied by water ligands. While the diverse chemistry catalyzed by non-heme iron enzymes is often attributed to the facial arrangement of these open coordination sites, the proposed catalytic mechanism for these enzymes calls for the initial binding of both substrate and the tetrahydrobiopterin cofactor as second coordination-sphere ligands. In our studies, we have used nitric oxide, NO, as a substitute for O₂ for the purpose of poising the high-spin Fe(II) site in an S=3/2 \{FeNO\}₇ form that is amenable to X-band EPR spectroscopy. Using ²H-Electron Spin Echo Envelope Modulation (ESEEM) and ¹H – Hyperfine Sublevel Correlation (HYSCORE) methods we have been able to measure ligand hyperfine couplings from substrate, cofactor and coordinated water ligands that have provided important details regarding the catalytic mechanisms of Phenylalanine and Tyrosine Hydroxylases.
**Cu²⁺ Based ESR as a Probe for Structural Morphology in Proteins and DNA**

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Electron spin resonance (ESR) spectroscopy in conjunction with site-directed spin labeling provides key insight into the structure and dynamics of biological macromolecules. Paramagnetic transition metal ions, such as Cu²⁺, have become important spin labels. Similar to nitroxide based radicals, Cu²⁺ can be site specifically incorporated into both proteins and DNA. In addition, paramagnetic metal ions often serve as natural cofactors within metalloproteins, or are used as linkers in oligomeric assemblies. This poster gives two specific examples of Cu²⁺ uniquely used in ESR: as a rigid reporter in proteins and as an accurate ruler of helical distance in DNA.

The double histidine motif is a unique Cu²⁺ binding methodology which uses two natural amino acid residues to selectively incorporate the divalent metal ion into either an α-helical or β-strand environment [1]. The Cu²⁺ is introduced complexed to the chelator iminodiacetic acid which improves selectivity to the binding site. This method requires no post-synthetic modification and provides easily interpretable, narrow distance distributions. The binding characteristics of both α-helical or β-strand binding sites have been characterized and ESR DEER sensitivity has thus been optimized.

Cu²⁺ can be introduced within the helix by use of a 2’2-dipicolylamine substituted nucleic acid [2]. Since the paramagnetic center is located within the interior of the helix, this spin labeling method reports most probable distances that match within 1 Å of predicted models of DNA backbone distances. The 2’2-dipicolylamine is easily incorporated into small DNA sequences and requires no post synthetic chemistry aside from the addition of Cu²⁺. Furthermore, this labeling method is structure- and nucleotide independent.

**References**


An EPR, ENDOR, ODMR Study of the Formation of a Bacteriochlorophyll g'/Chlorophyll aF Special Pair in a Homodimeric Photosynthetic Reaction Center

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Abstract

The heliobacteria are a family of strictly anaerobic, gram positive, photoheterotrophs in the Firmicutes. They make use of a homodimeric Type I reaction center (RC) comprised of two identical protein subunits that binds cofactors for light absorption and energy conversion via electron transfer. The primary donor in the electron transfer is a special pair of bacteriochlorophyll (BChl) g' molecules referred to as P800. It is known that in presence of light and oxygen BChl g is converted to Chl aF and the RC ceases to function1. However, the loss of P800 activity not linear with the loss of BChl g' suggesting that the RC remains functional if only one of the two BChl g' molecules of the special pair is oxidized. Here, we present EPR, ENDOR and ODMR data characterizing the lifetime and spin distribution of the cation radical of the BChl g'/Chl aF special pair. Tranient EPR data show that in anaerobic RCs the lifetime of P800+ is 4.2 ms but after exposure to oxygen the lifetime is shortened to 0.3 ms and the line-width of P800+ increases. The rate of electron transfer from the primary acceptor A0 to the iron sulfur cluster FX, as estimated from the net polarization generated by singlet-triplet mixing during the lifetime of P800+A0+, is unchanged. The ODMR data also show that conversion of the BChl g results in increased formation of triplet states of both BChl g and Chl aF but no evidence for formation of P800+ by charge recombination is found. Q-Band pulse ENDOR data show that in anaerobic RC's the unpaired electron is equally shared between the two BChl g' molecules of the special pair. However, upon exposure to oxygen, the proton hyperfine couplings increase, suggesting that the unpaired electron experiences greater localization in the BChl g'/Chl aF special pair.

References

Cytochrome P450 enzymes (CYPs) are heme-containing monooxygenase enzymes that exist in nearly every living organism. They are responsible for oxidizing a wide variety of substrates in biosynthetic and detoxification pathways and are a common target for drug design. In the resting state CYPs have a water molecule bound as the sixth axial ligand to the heme. Typically, potential drugs are thought to displace this water and either bind directly to the heme or occupy the active site without binding the heme, leaving the heme in a high-spin state. However, in some cases, the drug does not displace the axial water; instead, it binds through a hydrogen bonding network to the heme. This binding mode is demonstrated in only a few crystal structures and is generally considered to be rare. We use electron paramagnetic resonance (EPR) to characterize CYP binding modes in more detail. We can distinguish between water-bridged binding and directly coordinated binding using the pulsed EPR technique hyperfine sublevel correlation spectroscopy (HYSCORE). We find that the water-bridged binding motif is more common than previously thought, and in several of the CYP/drug combinations we have looked at, both water-bridged and directly coordinated complexes exist simultaneously. In this study, we look at how the CYP binding modes we observe with EPR change when multiple drugs are added. In particular, we study acetaminophen (APAP) and caffeine binding to CYP3A4, a known drug-drug interaction where APAP and caffeine bind simultaneously in the CYP3A4 active site, which leads to altered metabolism and unintended metabolic products. We find that the axial water protons are still present in the HYSCORE spectrum when saturating amounts of APAP are added, which suggests APAP binds to CYP3A4 via a water bridge. The addition of caffeine does not cause a loss of water protons in the HYSCORE spectrum, but it does cause an overall loss of signal, most likely because the heme is converted to a high-spin state. We also use deuterium-labeled drugs to determine whether acetaminophen and caffeine are occupying the CYP active site simultaneously. These results aid our understanding of how dynamic CYP/drug interactions are and might help shed light on cases where CYP drug metabolism results in unanticipated products, such as in cases of drug/drug interactions or toxic product formations.

References
Interactions and conformational changes of Bcl-2 proteins regulating cell death

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Proteins of the Bcl-2 family are essential regulators in the mitochondrial pathway of apoptosis. They form a complex regulatory network whose understanding is a central objective in cancer research. Members of the Bcl-2 family share Bcl-2 homology (BH) domains, forming three distinct groups: Bax and Bak constitute the group of pro-apoptotic effectors whose activity is mediated by anti-apoptotic regulator proteins like Bcl-2 and Bcl-xL. The group of proapoptotic BH3-only proteins (e.g. Bid, Bim, Bad) can inhibit the activity of the anti-apoptic regulators and/ or activate the pro-apoptotic effectors Bax and Bak [1].

Upon activation, cytosolic monomeric Bax undergoes major conformational changes, oligomerizes and enters the mitochondrial outer membrane (MOM) [2]. Pore formation and the subsequent release of cytochrome c lead irreversibly to cell death. Here, we show how it is possible to monitor interactions in a minimal regulatory Bcl-2 network comprising of Bax, cBid and Bcl-xL via site-directed spin labeling EPR. To this end, we performed continuous wave EPR kinetic experiments to follow the mobility of spin labeled Bax, cBid or Bcl-xL in the presence of the other Bcl-2 regulators and liposomes mimicking the MOM. Furthermore, we performed DEER experiments to unveil how the interactions modify the structure of the proteins at the membrane and ODNP experiments giving insight in the membrane-water interfaces of the proteins.

References
DUAL RADICAL CAPTURE AND REARRANGEMENT-ENABLING FUNCTIONS OF THE SUBSTRATE RADICAL MACROSTATE IN B<sub>12</sub>-DEPENDENT ETHANOLAMINE AMMONIA-LYASE REVEALED BY TIME-RESOLVED EPR SPECTROSCOPY

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Adenosylcobalamin (coenzyme B<sub>12</sub>)–dependent ethanolamine ammonia-lyase (EAL) plays a key role in aminoethanol metabolism involved in Salmonella- and Escherichia coli-induced disease conditions and microbiome homeostasis in the human gut. To gain fundamental insight into enzyme function toward therapeutic control, decay kinetics of the cryotrapped 2-aminopropanol substrate radical in EAL from Salmonella typhimurium are measured over a temperature (T) range of 220-250 K by using T–step reaction initiation and time-resolved, full-spectrum continuous-wave electron paramagnetic resonance (EPR) spectroscopy [1]. The observed substrate radical reaction kinetics are characterized by two pairs of biexponential processes: native decay to diamagnetic products, and growth of a non-native, uncoupled radical species and Co<sup>2+</sup> in cobalamin. The multi-component kinetics are simulated by using a minimal model, in which the substrate radical macrostate, S<sup>•</sup>, is partitioned by a free energy barrier into two sequential microstates at low-T: (1) S<sub>1</sub><sup>•</sup>, a relatively high-entropy/high-enthalpy microstate with protein configuration that captures the nascent substrate radical in the terminal step of radical pair separation, and (2) S<sub>2</sub><sup>•</sup>, a relatively low-enthalpy/low-entropy microstate with protein configuration that enables the rearrangement reaction. The bi-partitioning of S<sup>•</sup> is also observed for substrate aminoethanol, with different T-dependence and functional manifestations [2]. The non-native, destructive pathway of reaction of S<sub>1</sub><sup>•</sup> at T≤250 K is caused by a prolonged lifetime in the radical pair capture state, owing to the S<sup>•</sup> partitioning barrier. The monotonic S<sup>•</sup> decay over 278–300 K indicates that the free energy barrier to the protein configurational fluctuations that conduct interconversion of S<sub>1</sub><sup>•</sup> and S<sub>2</sub><sup>•</sup> is latent at physiological T values (lies significantly below the barrier of the chemical, rearrangement step). Overall, the results resolve the contributions of protein configurational microstates, and specific protein configurational fluctuations, to the dual radical–capture and rearrangement–enabling functions of the formal substrate radical macrostate in EAL catalysis. Supported by NIH NIDDK R01 DK054514.

References
Experimental and Theoretical investigations of EPR and optical spectra of Cu$^{2+}$ spin probe in BaO-TeO$_2$-B$_2$O$_3$ glasses

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Electron Paramagnetic Resonance (EPR) spectroscopy is a powerful and useful magnetic resonance phenomena to investigate the local structure of the host glass network containing transition metal (TM) ions such as Cu$^{2+}$. Under suitable magnetic field and microwave frequency resonance phenomenon takes place. The analysis of the optical and EPR spectra gives information about the local structure distortion of Cu$^{2+}$ ions in the host glass matrix. In the present investigation the glass system $x$BaO-$(30-x)$TeO$_2$-69B$_2$O$_3$-1CuO was prepared by the conventional melt quenching technique. The spin-Hamiltonian parameters and the energy differences between the excited $^2$B$_{2g}$ and $^2$E$_g$ to the ground $^2$B$_{1g}$ states were calculated from both experimental and theoretical studies. In the theoretical calculations, the tetragonal field parameters D$_s$ and D$_t$ are taken into consideration with the estimation of relative elongation of the ligand octahedron around the Cu$^{2+}$ ions. Experimental spin-Hamiltonian parameter values were used to obtain the simulated EPR spectra. Both the EPR spectra (experimental and computer simulated) are in good agreement with each other. The number of copper spins (N) participating in the resonance, paramagnetic susceptibility ($\chi$) values and normalized covalencies were evaluated. The peak-to-peak linewidth ($\Delta B$) and line intensity (I) are changing nonlinearly with the alkaline-earth content in the glass network. The values of $\alpha^2$ and $\beta_1^2$ which designate in-plane $\sigma$ and in-plane $\pi$-bondings were found to be moderately ionic nature in the present glass system. Whereas $\beta^2$ which represent out-of-plane $\pi$-bonding is completely ionic in nature.
The Li⁺ insertion/extraction mechanism study of MOFs-based electrodes via SSNMR, EPR, and sXAS techniques

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The in-depth Li⁺ insertion/extraction behavior study of metal-organic frameworks (MOFs) is of great significance in boosting their application in rechargeable lithium-ion batteries [1, 2, 3, 4]. Here we present a systematic study to this regard via SSNMR, EPR, and synchrotron-based soft X-ray spectroscopy (sXAS) techniques.

NMR signals from paramagnetic MOFs are generally difficult to detect because of the extremely strong hyperfine interactions between unpaired electron and nucleus. We apply a rotor-synchronized spin echo pulse, with typical τ duration of one rotor period (~16.67 μs), a very short recycle interval of 20 ms, and a MAS rate of ~60 kHz to collect the ex-situ ¹³C spectra of Co₂(OH)₂BDC MOF. The results suggest that Li-ions are reversibly inserted/deinserted to the carboxyl groups and benzene rings of Co₂(OH)₂BDC, which leads to a high reversible Li⁺ storage capacity [3].

Furthermore, we also carry out the ex-situ EPR study of CoHNta MOF. The localized high-spin Co²⁺ can only be observed below ~40 K due to strong dipole-dipole couplings between three parallel electrons, hence, all the EPR spectra are recorded with liquid helium. The results imply that the localized high-spin Co²⁺ with three localized electrons would convert to high-spin Co²⁺ with delocalized conducting electron upon cycling [4].

Fig. 1. High resolution ¹³C MAS NMR spectra of the Co₂(OH)₂BDC materials at the fresh, fully discharged and fully charged states and the suggested organic-moiety-dominated Li⁺ insertion/extraction mechanism.

Fig. 2. X-band EPR spectra at 2 K recorded on the CoHNta electrode materials cycled to various states-of-charge (SOC).

References:
Cu/radical systems are very important catalysts not only for selective oxidation of alcohols to aldehydes but also in biology. One of the most efficient and selective catalytic systems for aerobic alcohol oxidation is a CuI/bipyridine (bpy)/N-methylimidazole (NMI)/2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) catalyst which works at room temperature using air as oxidant. The reaction mechanism has been the subject of extensive recent investigations, however, it was still unclear until recently. This was mainly due to the lack of coupled spectroscopic techniques that allow a deeper insight from different perspectives to distinguish individual reaction steps and intermediates while measuring the catalytic activity. Recently, we have developed simultaneously coupled operando EPR/UV-vis/ATR-IR spectroscopy that allows comprehensive mechanistic studies of this catalyst system. The results show that TEMPO, in contrast to previous proposals [1], is acting as a stabilizer for the active (bpy)(NMI)CuII−O2•−−TEMPO intermediate without participating in the redox cycle itself [2]. The proposed reaction mechanism includes two major steps: i) formation of the active species by oxidation of the CuI precursor to CuII and ii) oxidation of the alcohol and re-reduction of the active CuII complex (Figure 1).

We have found by operando EPR and UV-vis investigations in combination with cyclovoltammetry that the rates of CuI oxidation and CuII reduction and, thus, the rates of benzaldehyde formation depend on R of the R−N moiety in the imidazole ligand (IM) [3]. For R = H and alkyl, CuI oxidation to CuII is fastest while re-reduction is slowest for R = H. Modification of the CuI/CuII redox shuttle by changing the R substituents of the IM ligand lowers the total benzaldehyde formation rates in the order R(+I effect) > R(conjugated system) > R = H.

Depending on the ligands, different structures of the active Cu complex have been proposed such as single, bi- or tetranuclear CuII species. However, we have found for this particular system that Cu single sites are the active species responsible for alcohol oxidation as evidenced from operando UV-vis/ATR-IR and XAS.

References:
EPR Study on Magnetic Properties of Nitroxide Radical Liquid Crystals

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All-organic liquid crystals (LCs) containing a five-membered ring nitroxide radical (NR) moiety in the mesogen core (NR-LCs) have attracted a great deal of attention as one of metal-free magnetic soft materials because they exhibit some fascinating properties in the externally applied magnetic fields; e.g., droplets of NR-LCs are attracted by a magnet and paramagnetic susceptibilities of the compounds increase at their crystalline (Cr)-to-LC phase transitions (magneto-LC effects) [1]. It has been reported that the inhomogeneity of intermolecular magnetic interactions would be one of the origins of the magneto-LC effects [2]. Here, we aim to quantitatively elucidate the contribution of the inhomogeneous intermolecular interactions to the magneto-LC effects by means of electron paramagnetic resonance (EPR) spectroscopy. We report the relationships between the Gaussian components of EPR spectra and the paramagnetic susceptibilities.

We designed and synthesized a new chiral NR-LC (2S,5S)-1 showing highly ordered chiral smectic A (SmA*) phase (Fig. 1), and observed its EPR spectrum at each temperature. Observed spectra were deconvoluted by derivative pseudo-Voigt function \( P'V'(H) \), which is a linear combination of a derivative Lorentzian function \( L'(H) \) and a derivative Gaussian function \( G'(H) \), to gain Gaussian components of the EPR spectra,

\[
P'(H) = \eta L'(H) + (1 - \eta)G'(H)
\]

where \( \eta \) is mixing parameter (0 < \( \eta \) < 1). While relative magnetic susceptibility value \( \chi_{rel} = \chi(T)/\chi(T = 30^\circ C) \) abruptly increases at phase transitions, mixing parameter \( \eta \) decreases at the same phase transitions (Fig. 2). The decrease of \( \eta \) indicates that the Gaussian components increase due to the inhomogeneous intermolecular interactions. Thus, we can conclude that the inhomogeneous intermolecular interactions considerably influence the magneto-LC effects. The analysis method of the correlation between the Gaussian linewidth of the spectra and the magneto-LC effects will be also discussed in detail.

Fig. 1. Molecular structure of (2S,5S)-1 with phase transition temperatures.

Fig. 2. Temperature dependences of relative paramagnetic susceptibility \( \chi_{rel} \) (closed circles) and mixing parameter \( \eta \) (open circles).

References

Gold nanomagnetism is a fascinating, yet controversial field: available results are contradictory and there is no full understanding and agreement of why a magnetic moment appears at the nanoscale.\textsuperscript{1} The main reason is, in our opinion, the lack of a precise dimensional and charge control of the systems. We overcame these issues by using atomically precise monolayer-protected gold clusters (MPCs) of controlled charge state. In particular, we used \ce{Au25(SR)_{18}} (SR = thiolated ligand) which has a core of only 1 nm and is the most stable and characterized molecular MPC. Several properties of the isolated clusters in solution have been studied in detail, such as its distinct electrochemical, NMR, and optical behaviors. Whereas \ce{Au25(SR)_{18}^-} and \ce{Au25(SR)_{18}^+} are diamagnetic, the neutral form, \ce{Au25(SR)_{18}^0}, is paramagnetic. We studied this type of cluster both in solution and in the solid state to see how the individual paramagnets interact. The \ce{Au25(SR)_{18}^0} clusters were protected by different thiolated ligands. The magnetic properties were studied by different continuous wave EPR and pulsed EPR and ENDOR techniques in combination with NMR.\textsuperscript{2,3,4,5} Whereas interconnection of individual \ce{Au25(SC4H9)_{18}^0} clusters through Au-Au bonds yields a polymer behaving as a one-dimensional antiferromagnetic system, the use of other ligands gives rise to different magnetic orderings, such as in the case of \ce{Au25(SCH2CH2Ph)_{18}^0}. Previous magnetometry studies only detected paramagnetism.\textsuperscript{6,7} We detected by EPR\textsuperscript{8} that a film, \ce{Au25(SCH2CH2Ph)_{18}^0} displays paramagnetic behavior but, at low temperature, ferromagnetic interactions are detectable. One or few crystals display ferromagnetism. Microcrystals show distinct paramagnetic, superparamagnetic, and ferromagnetic behaviors. The magnetic properties were rationalized using a superatom-model approach that pointed to the importance of considering both spin-orbit coupling and crystal distortion in understanding the properties of molecular Au clusters. The results were confirmed by the DFT and MD calculations.

References

Estimation of the mixing ratios of the possible source rocks in the present river bed sediments from ESR signals of quartz

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Bedrock is broken down by weathering and mixes with other materials on the Earth surface. The broken and weathered bedrocks change into unconsolidated materials, which are then carried downstream by rivers. When a new procedure for clarifying provenance of sediment is established, it can give significant information on the erosion processes, river contention, and crustal movement suggesting the environments at the time of sediment transportation.

Recently, Electron Spin Resonance (ESR) signal intensity of the E₁’ center of quartz is shown to be a useful parameter to investigate the provenance of aeolian dust [1] - [3]. ESR signal intensities of the Al, Ti–Li, and E₁’ center of quartz showed useful in estimating sediment provenance [4]. It is possible to estimate the mixing ratios of the possible source rocks in the present river bed sediments from the Al and Ti–Li center signal intensities of quartz [5].

In this study, we will report estimation of the mixing ratios of the possible source rocks in the present river bed sediments of the Kizu River basin (western Japan) from the Al, Ti–Li, and E₁’ center signal intensities in quartz grains. All quartz samples were irradiated by gamma ray to a dose of 2.5kGy. ESR signals were observed by ESR spectrometers (JES-X320; X-band JEOL RESONANCE Inc.). It is suggested that measurements of ESR signals intensities of multiple impurity center is effective in identifying the source of quartz and in estimating the sediments provenance.

References
Multiple Field and Frequency Spin Dependent Charge Pumping in Silicon Germanium Metal Oxide Semiconductor Field Effect Transistors

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We utilize a novel combination of electrically detected magnetic resonance (EDMR) techniques to study defect centers at the heterointerface between silicon germanium and HFO₂ based dielectrics in 0.75 x 50 µm² metal oxide semiconductor field effect transistors (MOSFETs). The interface defects under study occupy a nearly atomically flat interface so the effective sample volume is roughly 0.01 µm³. The replacement of silicon with silicon germanium alloys, (SiGe) and the replacement of SiO₂ gate dielectrics by HFO₂ based dielectrics provide a path to extend Moore’s law beyond the fundamental physical limits of Dennard scaling. We show that one may explore point defects at the SiGe/dielectric interface in SiGe MOSFETs utilizing the EDMR technique of spin dependent charge pumping (SDCP).¹² In SDCP, a trapezoidal voltage waveform is applied to a MOSFET gate. In a p-channel device, the waveform sweeps the SiGe/dielectric Fermi energy from slightly below inversion (near the bottom of the SiGe bandgap) to slightly above flat bands (near the top of the SiGe bandgap).

The periodic filling up and emptying out of defect levels at the SiGe/dielectric heterointerface generates a current measured from the source and drain to body. Since charge capture events at paramagnetic defects are spin dependent, this current can be utilized as an extremely sensitive probe of paramagnetic centers at the interface. The transistor area is approximately 50 µm by 0.75 µm. The SiGe composition is approximately 40% Ge and 60% Si; the effective oxide thickness is 0.9 nm. We have made SDCP measurements at X-Band (9.74 GHz) and ultra-low frequency (368 MHz) utilizing 1 MHz trapezoidal gate wave forms with 120 ns rise and fall times. The X-Band measurements yield a single line SDCP spectrum with an isotropic g=2.0055 with a somewhat orientation dependent linewidth of up to about 15 Gauss. We have made SDCP measurements of comparable sensitivity at a resonance frequency of 368 MHz at which the g resolution is, of course, much lower. In addition we observe a quite strong response of the charge pumping current when the magnetic field vector is swept through zero. By this we mean the magnetic field vector oriented along the z axis is swept from a negative value, through zero, to a positive value. Our preliminary results suggest that these heterointerface defects primarily involve silicon atoms back bonded to various combinations of silicon and germanium atoms.

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The Quintet State in Singlet Fission

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There has been a renewed interest in singlet exciton fission (SF) due to its potential for increasing the maximum efficiency of photovoltaics.\textsuperscript{1} SF is the spin allowed process in which a high energy photogenerated singlet exciton rapidly down converts into two low energy triplet excitons. The correlated triplet pair formed through SF is initially in a singlet state, \(1(T_1T_1)\), this triplet pair can undergo spin-mixing to form a quintet state, \(5(T_1T_1)\), which upon decoherence can produce two non-interacting triplets.\textsuperscript{2-4} Despite its long history\textsuperscript{1, 5} there are still many aspects of the mechanism of SF that are not well understood.

Molecular dimers provide an ideal platform to study the mechanism of SF. They contain the minimum number of chromophores necessary for SF and in solution due to their isolated nature, interchromophore effects such as diffusion can be neglected.

We will present a series of non-conjugated pentacene dimers, the non-conjugated linker provides just enough coupling between the pentacene units to allow for efficient SF but weak enough for long lived triplet states. Time-resolved electron paramagnetic resonance spectroscopy and complementary time-resolved optical spectroscopy will be used to study both the generation of \(1(T_1T_1)\) and its spin evolution to form \(5(T_1T_1)\) and \(T_1+T_1\). The role that the para-, meta- or ortho- linker has on the triplet yields will be demonstrated.

References

Influence of Ultraviolet Rays in In Vivo EPR Tooth Dosimetry

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INTRODUCTION

In vivo Electron Paramagnetic Resonance (EPR) tooth dosimetry using the L-band has been developed to allow incisors to be measured in situ in the mouth [1]. Sunlight contains ultraviolet rays (UV) and it was confirmed that UV irradiation to a tooth causes significant EPR signal of in vitro destructive EPR tooth dosimetry using the X-band. We therefore studied the contributions of UV to in vivo EPR tooth dosimetry.

MATERIALS AND METHODS

Three human extracted teeth were exposed to UVC by using FS-800 (Funakoshi Co., Ltd.) and the three other teeth were exposed to UVB by using LAX-103 (Asahi Spectra Co., Ltd.). Irradiated teeth were measured by using the 1.2 GHz L band EPR [1].

RESULTS

Linear exposure time and EPR signal responses were confirmed both for UVC and UVB. Detectable signals were observed with 1 hour exposure for UVC. Assuming 2 hours’ exposure per day to sunlight and 40 years’ exposure duration, about 20% of samples were above the 2 Gy exposure by 150 kV X-rays (Fig. 1).

DISCUSSIONS AND CONCLUSIONS

The dose response relationships of UVC and UVB exposures to L band EPR tooth dosimetry were investigated. The results suggest that daily UV exposure has a limited influence on in vivo tooth EPR dosimetry for triage in emergency cases.

FUNDING AND ACKNOWLEDGEMENTS

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REFERENCES

Streptococcus pneumoniae (S. pneumoniae) is the world’s foremost bacterial pathogen. Diseases arising from S. pneumoniae infections are responsible for more than 1 million deaths every year with an economic health burden of more than US$4 billion annually. Essential to the colonization of S. pneumoniae is its ability to acquire essential nutrients from the host environment, including trace metals such as zinc (Zn) [1]. In S. pneumoniae Zn is acquired by the ABC transporter AdcCB and the Zn-binding protein AcdA [2]. The AdcCBA permease complex acts in concert to facilitate unidirectional Zn import into the bacterial cytosol. Here we sought to examine the conformational landscape of AdcA in solution during its interaction with Zn.

In this study we used four (4)-pulse Double Electron Electron Resonance (DEER) with spin-labelled AdcA. Here, we measured distances between distinct residues of the protein in the presence and absence of Zn. We then combined this approach with Molecular Dynamics simulations of AcdA to generate a series potential conformations sampled by the protein. Here, we will discuss the comparison of the experimental DEER data with simulation data and its use in defining the solution structure of AdcA in the presence and absence of its cognate ligand.

References
High Sensitivity DC Magnetometry at the $T_2$ Limit

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Sensing static or slowly varying (DC) magnetic fields has wide ranging applications in fundamental physics, bioimaging, and materials science [1-2]. Several versatile magnetometry platforms have emerged over the past decade, such as electronic spins associated with Nitrogen Vacancy (NV) centers in diamond. However, their high sensitivity to external fields also makes them poor sensors of DC fields. This is because low frequency noise in environment -- the dominant class of noise – masquerades as the signal to be measured and degrades the sensor. Indeed, the usual method of Ramsey magnetometry leaves them prone to environmental noise, limiting the allowable interrogation time to the short dephasing time $T_2^*$. New methods that improve DC field sensitivity would have tremendous impact in many applications.

In this work we introduce a new platform, consisting of a quantum sensor and an ancilla, which selectively filters out the noise and opens door to high sensitivity DC magnetometry [3]. This hybrid magnetometry platform allows sensing static magnetic fields with interrogation times up to the much longer $T_2$ coherence time, allowing significant potential gains in field sensitivity. The ancilla serves to up-convert the DC signal to an AC signal, which can then be measured through quantum lock-in techniques with high noise rejection. This consequently allows longer interrogation times for the quantum sensor, leading to enhanced sensitivity.

We demonstrate the method for an electronic NV sensor and a nuclear $^{14}$N ancilla. In our experiments, we demonstrate sensitivities better than 6µT/νHz, comparable to the Ramsey method, and narrow-band signal noise filtering better than 64kHz. With technical optimization, we expect more than an one order of magnitude improvement in these parameters. Since our method measures transverse fields, in combination with the Ramsey detection of longitudinal fields, it ushers in a compelling technique for sensitive vector DC magnetometry at the nanoscale.

Figure 1: Schematic of the method. A) A magnetic field $B_1$ with low-frequency noise is up-converted by the ancillary $^{14}$N nuclear spin of an NV center (inset) to an AC signal with frequency $\omega_0$. Rectification by a CPMG pulse sequence (black lines indicate π pulses) performs a quantum lock-in detection at $\omega_0$, measuring the DC signal and rejecting noise. (B) Frequency-domain picture of the signal frequency upconversion and noise filtering. Ancilla assisted upconversion leads to a dual band-pass filtering of both signal and sensor noise thanks to the narrow DD filter bandwidth, $\sim 1/T_2$, with a consequent gain in magnetometer sensitivity.

Figure 2: Experimental results showing ancilla assisted DC magnetic field sensing at long coherence times (60us) and high sensitivity (<6µT/νHz).

References:
240 GHz high-power pulsed EPR of aqueous solution samples

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High-field EPR offers advantages in sensitivity and spectral resolution for a wide range of systems. Experimentally realizing these advantages can present challenges, however, due to constraints on sample size, microwave power, and microwave field homogeneity. One broad class of samples of particular interest that presents challenges is room temperature aqueous solutions, where large microwave losses can easily dominate the interaction of interest between the sample and the microwave field. Resonators can often be employed to overcome sensitivity challenges, but are impractical for many applications due to their restrictive geometry and limiting bandwidth. Here, we present a simple approach based on transfer matrix calculations to efficiently design and optimize non-resonant sample geometries, which both minimize microwave losses and maximize the reflection-mode EPR signal. Using a flat, layer-by-layer, geometry optimized for aqueous samples at room temperature, we demonstrate signal enhancements in CW-EPR measurements of GdCl₃ of more than a factor of 30 relative to an un-optimized geometry for the sample holder, while at the same time reducing the required sample volume by more than a factor of 10, to 0.6 uL. This dramatic increase in sensitivity has made accessible room temperature measurements of samples in the 50 uM concentration range, a regime of great importance for measurements of, for example, spin-labeled biomolecules.

We take advantage of the microwave enhancement and broad bandwidth provided by the optimized sample design to perform high-power pulsed EPR measurements of aqueous samples using the UCSB mm-wave free electron laser (FEL) (Fig. 1). We employ a recently implemented quasi-optical phase cycling procedure, wherein we use precision-machined dielectrics to shift the phase of a 240 GHz pulse, to efficiently carry out 2- and 4-step phase cycling, allowing for the efficient separation of EPR coherences. Phase cycling, together with sample geometry optimization, enables room temperature measurements of $T_1$ in aqueous solutions—a first of such measurement by FEL-EPR—which we demonstrate on solutions of trityl (Fig. 1).

Fig.1. Left: Fourier transform EPR lineshape of 3.2 mM trityl in D₂O at 8.57 T, 280 K, acquired using the UCSB mm-FEL. Right: $T_1$ saturation recovery curve of 3.2 mM trityl in D₂O at 8.57 T, 280 K.
Frequency-Stepped Measurements of Electron Spin Relaxation

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Electron spin lattice relaxation (T1) reflects the interaction of electron spins with their environment, and has important applications, including for in vivo oximetry. The Bloch equations describe the net magnetization of electron spins as they return to equilibrium with rates determined by T1 and T2. An analytical solution to the Bloch equations can be obtained for special cases 1,2,3. For instance, an analytical solution can be obtained for ∆ω = 0, where ∆ω is the offset from the center of the line. This is the case for conventional continuous wave saturation recovery, in which the microwave magnetic field, B1, is stepped from constant high value during the pump time to lower constant value for the observe time. There is also an analytical solution for ∆ω ≠ 0, when ∆ω and B1 are constant. This second case is the topic for this study. In this new way of measuring T1, the spins are saturated on resonance with continuous waves and constant B1. Then, in sub-nanosecond time resolution, the frequency of the continuous waves is switched to a frequency well away from resonance, but still within the EPR lineshape, without changing B1. Since the effective B1 felt by the on-resonance spins has decreased, this allows for the on-resonance spins to recover from saturation with time constant T1. A cross-loop resonator is particularly useful for this experiment, because the excitation and detection resonators can be tuned to the excitation and detection frequencies, respectively. An arbitrary waveform generator is essential to achieving sub-nanosecond frequency switching. Samples with a range of relaxation times were measured.


Comparison of AWG-based saturation recovery with conventional saturation recovery and inversion recovery

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A saturation recovery EPR system has been constructed that uses an arbitrary waveform generator (AWG) as the microwave source. Spin-lattice relaxation times (T1) are obtained with the new system and compared with those measured with a previously-built conventional X-band saturation recovery spectrometer that has a diode source [1]. Relaxation times are also compared with those obtained by inversion recovery on a Bruker E580 to test for spectral diffusion processes. Results for samples with a wide range of relaxation times will be presented, showcasing the flexibility of the new bridge design. The AWG-based system measures T1 times in the microsecond to several hundred microsecond range. The simplicity of the AWG bridge will allow this technique to be more widely implemented as waveform generators become commonplace in the laboratory setting. The AWG-based system uses a Bruker dielectric resonator with a narrow frequency tuning range. Other components of the system, including the AWG, have a wide range of operating frequencies, which will permit operation over a much wider range of frequencies than are possible with diode sources, by changing only the narrow-band, frequency sensitive components. As AWG source noise is addressed in future product designs, AWGs are expected to become the standard of RF generation.

References
Quantum Coherence in Electron Spin-Correlated Radical Pairs

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As early as magnetic resonance (MR) spectroscopy was proposed as a candidate to address a useful number of qubits for quantum information processing (QIP), so too was its candidacy jeopardized by two major inadequacies: (1) the number of quantum gates required to prepare (pseudo)pure quantum states in most proposals using MR spectroscopy for QIP is a challenge to scalability [1], and (2) the separability of these states is contrary to the quantum entanglement believed responsible for exponential (per qubit) computational speed-up [2].

With regard to these two respective weaknesses, the strength of quantum coherence in photo-initiated SCRP is two-fold: (1) it can be generated by a single, 8 ns laser pulse and (2) the spin system is born from a pure, highly polarized state. Specifically, SCRPs with minimal spin-orbit coupling like organic molecules are synthetically tunable such that a series varying the donor (D, primarily radical cation) and acceptor (A, primarily radical anion) was prepared (Fig. 1). The laser flash excites the chromophore (C) to initiate stepwise electron transfers monitored by transient absorption spectroscopy that result in the terminal SCRP in 5 ns or less in 1-4; the g-anisotropy and hyperfine differences between the D and A drive state-mixing.

In a four-state spin basis, the populations and coherences for the electron spin ensemble can be described by a density matrix. The coherences on the off-diagonal are ranked by the change in magnetic moment along the static magnetic field of an EPR spectrometer: the allowed, transverse magnetization of single- (SQC, \( \Delta m_s = 1 \)), and forbidden double- (DQC, \( \Delta m_s = 2 \)) and zero- (ZQC, \( \Delta m_s = 0 \)) quantum coherence.

By incrementing the dwell time after the laser flash, the oscillations of the forbidden coherences can be indirectly measured. ZQC was measured in the free-induction decay of SQC created by a single microwave pulse (Fig 1II). Strikingly, the SQC and ZQC can be addressed individually using specific microwave pulse turning angles (Fig 1IV). Identified by its unique time- and phase- dependence, the DQC requires a second pulse to be read out in echo-detected experiments (Fig 1III). In this regime, the ability to address all the elements of a 2 qubit density matrix in a single two-dimensional time-domain experiment with only two, short microwave pulses is unique to SCRPs. The effect of electron delocalization by creating molecular analogs with degenerate A is investigated in 3 vs 2. Additionally, 3 when one of the A units is pre-reduced, lends itself to future studies on 3-spin systems such as the teleportation of spin polarization of stable radicals to the spatially and magnetically resolved SCRP.

References

Fig. 1. I. Molecules studied in this work, with D in blue, C in green, and A in red. B. II and III. Laser (purple) and microwave pulse (blue) sequences used to measure ZQC (green) and DQC (red) indirectly through the FID or echo of the SQC. IV. Nutation curves for 1, 2, and 4, at early times after the laser flash, (top, ZQC dominates), vs late (bottom, ZQC relaxed). See [3] for more details.
Protocols for Imaging Individual Nitroxide Electron Spins with Atomic Precision

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Imaging individual electron-spin labels attached to a single protein or protein complex with atomic precision would be a powerful tool for structural biology. Electron spin resonance from individual electron spins has been detected and imaged previously using magnet-tipped cantilevers [1] and nitrogen-vacancy centers in diamond [2], but these experiments required extraordinarily long signal-averaging times — 13 hr/point in Ref. 1 and 40 min/point in Ref. 2 [3]. A per-spin sensitivity sufficient, in principle, to quickly detect single electron spins has been demonstrated in an experiment in which nuclear magnetic resonance was observed as a force acting on a high-compliance microcantilever carefully prepared with an integrated nanomagnet tip [4]. In this poster we present protocols designed to enable these cantilevers to, in practice, (1) detect electron spin resonance from individual nitroxide-based spin probes in reasonable detection times of 1 minute/point and (2) reconstruct with atomic precision the location of the individual spins probes from the detected signal map.

Detection protocol: To detect electron-spin resonance we will observe electron spin magnetization as a change in the spin-force gradient acting on a magnet-tipped cantilever [5]. This approach has the advantage of allowing us to observe the average, Curie-law magnetization of the electron spins instead of requiring us to detect electron-spin fluctuations as in previous experiments [1, 2]. The spin-force-gradient shifts the mechanical oscillation frequency of the cantilever. To detect the resulting frequency shift with high signal-to-noise ratio the cantilever amplitude should be set equal to the tip-sample separation (10 to 100 nm) [6]. A signal map is acquired by scanning the cantilever in (x, y) and an image-reconstruction protocol is applied to obtain an estimate of the sample’s electron-spin density. To avoid blurring the resulting electron-spin image, we propose to synchronize very short spin-flipping microwave pulses to the cantilever oscillation, where the microwave pulse duration is much shorter than the cantilever’s period of oscillation.

Image-reconstruction protocol: We have developed a fast novel, image-reconstruction protocol using Bayesian Markov-chain Monte Carlo methods. This protocol obtains the electrons’ coordinates with error bars. A surprising finding is that a three dimensional image can be obtained from two dimensional data, accelerating the data acquisition by a factor of 32 to 64. Simulations employing currently-achievable signal-to-noise ratios indicate that the algorithm can reconstruct the (x, y, z) locations of individual buried electron spins to within a resolution of just a few Å in experiments requiring under a day of signal averaging.

References:
Measurement of thiol-disulfide redox status is crucial for characterization of tumor physiology. Disulfide-linked dinitroxides, such as I, are being developed as in vivo probes of that redox status. Reaction of the disulfide with glutathione or other free thiols cleaves the disulfide linkage, forming monoradicals. EPR spectra of the disulfide-linked dinitroxides are readily distinguished from those of the corresponding monoradicals\(^1,2\) so EPR spectra can be used to monitor the rate of cleavage and the thiol redox status. The time dependence of the spectrum of I during reduction with glutathione is shown in Figure 1. The broad peaks labeled 2 and 4 are characteristic of a flexible diradical for which conformations with large and small values of the exchange interaction are interconverting rapidly. Lines 1, 3, and 5 have contributions both from the diradical and the monoradical that is formed by cleavage of the disulfide linkage. Sankarapandi et al. reported a rapid method to simulate the dinitroxide spectra\(^3\) and shared that Fortran code with us. The code has been converted to MatLab that can be used with the EasySpin program\(^4\) for EPR simulations. These calculations permit lineshape simulations to determine the relative populations of monoradical and diradical species.

References


A procedure is developed to calculate pulsed electron paramagnetic resonance (EPR) signals by the use of stochastic Liouville equation (SLE), taking into account relaxation rigorously. The coherent pathway $S_c^-$ has been taken into account by selecting the elements of the density matrix appropriately. It can be carried out on a PC using Fortran or Matlab. The flow chart for this simulation is included. It is illustrated here numerically, as coded in Matlab, to calculate the two-dimensional spin echo correlation spectroscopy (2D-SECSY) and two-dimensional electron-electron double-resonance (2D-ELDOR) signals for two cases: (i) a coupled electron-nuclear system with the electron spin ($S = \frac{1}{2}$) and nuclear spin ($I = \frac{1}{2}$) corresponding to the experimental results of Lee, Patyal and Freed [1] in a malonic acid single crystal, and (ii) 2D-ELDOR signal obtained for an electron-electron dipolar-coupled system of two nitroxide radicals in a malonic acid single crystal, with hyperfine interactions between the two electrons included, for a proposed experiment, intended for distance measurements, important for biological systems.
The future of MAS-DNP: towards higher fields and faster spinning

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Dynamic nuclear polarization (DNP) has the potential to substantially enhance the sensitivity of NMR spectroscopy. Although the DNP effect has been known since the 1950s, its application for high-resolution solid-state NMR spectroscopy – implying the use of magic angle spinning (MAS) and strong magnetic fields – had been minimal until the last decade due to technological limitations. Since some of these limitations have now been overcome, leading to the availability of commercial MAS-DNP apparatus, the pertinence of this technique is beginning to be realized. As a demonstration of the potential power of MAS-DNP, two examples of experiments that would have been previously impossible will be presented. First, ²⁹Si-²⁹Si correlation experiments, recorded at natural isotopic abundance for functionalized nanoparticles; these provide the surface grafting scheme [1]. Second, ⁴³Ca NMR experiments, recorded at natural isotopic abundance (0.14 %) for both synthetic and natural bone/teeth, allowing the discrimination between surface and core species (see Fig. 1) [2].

Although much progress has been made, it will also be shown in this presentation that there is still a lot more to understand, improve upon, and thus achieve. The methodology to obtain the greatest amount of absolute sensitivity for MAS-NMR experiments will be discussed, as will be the common pitfalls. Using bespoke numerical simulations we are able to understand the properties and associated efficiencies of various polarizing agents and their mechanisms of DNP. For example, the current “best” polarizing agent, AMUPol, appears to work over 3 times better than an alternative. However, it will be shown that this alternative is actually a more efficient polarizing agent in terms of provided overall sensitivity, and it becomes even more superior at higher magnetic fields as well as at faster MAS rates. As such, the way in which “efficiencies” of polarizing agents is determined needs to be reevaluated. In light of these developments, exceptionally successful high-field (19 T) and fast MAS (40 kHz) DNP experiments will be presented, showing the suitability of this regime for catalytically-relevant materials (see Fig. 2). Finally, our work towards even greater increases in NMR sensitivity through lowering the sample temperature for MAS-DNP will also be presented. Specifically, results from our home-built system that is used to sustainably cool (to 30 K) and spin the sample with cryogenic helium will be shown (see Fig. 3).

Fig. 1. ¹H-⁴³Ca DNP-HETCOR of HAp  Fig. 2. ²⁷Al MAS-DNP  Fig. 3. ULT-MAS-DNP

DNP NMR of adsorption and exchange at a silicon surface

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We use DNP-enhanced NMR to directly detect hydrogen attached to the surface of silicon microparticles, using the endogenous dangling-bond electron spins present at the silicon surface. The frequency-modulated DNP experiments were performed at liquid helium temperatures using a lab-built 94 GHz system. The proton NMR spectrum from a dry sample of polycrystalline silicon powder (1–5 µm) shows a distinctively narrow Lorentzian-shaped resonance with a width of 6.2 kHz, indicative of a very sparse distribution of protons attached to the silicon surface. These protons are within a few atomic monolayers of the silicon surface.

![Comparison of the DNP-enhanced proton spectra for freshly prepared and aged samples consisting of silicon microparticles suspended in a solvent consisting of 80% H\(_2\)O and 20% D\(_2\)O; and 20% H\(_2\)O and 80% D\(_2\)O.](image_url)

**Fig. 1.** Comparison of the DNP-enhanced proton spectra for freshly prepared and aged samples consisting of silicon microparticles suspended in (a) 80% H\(_2\)O and 20% D\(_2\)O; and (b) 20% H\(_2\)O and 80% D\(_2\)O.

Figure 1(a) shows the growth of this narrow peak following suspension of the particles in a solvent consisting of 80% H\(_2\)O and 20% D\(_2\)O, indicating growth of the sparse surface proton layer. However, when the particles were suspended in a solvent with 20% H\(_2\)O and 80% D\(_2\)O, the narrow bound proton peak was observed to shrink due to exchange between the surface protons and the deuterium in solution, as seen in Figure 1(b). This decrease was accompanied by a concomitant growth in the intensity of the frozen solvent peak. When the particles were suspended in the organic solvent hexane, the proton NMR spectra remained unchanged over time. These results are consistent with the known chemisorption of water on the silicon surface resulting in the formation of hydride and hydroxyl species.

**References**

FIELD SWITCHING IMPROVES SABRE-SHEATH BY COHERENTLY PUMPING HYPERPOLARIZATION

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The most common hyperpolarization method (dynamic nuclear polarization or DNP) can dramatically increase magnetization in virtually any organic molecule, but the apparatus is complicated and expensive (ca. $2.5M for clinical systems) and polarization grows slowly. Another approach, called SABRE, was conceived about a decade ago[1]; our work has extended this in the development of LIGHT-SABRE[2], which permits efficient generation of hyperpolarization directly in a high field magnet, and SABRE-SHEATH[3], an approach to directly polarize heteroatoms such as ¹³C or ¹⁵N using an extremely simple low field apparatus. These papers have led to a wide range of applications: our group alone has polarized about 50 different reagents, including many that are biologically interesting.

This inexpensive, general, and simple method has a few current limitations. The most significant is that hyperpolarization levels are typically lower than in DNP. Here we show that a simple modification—pulsing the low matching field to cause a coherent transfer—can produce very substantial magnetization enhancements. In essence, this is the difference between saturating a transition and giving $\pi$ pulses. In Figure 1, we show the ¹⁵N magnetization induced in acetonitrile by changing the field to the exact SABRE-SHEATH resonance for x ms, moving it off resonance for (100-x) ms, and repeating many times. The case of x-100 ms (the right side of the curve) corresponds to normal SABRE-SHEATH. In fact, however, magnetization flows through $^2J_{NN}$ couplings with a predicted near-complete transfer at 20 ms, so pulsing the field (and then allowing time for exchange) generates large signal increases—60% increase in our first experiments. Theory and experiment are in excellent agreement (Figure 1) and the approach also lets us unravel important details about the magnetization transfer process, including the presence of inactive states in the catalytic process. Extensions to more complex “field pulses” and to arbitrary field profiles will be discussed.

![Fig. 1. SABRE-SHEATH intensity verses pulse length in acetonitrile(experimental red, simulated blue). The field is cycled between the correct SABRE-SHEATH matching field and a mismatched field, with the sequence repeated after 100 ms. Thus the right hand side corresponds to normal SABRE-SHEATH. The maximum occurs at much shorter times, because scalar couplings across the iridium bond cause coherent transfer.](image)

References

Study of DNP mechanisms by ELDOR detection of electron depolarization at 7T

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Radical design to improve dynamic nuclear polarization (DNP) signal enhancement has led to a slew of new radicals for MAS and dissolution DNP applications. The primary design goals for these new radicals were (i) elongating $T_{1e}$ and (ii) optimizing the electron-electron coupling and resonance frequency difference between the two electrons to equal the $^1$H Larmor frequency to facilitate cross effect (CE) DNP. Empirically the use of these designer radicals has improved DNP performance; however, true understanding of the mechanism(s) behind the improvement in DNP performance can only be obtained from EPR experiments performed under “DNP” conditions. It was recently shown that electron depolarization profiles as obtained from electron-electron double resonance (ELDOR) experiments allow for direct analysis of the underlying DNP mechanism(s) for various radicals and experimental conditions. The shape and extent of electron depolarization depend on the microwave power intensity ($B_1$), the microwave irradiation time ($t_{sat}$), the electron relaxation rates ($T_1$, and $T_2$), and the electron-electron spectral diffusion rate ($\lambda$), where $T_1$, $T_2$, and $\lambda$ are dependent on the radical type and concentration, and the experimental temperature.

Our development of a 7T dual DNP / EPR spectrometer operating at 200 GHz / 300MHz allows for the collection of both DNP and the corresponding EPR and ELDOR data under identical conditions. We show that the electron depolarization profiles of nitroxide based mono-, bi-, tri-, and spin-labelled dendrimer radicals differ significantly at 4 K despite the global unpaired electron concentration being held constant. Our results indicate that the extent of electron-electron spectral diffusion increases significantly when increasing the number of dipolar coupled electron spins from two to three, suggesting that efficient indirect CE (iCE) DNP may require more than two efficiently coupled electron spins (Fig. 1). In addition, we show that the DNP efficiency is directly related to the extent of electron depolarization as determined in our ELDOR measurements.

Figure 1. ELDOR electron depolarization profiles for mono-, bi-, tri-, and spin-labelled dendrimer at 4 K. The DNP signal enhancements after 60 s of signal build-up is given in parenthesis in the figure legend.
Integrated micro-devices for para-hydrogen gas delivery: applications to hyperpolarisation at the micro-scale

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While the bulk of the recent efforts on para-hydrogen induced polarization (PHIP) focuses on preparation of large sample quantities, there is a growing demand for low-volume NMR/MRI experiments requiring the use of micro-sized coils such as, e.g., portable NMR analytics devices or in-vivo metabolomics on small organisms\cite{Badilita2012}. Micro-coils provide higher mass sensitivities than standard coils, unlocking opportunities for miniaturized and application-specific NMR systems. However, the reduced sample volume translates to a lower absolute signal picked up by the detector. As a convenient solution to the signal-enhancement problem we addressed Signal Amplification by Reversible Exchange (SABRE). This hyperpolarization method holds the potential of becoming a NMR micro-coil friendly technique, given its reversible character that enables continuous polarization of limited sample amounts \cite{Hövener2013}. By designing an integrated SABRE polariser and NMR micro-detector, and by developing a methodology to allow for continuous in situ hyperpolarisation at the micro-scale, we aim to effectively merge these two aspects of NMR technological development.

We present a series of NMR-compatible lab-on-a-chip designs for \textit{in-situ} pH\textsubscript{2} hyperpolarization that can be easily fabricated with standard wafer-scale soft photolithography techniques. To maximize contact surface between gas and liquid and to avoid the need for turbulent mixing we implemented two-phase contact of parahydrogen and target solution by employing highly gas permeable materials, such as polydimethylsiloxane (PDMS) and hollow-fiber polypropylene membranes (Fig. 1). We provide demonstration of integration of the developed systems with a Helmholtz pair micro-detector\cite{Spengler2014} on a 500 MHz Bruker NMR scanner, targeting a sample volume of 0.5 µL.

References
Modeling of Polarization Transfer Kinetics between Hyperpolarized Water and Protein

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The interactions with water are important in determining protein structure, dynamics, and function [1]. Using NMR, these interactions can be studied by observing polarization transfer from water to protein via the nuclear Overhauser effect (NOE), as well as through proton exchange. Since the hyperpolarization creates highly non-equilibrium spin systems, the transfer of polarization from hyperpolarized water to the target protein by NOE or exchange is also enhanced. Previously, our group demonstrated that dissolution dynamic nuclear polarization (D-DNP) can enhance the NOE intensity from water to small molecules, allowing measurement of the cross-relaxation rate [2]. Here, we utilize D-DNP to hyperpolarize $^1$H spins on water, and then observe a large enhancement in the amide proton region of the protein spectrum due to polarization transfer from water. These transferred signals show a pH dependence, suggesting that they are mainly caused by exchange processes. The hyperpolarization can be further passed to aliphatic protons by intramolecular NOE. The time dependence of polarization was measured using a series of NMR acquisitions with a 30° flip angle selective pulse (Fig. 1). Finally, the buildup curves of amide and aliphatic proton signals of the protein were obtained. A three-site model describing the time dependence of water, amide and aliphatic proton signals was derived for quantitatively determining signal transfer rate constants. From the initial buildup rate of amide signals, the overall exchange rate was determined. Further, the overall cross-relaxation rate between amide and aliphatic protons was determined by fitting the model to the observed signal intensities. The obtained kinetic parameters show good agreement with the values from conventional EXSY and NOESY measurements. Because the magnitude of polarization transfer is directly related to solvent accessibility, a rapid measurement of these rates can be applied to the study of water-protein interactions, even when structural changes of the protein occur.

![Fig. 1. Stacked plot of (a) $^1$H amide and (b) aliphatic signals of trypsin, successively acquired using a pulse sequence with 30° selective excitation pulse, after mixing with hyperpolarized water at pH 7.5. The time between scans was 70.1 ms. The final samples contained 0.33 mM trypsin with 3.26 M water in deuterium oxide for (a), and 0.38 mM trypsin with 3.18 M water for (b).](image)

References


With this contribution, we present liquid state Overhauser effect Dynamic Nuclear Polarization (ODNP) performed in a microfluidic chip at X-band frequencies. First results of the novel ODNP chip (Figure 1 (B)) as well as its performance will be discussed.

ODNP is sensitive to the rotational and translational correlation times of water and can be used to study water accessibility or the hydration dynamics of solvent molecules close to a surface [1], e.g., membrane molecules.

Based on our previous work [2], we employ a microfluidic chip (13 mm x 10 mm) designed for volume limited samples (50…500 nL), that features a stacked split ring-type MW resonator. The MW resonators (Figure 1 (C)) are much more compact compared to traditional setups, that require conventional EPR resonators. Microfluidic channels allow precise handling and positioning of nano-liter sized liquid samples. The self-resonance frequencies $f_i$ mainly depend on $\theta$ and the outer diameter of the ring and can be adjusted over a wide frequency band (Figure 1 (A)).

References


Whole cell $^{13}$C solid-state NMR of microalgae

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Microalgae are at the basis of the aquatic food chain and it is thus essential to understand the toxicity of external agents towards them. Indeed, any impact on phytoplankton can cascade up to higher organisms; therefore microalgae are sensitive sentinel species which can report on the health of their environment. In vivo and whole-cell NMR are currently being developed to circumvent the caveats of working with in vitro model systems. While specific labelling of a single biological target avoids the problem of assigning a multitude of NMR resonances (a crucial problem in whole-cell NMR), this strategy is limited to certain family of compounds by the organism’s biochemistry. Herein we will describe our recent results on the NMR study of intact *Chlamydomonas reinhardtii* microalgae which were fully $^{13}$C labelled.

The complexity and heavy spectral overlap of whole-cell NMR spectra is here reduced by applying different polarization transfer excitations (ultrashort CP, NOE and INEPT) which differentially excite rigid, semi-rigid and mobile species in the microalgae. Resolution is further enhanced by dipolar (DARR or PDSD) or J-mediated 2D polarization transfers. This approach allowed us to discriminate and characterize the highly crystalline starch reserves in situ and to assign the highly mobile and abundant galactolipid headgroups in the lipid membranes. Our results also show that microalgae cell walls appear to be softer than those of higher-order cellulose producing plants. Indeed, while some saccharides in the glycoproteins which form the cell wall of *Chlamydomonas reinhardtii* appear to be rigid, others, possibly the external part of the cell wall, are highly mobile. The NMR strategies herein described enable a simplification of spectra and identification of the main constituents of *Chlamydomonas reinhardtii* which pave the way for the in vivo NMR study of the molecular-level interaction of toxic agents with microalgae.
IP-ISQC: In Phase Ultra High Resolution In Vivo NMR

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Current NMR techniques allow for organisms to be studied in vivo, however magnetic susceptibility distortions prevent the acquisition of high-resolution data. Intermolecular single quantum coherence NMR[1] is a technique that involves breaking the sample symmetry to form long range dipolar couplings, which can be exploited to extract “solution-like” chemical shift information, largely free of these susceptibility perturbations.[2] While this approach holds vast potential for studying intact samples, present practical limitations include radiation damping, non-phase sensitive data and significant relaxation losses which hinder its wide spread application. Here, all these drawbacks are addressed and a new technique termed In-Phase Intermolecular Single Quantum Coherence is developed.

![Fig. 1. A = ¹H NMR in-vivo, B = IP-ISQC NMR in-vivo, C = ¹H NMR aqueous buffer extract of a worm.](image)

To demonstrate the applicability of the technique to remove magnetic susceptibility distortions in vivo, the approach is applied to a living earthworm in a 5 mm NMR probe. Figure 1A shows the conventional NMR spectrum, which is largely broad and featureless preventing the extraction of high-resolution metabolic profiles. Figure 1B demonstrates the IP-ISQC spectrum from the same worm and Figure 1C shows an aqueous buffer extract from a separate worm for comparison. A comparison of Figure 1B and 1C shows that the IP-ISQC recovers an in vivo metabolite profile that is extremely similar to an aqueous buffer extract. It also confirms that the real line-shape, intensities and general spectral profile are well represented in the IP-ISQC. The ability to “look inside” a living organism and isolate solution-state NMR-like metabolic information free of distortions is quite profound and should find application in a wide array of fields where in vivo metabolism is of interest. The presentation will discuss the development of the pulse program and demonstrate its application to a wide range of living organisms and inhomogeneous samples.

References

Understanding mass transport phenomena with flow MRI

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Novel techniques such as 3D-printing enable manufacturing of reaction vessels with channel dimension from a few to a few hundred micrometers [1,2]. These reactors are characterized by a good surface-to-volume ratio and therefore feature good liquid-wall interactions. However, mass transport is limited by the laminar flow pattern typical for these reactors. By introducing dedicated geometries this limitation can be reduced and flow patterns can be adapted to the needs of the reaction. The experimental characterization of these patterns is challenging due to the necessity of determining three-dimensional vector fields and the small dimensions. Magnetic resonance imaging velocimetry (MRI velocimetry) acquires three-dimensional vector fields without requiring special tracer particles which could block the channel. If the flow is stationary, it is possible to resolve flow patterns with good spatial resolution and accuracy in three dimensions.

![Three-dimensional velocity vector map](image)

In this study a microfluidic reactor with herringbone structure, manufactured by 3D-printing, was examined. The herringbone structure enables more complex flow in the reactor [2]. To examine these flow patterns, the Flow Imaging Employing Single Shot Encoding (FLIESSEN) pulse sequence was expanded to the third dimension [3,4]. A three-dimensional velocity vector field plot (Figure 1) emphasizes the ability of the herringbone structure to break up the laminar flow pattern. Furthermore, the surface shear rate was calculated to investigate the interactions of the liquid with the glass plate. It can be calculated from three dimensional velocity data using finite differences [3]. For a slice near the glass cover plate, the induced patterns are clearly visible and reveal the improved liquid-wall interactions favorable for solid-liquid reactions at these positions.

References


Better Accelerated MRI Using Nonlinear Gradient Encodings

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This abstract presents the first experimental demonstration of an imaging method where the addition of nonlinear gradients to an undersampled sequence dramatically improves image quality, as demonstrated in both phantoms in humans. FRONSAC encoding is a general approach that adds rapidly oscillating, low amplitude nonlinear gradients to standard undersampled scans, such as spiral or Cartesian. [1] Our results show that Cartesian-FRONSAC retains many desirable features of Cartesian imaging, namely insensitivity to unknown gradient delays and off-resonance, while greatly improving undersampling artifacts.

Imaging parameters were TR = 1000 ms; TE=18 ms; bandwidth = 50 Hz/pixel; matrix size = 256 x 256; flip angle = 30 degrees; slice thickness = 3 mm. The gradient coil is capable of achieving a maximum C3, S3, and Z2 fields of 138.56 Hz/cm³ (3254.8 mT/m³), 134.33 Hz/cm³ (3155.4 mT/m³) and 2022.4 Hz/cm² (475.08 mT/m²). For gradient echo images, nonlinear gradient oscillation frequency of \( \frac{w_0}{2\pi} \) = 3.2 kHz the slew rates for C3 and S3 are 3.639 T/s and 1.8195 T/s and for Z2 is 12.75 T/s. All experiments were approved by the IRB, and no PNS was reported.

**Fig. 1.** Reconstructions of Cartesian and FRONSAC-Cartesian data at R=4 and R=8 demonstrate that the addition of FRONSAC encoding greatly reduces undersampling artifacts. As shown in previous simulations, the brain images also demonstrate insensitivity to gradient delays, as well as Cartesian type behavior in the presence of off-resonance spins.

FRONSAC is a widely applicable approach to further reducing acceleration factors while maintaining image quality. Results suggest that a single FRONSAC waveform (not optimized for a particular geometry, orientation, resolution or contrast mechanism) dramatically improves undersampling artifacts while maintaining features of the base imaging modality.

**References**

Structure and dynamics of protein, pigment and lipids of native light harvesting complex II in lipid membranes investigated by solid-state NMR

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Photosynthetic organisms are vital for life as they provide us food and oxygen. In oxygenic organisms, Light Harvesting Complex II (LHCII) plays a key role to gather light and transfer it to reaction center for photo-induced processes. However under extreme high light conditions LHCII turns into a photo protective state via non-photochemical quenching (NPQ) process, preventing plants and algae from photo damages. In this study, we performed Magic-Angle spinning solid-state NMR (MAS) and biosynthetic isotope labeling on native LHCII complexes that were isolated from Chlamydomonas reinhardti green algae, and reconstituted in liposome membranes. Resonances of proteins, pigments and protein-associated glycolipids were identified by using J-coupling (INEPT-TOBSY) and dipolar coupling (CP-PARIS, NCC) based experiments. We discuss the molecular dynamics of LHCII and make a comparison between the NMR analysis and the crystal structure of LHCII. Results show a similar NMR and X-Ray coil/helix ratio for alanine, glycine, threonine and serine. In addition of protein analysis, a specific assignment of lipid signals (both head group and tail) and observed chlorophylls is reported. We compared the protein and lipid signals with NMR spectra of whole photosynthetic WT thylakoid membranes and chlorophyll signals of LHCII aggregates. NMR experiments were recorded at ultra-high field 950-MHz Bruker spectrometer (uNMR-NL) equipped with a 3.2 mm CP/MAS probe and 750-MHz Bruker Advance-II with a 4mm CP/MAS trip-probe.

Fig. 1. Shematic illustration of LHCII in liposome membranes studied by solid state NMR experiemnts.
$^2$H-$^{13}$C Correlation SSNMR for Investigating Biomolecular Dynamics and Structure

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$^2$H spins are increasingly incorporated into proteins and other biomolecules for $^1$H-dected fast MAS solid-state NMR experiments. These $^2$H spins also serve as an excellent probe of molecular motion and structure. Here we have investigated $^2$H-$^{13}$C polarization transfer and the applicability of $^2$H-$^{13}$C correlation spectroscopy on perdeuterated and H/D exchanged carbohydrates and proteins. An essential element of our experiments is the Rotor Echo Short Pulse RRAdiation mediated Cross Polarization ($^{1}$R$^{2}$A$^{1}$D$^{2}$T$^{1}$ION) sequence [1], which excites and transfers $^2$H magnetization to other spins in a broadband fashion. We show that $^2$H-$^{13}$C polarization transfer is possible not only for directly bonded $^{13}$C-$^2$H spin pairs but also for long-range spin pairs where the deuterons are introduced by $^1$H/$^2$H exchange, and this long-range $^2$H-$^{13}$C transfer has sufficient sensitivity for 2D $^2$H-$^{13}$C correlation experiments. We demonstrate this $^2$H-$^{13}$C correlation technique on bacterial cellulose, plant cell wall polysaccharides and the model protein GB1. For $^{13}$C, $^2$H-labeled bacterial cellulose, most functional groups exhibit quadrupolar couplings (CQ) close to the rigid limit of 170 kHz, except for the hydroxymethyl C6 group, which shows a motionally averaged CQ of 80±20 kHz, indicating trans-gauche isomerization of this group. On H/D exchanged Arabidopsis thaliana primary cell walls, we measured quadrupolar couplings of deutooxyl groups via $^{13}$C detection. The spectra indicate that the main polysaccharide signal at 72 ppm is a superposition of a rigid-limit CQ of 187 ± 10 kHz and a motionally averaged coupling of 50 ± 10 kHz, which can be attributed to the rigid cellulose and the highly dynamic matrix polysaccharides, respectively. The $^2$H-$^{13}$C correlation approach and its variants were also applied to H/D exchanged versus perdeuterated protein GB1 to compare polarization transfer efficiencies and to achieve spectral editing.

Figure 1. (a) $^2$H-$^{13}$C polarization transfer of $^{13}$C, $^2$H-labeled bacterial cellulose and H/D exchanged glucose. (b) $^2$H-$^{13}$C correlation spectrum of bacterial cellulose. The C6 cross sections of interior and surface cellulose C6 show distinct quadrupolar couplings, indicating different mobilities. (c) The C2, C5 cross section of the 2D $^2$H-$^{13}$C correlation spectrum of $^{13}$C-labeled and H/D exchanged Arabidopsis thaliana plant cell wall. The $^2$H cross section shows the coexistence of a rigid and a dynamic component.

Reference
NMR-led integrated structural biology approach reveals a dynamically regulated allosteric mechanism in natural product synthesis

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Many structurally complex antibiotics are synthesized inside bacterial cells by large modular protein machineries such as polyketide synthases (PKS) or non-ribosomal peptide synthases (NRPS). Because the components that are responsible for a single synthesis step in these assembly lines are often not encoded in one polypeptide, the quality of the product relies on the flawless interplay between different modules and enzymes [1,2].

The antibiotic enacyloxin IIa, shown to be active against a common hospital-acquired drug resistant pathogen A. baumannii, is produced by a hybrid PKS/NRPS and its synthesis requires the interaction of a stand-alone condensation domain (C domain; 57 kDa) with a peptidyl carrier protein (PCP; 11kDa) to terminate polyketide chain elongation [4]. We used an integrated structural biology approach combining solution and solid state NMR with carbene footprinting and X-ray crystallography to elucidate the atomic details of this interaction. Solution NMR titrations identified an intrinsically disordered docking domain located on the C-terminus of PCP that is essential for initiation of the interaction and consequently successful substrate transfer to the C domain. Formation of the complex between PCP and the C domain leads to a considerable loss of flexibility in this region but not complete immobilization, although the binding is strong and has low micro molar affinity. Solid-state NMR at fast 60-100 kHz spinning of PCP in complexes with the C domain and an upstream keto-synthase domain reveals distinct structural changes in PCP upon binding to the much larger enzymes. Moreover, data indicate that binding of PCP induces conformational changes in the C domain that enable the acceptor substrate to access the active site.

These findings suggest an intriguing dynamically regulated allosteric mechanism for condensation domain activity and provide a basis for rational engineering approaches of biosynthetic pathways to yield novel antibiotics.

References
Diabetes: the role of sugar in tissue and organ damages

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A large and growing number of people are suffering from diabetes with constantly high sugar content circulating in blood, and accompanying complications, affecting heart, blood vessels and other essential organs. This abnormally high sugar level could potentially result in high degree of glycation reaction and consequential accumulation of advanced glycation end-products (AGEs), including amino acid sidechain modifications and crosslinks, which are generally accepted as structural modifiers of proteins. Extracellular matrix (ECM), mainly composed of collagen, has been proved to participate in regulating cell behaviours, such as adhesion, proliferation and differentiation, and thus, glycation-modified ECM lead to abnormal cell behaviours and widespread cell necrosis, potentially causing numerous diabetic complications [1].

This project explores glycation induced ECM structural and functional changes using an experimental model from vascular smooth muscle cell ECM, to mimic the pathological condition of diabetic blood vessels. Cell culture and isotope labelling scheme allow us to obtain a range of 13C and 15N enriched ECM. Reacted with 13C labelled sugars, ribose and ribose-5-phosphate, ssNMR enables us to look at specific amino acids in ECM selectively, probe the glycation site, identify the major glycation products derived from sugars and explore the effects of glycation on ECM structure and molecular dynamics down to atomic level. Such knowledge may advance approaches to inhibit the consequences of diabetic glycation, and ultimately more effective treatments for diabetes and diabetic complications.

Primary glycation sites are on lysine and arginine. We identify some lysine and arginine derived AGEs in our ssNMR spectra (Fig. 1). Though stiffer ECM in diabetes, normal ageing and even cancer has been attributed to collagen crosslinks [2], our results show that crosslinks are relatively insignificant and unlikely to make a huge difference on ECM properties. Whilst, the major glycation products observed is lysine and arginine sidechain modifications. Thus, we propose that disruption of charge balance and addition of AGE substituents, especially in gap zones, makes a considerable contribution to increases in collagen stiffness and other ECM property changes.

References
Proton-detected Solid-state NMR to Distinguish the Hairpin and the Extended Duplex Structure of a Dimeric RNA in Crystals

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RNAs, including coding RNAs and non-coding regulatory RNAs, are critical molecules with versatile functions and have received considerable attention for its miscellaneous biological roles. Knowledge of three-dimensional structures is requisite for understanding the physiological roles of RNA. However, the structural flexibility often hinders the formation of RNA crystals, while the application of solution NMR into RNA is limited by the molecular size. Alternately, SSNMR is emerging as an attractive technique for RNA characterization, with particular merits of no limitation of molecular size and no need of large 3D crystals.

Here we describe a set of new proton-direct detected magic-angle-spinning solid-state NMR (SSNMR) experiments towards characterization of H-bond networks of microcrystalized RNAs, which are evaluated on a dimeric 23-mer RNA of human immunodeficiency virus type-1 (HIV-1). The 1H-direct detected 1H-15N correlation spectra are acquired on fully protonated RNA at a magic-angle-spinning frequency of 40 kHz. The newly designed SSNMR experiments, which include a period of 15N-15N protons assisted recoupling (PAR), yield direct information of the inter-nucleotide NH…N hydrogen bonds within the canonical Watson-Crick base pairs (Fig. 1). Comparison of spectral patterns between the uniformly labeled sample and the isotopically diluted sample with nature abundance RNA confirms the kissing-loop structure of the dimeric RNA. It is much anticipated that the strategy present here would contribute the progress of structure determination on RNA microcrystalline.

Fig. 1. The 600 MHz SSNMR spectra of 2D hCNH and 2D hCN(PAR)NH to visualize the NH…N hydrogen bonds of Watson-Crick base pairs. (A-B) The hCNH (A) and hCN(PAR)NH (B) spectrum recorded on (G,C)Lab-RNA. (C-D) Comparison of the hCNH and the hCN(PAR)NH spectra of (G,C,A,U)Lab-RNA. Gray dashed lines highlights the NH…N hydrogen bonds.
Proton-detected solid-state NMR on large proteins

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Solid-state NMR for protein structure research and other biological applications has traditionally been primarily concerned with carbon-detected experiments. In recent years, the development of magic-angle spinning (MAS) frequencies over 100 kHz has evoked further interest in proton-detected experiments since, in this MAS frequency regime, the 1H dipolar couplings begin to be averaged out sufficiently for spectral peaks of reasonable linewidth to be observed. Consequently, structure determination by site-specific chemical shift assignment of protons in addition to carbon and nitrogen shifts is possible. Moreover, detection of protons potentially allows for investigation of the system in interaction with ligands, cofactors or other compounds of interest, where often protons are directly involved in said interactions, e.g. through hydrogen bonding. A particular boost for this type of experiments followed from the commercial availability of a 111 kHz MAS probe.

This work presents data acquired for large systems, such as the HpDnaB helicase that is involved in unwinding dsDNA ahead of the replication fork to prepare a ssDNA template for DNA replication. The full-length dodecameric complex contains 12x488 residues with a molecular weight of 672 kDa and its spectral resolution is good enough for acquisition of 3D experiments such as an HNCA or HNcoCA. Work is ongoing to assign the chemical shifts of HpDnaB in complex with DNA.

In addition, this work addresses technical issues that are involved with these experiments, both during sample preparation and at the spectrometer. For instance, it is rather easy to imagine that filling a rotor with 0.5 mm inner diameter and 0.59 µL inner volume with microcrystalline or sedimented protein is a somewhat precarious task. The commercially developed tools and work-flow are complemented here by in-house designed tools and methods.
SOLID-STATE NMR INVESTIGATIONS OF PROTEIN DYNAMICS IN SPIDER SILKS

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Spider silk is a biopolymer of great interest in materials science due to its unique mechanical properties and biocompatibility. More specifically, major ampullate silk (MaS) provides a combination of elasticity, strength and toughness unmatched among the best industrial fibers.[1] Furthermore, orb-web spiders can also produce six other varieties of silk, each of them characterized by a unique set of functional properties giving a specific biological function.

Previous study has demonstrated that MaS, minor ampullate silk (MiS) as well as cocoon silk exhibit similar secondary structure despite their different amino acid sequence.[2] At the molecular level, these silks are composed of fibrous proteins organized into highly oriented crystalline β-sheets embedded in an amorphous matrix of other slightly oriented structures (31- helices, random, turns).[3] However, the current structural data does not explain the differences observed in the mechanical properties of the MaS, MiS and cocoon silk. In addition, although the spinning speed seems to be determinant on the structural organization of silk,[4] this aspect is sparsely documented in literature. Thus, the aim of the present study is to examine the effect of the reeling rate and primary structure on the silk protein secondary structure and dynamics by solid-state NMR.

Our NMR studies[5] have confirmed that different types of silk (MaS, MiS that belong to the same structural class) have differences in their relaxation dynamics for certain residues (Ala, Gly and Gln). Moreover, MaS reeled at different speeds have been analyzed. The results show that higher is the reeling speed, lower is the organization of the fiber. Finally, those findings have been correlated to previous structural and orientational results obtained by Raman spectromicroscopy in order to expand our structural knowledge of spider silk.[6]

References
Accurate Measurement and Prediction of $^{15}$N$^\text{H}$ and $^{13}$C$^\text{a}$ Chemical Shift Tensors in Proteins

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The chemical shift tensor (CST) is a rich probe of structure and dynamics, dependent on electronic environment, local geometry, H-bonding, and dynamics. Isotropic chemical shifts and chemical shift anisotropies can be employed as structural restraints in biomolecular structure determination, supplementing distance restraints.\textsuperscript{1,3} To establish robust protocols for using chemical shifts in structure determination, accurate experimental measurement approaches as well as computations of CST parameters need to be established for well-characterized proteins. Here, we present an analysis of experimental and computed $^{13}$C and $^{15}$N chemical shift tensors using a 132-residue lectin from *Oscillatitoria agardhii* (OAA). OAA is an ideal benchmark system because it crystallizes readily and reproducibly (thus X-ray crystallography and MAS NMR studies are done on the same crystal form), its crystal structure has been determined at high (1.2 Å) resolution, and it has been extensively characterized by solution NMR.

We explored a hybrid quantum mechanics/molecular mechanics (QM/MM) approach\textsuperscript{4} for calculation of chemical shift tensors and observed excellent agreement between MAS and solution NMR isotropic shifts. We recorded residue-specific CSA lineshapes using RN symmetry sequences\textsuperscript{5} and correlated those to the computed tensors. We examined the influence of crystal contacts, H-bonding, ligand molecules, and dynamics on the accuracy of chemical shift tensor calculations\textsuperscript{6}. We will present the results of this study and discuss the potential of this approach for structure refinement.

Figure 1. a) OAA structure highlighting (in stick representation) a sample fragment used in the hybrid QM/MM approach. A15 (yellow) and surrounding residues (pink) are treated at the QM level while the rest of the protein is treated at the MM level. b) First 2D NCA plane from the 3D R14\textsuperscript{5} $^{15}$N CSA spectrum of uniformly $^{13}$C/$^{15}$N labeled OAA. c) RN $^{15}$N chemical shift anisotropy lineshapes obtained using the R14\textsuperscript{5} symmetry sequence. Spectra were collected at 600 MHz and a MAS frequency of 14 kHz.

References
Magic angle spinning (MAS) solid-state NMR can exhibit tremendous power for structural and dynamical analyses of large and/or insoluble proteins. However, the analyses are often prevented due to difficulty of peak picking in chemical shift assignment. Recently, Harden and co-workers developed a novel approach, COSCOMs, that does not require preliminary peak picking. This approach is based on so-called covariance NMR. The covariance can be used as a score of strip matching. Then, the map of intra- and inter-spectral covariance reflects the sequential correlation. Therefore, the sequential correlations can be collected by only peak picking on the covariance map[1]. There is no doubt COSCOMs approach is also attractive in MAS solid-state NMR.

We extended COSCOMs to assign standard MAS solid-state NMR of the backbone using 3D-CANCO, NCACX and NCOCX. The most concerned problem was the extension requires the covariance map calculation in a six-dimensional space, which is extremely huge (order of terabyte) to calculate and store. Then, we applied Monte Carlo integration to obtaining the covariance map. The approach was verified using 3D-CANCO, NCACO and NCOCA spectra of GB1 microcrystal. We could construct the 6D covariance map within few minutes using a laptop computer. And, we successfully assigned the backbone chemical shifts of GB1 derived from the approximated 6D covariance map without human intervention. The N, Ca and C’ chemical shift RMSDs from the shifts obtained by a conventional method are 0.081±0.004, 0.063±0.005 and 0.115±0.089 ppm, respectively, as shown in Fig. 1. These values indicate the fidelity of the assignment results. Thus, our Monte Carlo integration based covariance NMR approach is helpful for backbone chemical shift assignments.

![Fig. 1. Differences between backbone chemical shifts obtained by conventional and Monte Carlo integration based covariance NMR approaches. Their deviations were computed from five runs.](image)

References

Towards understanding the factors contributing to membrane protein stability

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Understanding the factors and interactions contributing to protein stability is critical for studying protein folding. Thermal denaturation represents a simple way to test how protein structure is destabilized, and how this destabilization is coupled to local changes in protein dynamics. In our research, we intend to use solid-state NMR (ssNMR) to probe the temperature-dependent dynamic transitions in retinal-binding membrane proteins of seven-helical (7TM) architecture.

At the initial preparatory stage we used UV-Vis spectroscopy to probe the stability of 7TM alpha-helical bundles. Preliminary experiments were carried out on proteorhodopsin in a temperature range of $20 \, ^\circ \text{C} - 70 \, ^\circ \text{C}$ for samples at pH 6 and pH 9, respectively. For both samples, an amplitude reduction and red shift of the retinal absorption peak were observed as the sample is heated and reversely cooled down to room temperature. Irreversible changes occurred at the temperature range around $50 \, ^\circ \text{C} - 70 \, ^\circ \text{C}$, indicating the denaturation onset is in the vicinity of $50 \, ^\circ \text{C}$. The samples were then incubated for 5 days at several elevated temperatures below $50 \, ^\circ \text{C}$ to further test the reversibility and stability for NMR experiments. $^{15}$N $R_1$ and $R_1\rho$ relaxation measurements at selected temperatures based on preliminary experiments are currently being planned to site-specifically probe protein dynamic transitions.
Structural study of α-synuclein 71-82, a peptide derived from a protein involved in Parkinson’s disease: interactions with model membranes

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α-Synuclein (AS) is an amyloid protein involved in Parkinson’s disease (PD). In pathological cases, aggregates of this protein form in the dopaminergic neuronal network, leading to its progressive degeneration accompanied with a dramatic decrease in the dopamine levels. Under physiological conditions, AS is disordered in solution or its progressive degeneration accompanied with a dramatic decrease in the dopamine levels. Under physiological conditions, AS is disordered in solution or weakly bound to neuronal membranes, via the formation of α-helices. The triggers and steps underlying the formation of insoluble β-sheet rich fibrils are still quite unclear. In our work, we focus on a central 12 amino acids stretch of AS in the amyloidogenic part of the protein that is believed to be responsible for the fibrillization of the whole protein [1, 2]: AS-71-82.

The interactions between AS and membranes are thought to be the starting point of the fibrillization process [3]. In order to investigate and probe the mechanisms responsible for this fibrillization, model membranes were used (DMPC for zwitterionic membranes; DMPG for negatively charged membranes). Interactions between peptides/proteins and membranes can take place at different locations across the bilayers so, in order to probe the interactions between AS-71-82 and model membranes, several complementary spectroscopic methods are needed. Infrared spectroscopy was used to study the peptide secondary structure, as well as the phospholipid acyl chain order and carbonyl group hydration. Solid-state NMR (ss-NMR) allowed the study of the impact of AS-71-82 on (i) the lipid phosphate headgroups (³¹P ss-NMR), and thereby its impact in the vesicles morphology, and (ii) the acyl chains via quadrupolar splitting measurements (²H ss-NMR).

Overall, negatively charged membranes have the highest impact on AS-71-82. They induce a complete change in the peptide secondary structure, from a random coil conformation, as observed in solution and in the presence of purely-zwitterionic membranes, to highly ordered β-sheets upon interactions with DMPG. However, AS-71-82 does not seem to perturb the acyl chains order, nor the hydration of phospholipid carbonyl groups, of either neutral, partially charged or fully charged model membranes. This suggests that electrostatic and/or polar interactions could immobilize the peptide at the membrane surface, thus acting as a fibrillization substrate for the peptide. Our experiments tend to confirm the resemblances in behavior between AS-71-82 and AS, as they both seem to act as peripheral membrane peptide/protein, [4]. This reinforces the interest in studying this peptide in order to gain insight into the fibrillization process of the parent protein.

References
Insight into the Chromophore of Rhodopsin and its Meta-II Photointermediate by $^{19}$F Solid-State NMR

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Rhodopsin is the visual pigment of the rod photoreceptor cells in the vertebrate retina, which mediate dim-light vision. The chromophore is a vitamin A derivative, 11-cis retinal, which is attached to Lys-296 in the binding site through formation of a protonated Schiff base. Here we present a solid-state NMR approach with fluorine-labeling in combination with computational chemistry to gain insight into the chromophore of the dark-state of rhodopsine and its Meta-II photointermediate. The advantage of the fluorine label as a NMR probe is its high gyromagnetic ratio, large chemical shift range and high sensitivity to its microenvironment. In addition, from the spinning side bands in solid state NMR the chemical shift tensors can be easily derived, which provides additional spatial information.
Identification of Oligo-Peptides Conformation by 3D $^{14}$N/$^1$H Double Quantum/$^1$H Single Quantum Correlation Solid-State NMR at Natural Abundance

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β-sheet structure of peptides can be formed in antiparallel (AP-) and parallel (P-) by hydrogen bonds between NH groups of one strands and C=O of adjacent strands. While $^{13}$C and $^{15}$N labelling approach are widely used for structural determination in NMR, the structural analysis for naturally abundant samples has been required. Although $^1$H/$^1$H internuclear correlation provides useful information at fast MAS, the poor $^1$H resolution limits its wide applications. Thus, it is essential to add more dimension in $^1$H NMR experiments. In the previous study, P- and AP-β-sheet of oligo-alanine in natural abundance were distinguished through $^1$H/$^1$H correlations with the assistance of two $^{14}$N dimensions. The interstrand NH/NH correlation is only observed in AP-arrangement by $^{14}$N/$^1$N correlations through $^1$H/$^1$H correlations. However, this method showed low sensitivity due to two filterings of $^1$H/$^1$N HMQC.[1]

In this work, we propose a novel approach to differentiate between P- and AP-β-sheet structures by using 3D $^{14}$N/$^1$H double quantum (DQ)/$^1$H single quantum (SQ) correlation experiment. The single $^1$H/$^{14}$N HMQC filtering with the aid of high DQ filtering efficiency offers high sensitivity. This approach was demonstrated by tri- (Ala$_3$) and tetra-alanine (Ala$_4$) in either P- or AP-structure. The overlapped $^1$H resonances of NH and NH$_3$ of AP-Ala$_3$ are separately resolved in the $^{14}$N dimension (Figure 1(A)), which selectively gives 2D $^1$H(DQ)/$^1$H(SQ) correlation between NH and the other functional groups (Figure 1(B and C)). NH/NH correlation of AP-Ala$_3$ appears on the diagonal line (Figure 1(C)), whereas very little diagonal-peak is observed in the case of P-Ala$_3$ (Figure 1(B)). This result confirms each conformation through presence (AP) and absence (P) of interstrand NH/NH correlations.[2]

![Fig. 1.](image)

Fig. 1. (A) $^1$H/$^{14}$N HMQC 2D spectra of AP-Ala$_3$, 2D planes of $^1$H DQ/SQ of (B) P-Ala$_3$ and (C) AP-(Ala)$_3$, with corresponding molecular structures. 2D planes are extracted from a 3D $^{14}$N/$^1$H/$^1$H dipolar correlation spectra at the NH signal in the $^{14}$N dimension under 70 kHz MAS.

References
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ISSUES WITH TISSUES: SAMPLE STABILITY IN HR-MAS

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Objective: The benefit of ex-vivo HR-MAS spectroscopy of intact biopsies alongside in-vivo MRI and MRS is unquestioned. However, fast spinning in order to suppress water sidebands destroys the sample and ongoing enzymatic reactions are able to heavily alter the metabolomic profile. Recent studies found that most spectral information of human tumor samples in HR-MAS derives not from the tissue itself but the surrounding liquid phase [1]. A short review summarizes current bottlenecks in HR-MAS [2]. We wanted to investigate these processes in more detail for two common tissue types in our lab: rat liver and murine breast cancer tissue.

Methods: The current state-of-the-art protocol from Beckonert et al. [3] claims that at 10⁰ C maximum, spectra can be recorded without significant changes within one hour of rotation at f = 4-6 kHz. We prepared under the guidelines of [3] from each tissue five technical replicates (~12-16 mg ) and recorded ten consecutive noesy (64 scans) spectra over a total time of ~80 min at f = 5 kHz (5 min tuning, matching, shimming, pulse setting and ~75 min acquisition).

Results and Discussion: An overlay of the processed noesy spectra of one liver sample shows that peaks in the aliphatic region strongly increase already within the first hour of measurement (Fig. 1 left), leading to integral enhancements by almost a factor of two. The PCA (Fig. 1 right) of the two tissue data sets compared to five “ideal” samples (copies of ten identical noesy rat liver extract spectra) show that both sample types strongly alter due to rotational and enzymatic effects. Slow-speed spinning [4] can be an alternative to avoid the effects of physical decomposition but cannot hamper enzymatic degradation. Therefore new methods and protocols for reliable tissue analysis by HR-MAS need to be developed.

Fig. 1: Overlay of 10 HR-MAS (5 kHz) liver noesy spectra (Δ=7 min) and PCA analysis of ideal vs real samples

References

Toward an Understanding of the Crystallization Mechanism of Si-ZSM-5 by Solid-state NMR Spectroscopy

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Solid state NMR techniques have been employed to study the crystallization process of Si-ZSM-5 prepared by solvent-free synthesis method. In this work, the defect sites in zeolites Si-ZSM-5, mainly arising from the SiO··H-OSi hydrogen bonds and the SiOH nests, have been identified by $^1$H and $^2$H MAS NMR spectra. Meanwhile the specific intermolecular proximities shown in 2D $^1$H DQ-SQ NMR spectra illustrate that the spatial coulombic interactions associated with the hydrophobic property of TPA$^+$ organic cations control the formation of channel systems in the individual zeolite case. SSNMR results informed that the van der Waals interactions promote the formation of inorganic-organic composites to separate the TPA$^+$ organic cations and the coulombic interactions between the SiO··H-OSi defect sites and the methyl groups in TPA$^+$ direct the inorganic-organic composites to form the specific ZSM-5 channel systems.

Figure 1. $^2$H MAS NMR spectra of as-synthesized samples heated at 180°C for different time: (a) 0 h, (b) 5 h, (c) 15 h, (d) 16 h, (e) 18 h, (f) 20 h.

$^2$H MAS NMR spectra demonstrate that the in-cage SiO··H-OSi hydrogen bonds have been created to counterbalance the positive charges from TPA$^+$ cations and the linear SiO··H-OSi hydrogen bonds help the crystallization process through holding the silicate species together. The existence of linear SiO··H-OSi hydrogen bonds explains why ZSM-5 have been taking the layer-by-layer grown-up process; and the formation of in-cage SiO··H-OSi hydrogen bonds is the key structure properties of nucleation phases, even though the long-range periodic structure of ZSM-5 is not be established yet. $^2$H MAS NMR spectra also show that the SiOH nests are existed in the structures even the zeolite crystals have been fully established. No evidences show that the formation of SiOH nest is for providing the additional stabilities to the in-cage SiO··H-OSi hydrogen bonds.
The highly resolved $^1$H 2D DQ-SQ NMR spectra display the spatial proximity between the methyl groups of TPA$^+$ cations and the in-cage SiO·H-Osi hydrogen bonds in the framework. These coulombic interactions are the key factors to organize the disordered inorganic-organic composites forming the ZSM-5 zeolitic channel systems. Our observations confirmed that, besides acting as the coordination centers for water molecules and silicate anions, TPA$^+$ cations provide the blueprint for the spatial architecture of ZSM-5 through the coulombic interactions.
Heteroatomically and polyatomically doped graphene and carbon nanotubes have been investigated with fervor recently, thanks in part to their positive effect on the electrochemical performance of new fuel cell and battery systems as catalyst stabilizers or metal-free catalysts. A direct link between structure and function in these materials is, as of yet, unclear; most microscopic analyses are done through SEM/TEM and EDX, and in many cases these methods may be too ambiguous for meaningful interpretation on the microscopic scale. Both these methods lack the atomic-scale resolution and extreme sensitivity of solid-state NMR; however the low dopant levels and electronic conductivity of these materials may have discouraged their thorough analysis with the technique. Nevertheless, with some experimental considerations meaningful ssNMR spectra of dopant sites in functionalized / doped graphene and CNTs can be collected.

Doped graphene and CNTs were synthesized using varied CVD-based methods, and ssNMR was used to unambiguously identify dopant atom sites, finding that these synthesis methods result in highly homogeneous populations of installed phosphorus and nitrogen atoms, in contrast to other published methods offering apparently higher doping levels with huge amounts of by-products and impurities. We present the first experimental $^{15}$N spectrum displaying graphitic N in N-doped graphene. $^{15}$N-labeled nitrogen doped graphene synthesized as reported here produces mainly graphitic nitrogen sites located on the edges of sheets and around defect sites. $^1$H-$^{15}$N 2D HMQC experiments reveal that the edge-based nitrogen site is correlated to a high-frequency broad proton site, at an expected chemical shift for sheet-edge hydrogen atoms in graphene. A second method of analysis through 2D $^1$H ssNMR is also presented for an unlabeled sample, which provides a slightly more ambiguous analysis at a fraction of the cost. A nearly homogeneous population of phosphorus in P-doped graphene is found, with > 90% graphitic phosphorus and a small amount of phosphate oligomer. Similar findings were noted for the phosphorus sites in phosphorus and nitrogen co-doped CNTs.

Experimental and theoretical comparison of $^{27}\text{Al}$, $^7\text{Li}$, and $^{13}\text{C}$ magic-angle-spinning NMR spectra for LiAlH$_4$ and LiAlPh$_4$ crystals with rotational symmetry in their unit cells

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The disproportionation reaction of aluminum monohalides creates new crystals with uncommon structures that have high rotational symmetry in the asymmetric unit cell. (1) NMR is particularly appropriate for detecting these symmetries and the corresponding local atomic coordination. The recently developed modern theory of polarization has led to the ability of both planewave-pseudopotential and Gaussian-based band-structure computer codes to predict features of these crystalline NMR spectra, including both shielding and interactions of quadrupolar nuclei such as Al and Li. This work compares theory and experiment for the relatively well understood crystal LiAlH$_4$. For the new LiAlPh$_4$ crystal this work establishes the level of agreement between experiment and theory that should be possible for this new class of materials.

![Structure of LiAlPh$_4$.](image)

Fig. 1. Structure of LiAlPh$_4$.

References

ABSOLUTE QUANTIFICATION IN ssNMR

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NMR spectroscopy is well-known for its non-destructive analytical aspects. As the absorption of electromagnetic energy by a given nucleus is independent of the type of chemical compound it is part of, NMR excels in relative quantification in complex mixtures.[1] 1H ssNMR is regularly used in food and pharmaceutical sciences to detect impurities and quantify their fraction relative to the main compound.[2] While quantification has been achieved using a referencing standard (internal, external or electronically synthesized (ERETIC)),[3-6] absolute quantification remains a challenge, especially in solid state.

NMR quantification relies on the relation between the applied and received voltages, a problem often referred to as the ‘reciprocicity theorem’.[7-10] Application of a voltage to an NMR circuit (sample loaded probe) generates an RF field distribution acting onto nuclear magnetic moments, which in turn generate a magnetic flux inducing a voltage over the ends of the coil when they return to equilibrium. The main losses in energy take place through dissipation by the coupling of electric field and sample. Excluding electric fields associated with the probe circuit from the sample volume, the voltage generated over the coil becomes independent of the sample.

Combining 1H MAS NMR detection with standard addition yields a convenient methodology for accurate, absolute quantification as for example water in microporous materials (Fig. 1). In the standard addition method, known amounts of a compound are added to the sample containing an unknown quantity of the same compound. The absence of an electric field component in the sample volume can be assessed by considering the variation of the quality factor (Q) of the probe with dielectric constant (ε) of the sample. Keeping the Q- and filling factor of the loaded probe constant, the probe can be calibrated with respect to a gravimetric device, enabling the application of an NMR spectrometer as an absolute quantification device. In MAS probes, accurate sample insertion and sample position are critical to magic angle spinning, implying an extremely high degree of reproducibility of the filling factor between measurements. Equipped with these properties the NMR spectrometer can perform absolute quantification keeping its advantage of its exquisite resolution.

References

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Surface characterization by DNP-surface enhanced spectroscopy on an alumina catalyst system that is designed for the synthesis of 5-hydroxymethylfurfural

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5-hydroxymethylfurfural (HMF) is a versatile precursor in chemical industry for the synthesis of various types of chemicals, solvents, surfactants, pesticides, and resins. HMF is obtained by catalytic dehydration of fructose, glucose, and sucrose in the presence of acid catalysts. A synthetically modified alumina catalyst is prepared for the production of HMF by grafting with a thiol group and a sulfonyl acid group to improve the catalytic ability, selectivity, and stability. The incorporation of sulfonic acids thioether groups in mesoporous materials has shown high conversion of fructose to HMF. Solid-state dynamic nuclear polarization (DNP) NMR spectroscopy was utilized for the characterization of sulfonic acids thioether groups on alumina surface. A 14.1 T solid-state NMR spectrometer equipping a 395-GHz gyrotron was utilized for our DNP NMR experiments at 100 K. The DNP-surface enhanced spectroscopy (SENS) method was found useful for monitoring the changes of surface functional groups on this catalyst system as the catalytic reaction proceeds. We have detected 13C and 29Si nuclides by both direct polarization as well as cross-polarizations (CPs) from protons of DNP juice under magic-angle-spinning (MAS) with and without microwave irradiation. \(^1\text{H}-^{13}\text{C}\) and \(^1\text{H}-^{29}\text{Si}\) CPMAS detection methods naturally become a unique means for the surface-selective \(^{13}\text{C}-\) and \(^{29}\text{Si}-\)detections. Various sampling conditions of utilizing bi-radicals, such as TOTAPOL, AMuPOL, and TEKPOL, were employed by considering the polarity of the sample to find optimal DNP conditions of obtaining the required spectral information with enhanced signal intensities. DNP signal enhancements of \(\varepsilon = 4\sim12\) was observed for both \(^1\text{H}-^{13}\text{C}\) and \(^1\text{H}-^{29}\text{Si}\) detections.
A Solid-State MAS NMR Probe for Materials Interacting with Gases

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In materials such as gas catalyst and gas capture, interaction with gas plays the major role in their functionality. For analysis of such materials with MAS NMR, it is required to avoid air in the whole process of the experiment from sample preparation to the end of observation with MAS. Here, we report on a solid-state NMR probe (Fig. 1), which allows to measure the functional materials under fast MAS with given gas species without exposing the sample to the air in the process.

The probe is airtight and separable to two parts; a probe head equipped with 1.6 mm MAS module and a body which contains electronic circuit and seal off valves (Fig. 1 (b)). The probe head (Fig. 1 (c)) is small to take in and out a glove box in keeping air-tightness by the seal assembly (Fig. 1. (d)) which controlled by a stem in the MAS gas tubes connected to the probe head by a VCO® connector.

Air-tightness of the constructed probe was demonstrated by observing $^7$Li NMR peak area fraction of LiI hydrate in $^7$Li MAS NMR of LiI which was ball-milled to enhance the deliquescence. Ball-milled LiI prepared in air-tight ball-milling equipment was packed into a 1.6 mm NMR sample tube in a glove box without exposing to the air. The tube was spun at 20 kHz in the closed probehead with pure N$_2$ gas obtained from vaporization of liquid N$_2$. Then the probe head was open during MAS with the pure N$_2$ gas. Fig. 2 shows the peak area ratio of LiI hydrate was kept approximately constant before breaking the air-tightness of the probe. After opening the probe head, the intensity continued increasing with the passing time. This result shows the probe has enough air-tightness for analysis of materials.

**Fig. 1.** (a) Overall view, (b) an inner structure without the cover, (c) the probe head, and (d) the seal assembly and VCO® connector.

**Fig. 2.** Transition of the peak area ratio of LiI hydrate to the whole spectrum before (red circles) and after (gray circles) breaking air-tightness.
HIGH CAPACITY SILICON ELECTRODES FROM A MULTI-
NUCLEAR-SOLID-STATE NMR STANDPOINT

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A change in the way we produce and store energy in the future is inevitable. Lithium-ion batteries (LIB) have a great potential to supersede conventional fuel-powered vehicles. One of the major drawbacks of conventional LIBs is their low energy density. Silicon-containing LIBs, however, can reach theoretical capacities over 4000 mAh/g and are therefore of great interest for high capacity electrodes. A major drawback of silicon-containing electrodes is their tendency to expand significantly upon lithiation. Additionally, the phase changes during lithiation and delithiation are still not fully understood [1].

The current study utilizes both ²⁹Si and ¹⁹F MAS-NMR (850 MHz, spinning speed 23kHz) to gain more insight into the electrochemical cycling process of silicon electrodes. Of particular interest are nano-structured silicon monoxide anodes, which show promising cycling behaviour compared to the well-studied silicon anodes. Interestingly, ²⁹Si MAS-NMR reveals that the lithiated silicon domains (Li_xSi_y) that are formed during the lithiation process appear at different chemical shift values in silicon (broad resonance at 300 ppm) compared to silicon monoxide anodes (broad resonance at -100 ppm). We hypothesize that the lithiated silicon domains in SiO have less of a metallic character than in the case of lithiation of pure Si due to its nano-sized particles and therefore show a less pronounced Knight shift [2].

Preliminary studies using ²⁹Si MAS-NMR have also revealed an unknown silicon species which differs from the lithiated silicon domains. ¹⁹F MAS-NMR showed the formation of an SiO_xF_y species, which originates from the reaction of the silicon surface with the electrolyte LiPF_6 [3]. Further studies using CP-MAS will clarify if the unknown species found through ²⁹Si MAS-NMR is in fact SiO_xF_y.

References
Molecular rotation in an aluminium based metal organic framework (MOF)

Metal organic frameworks have achieved extremely high surface areas and pore capacities, which has allowed many developments in the areas of photochemistry, gas separation and capture\(^1\) as well as catalysis. The building blocks of these materials can have local rotational motion and this motion can be tuned and potentially put to use for molecular sensors or actuators. NMR is a useful tool to study this rotation as \(1/T_1\) is related to the molecular correlation time\(^2\). In this study we investigate the local motion of benzoate groups in an aluminium based MOF, known as Sion-7, with a pyrene-benzoate organic linker. The local reorientation is studied by following \(^1\)H \(1/T_1\) as a function of temperature with a clear phase transition visible, we also show \(^2\)H line-shape data and \(^{13}\)C linewidths to elucidate the behaviour alongside pXRD and molecular dynamics simulations.

![Image: Rotation of benzoate groups in Sion-7](image)

Figure 1: Rotation of benzoate groups in Sion-7

INTERACTION OF BENZENE AND BENZENE DERIVATIVES WITH UIO-66(ZR) BY SOLID-STATE NMR

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Metal organic frameworks (MOFs) consist of metal oxide cores linked by multifunctional organic linkers, forming crystal-like structures with well-defined pore size. They are highly promising materials in a large variety of applications including gas storage, solvent separation, drug delivery, and catalysis. In particular, MOF materials show great potential for an improved separation of benzene derivatives. Benzene and its derivatives are of key importance for many practical applications due to their usability as base chemicals and scaffold building primers and ligands. Thus, the characterization of the guest-host interaction for identifying the factors which govern the separation of various benzene derivatives is crucial for designing efficient MOF materials. Among existing analytical methods, NMR is well suited to gain such information. NMR methods have been already successfully applied to the study of structure, dynamics, and flexibility of different MOFs but they have been employed up to date only in very few cases for characterizing their interaction with liquid organic compounds.

Therefore, in this study, the interaction of benzene and various benzene derivatives with UiO-66(Zr) is investigated by a combination of $^1$H and $^{13}$C NMR MAS spectroscopy and $^1$H site-selective relaxometry. For this, various solvent concentrations corresponding to different amounts of solvent molecules per MOF unit cell were studied.

The obtained results show that the interaction of benzene with UiO-66 is strongly concentration dependent. At very low concentrations a single benzene signal is observed at a position shifted towards lower ppm values with respect to the position of the signal of the free benzene. With increased benzene concentration above a well-defined concentration, a second and then a third signal are observed, both at the same chemical shift as that of free benzene. Supported by the relaxometry results and results obtained in our group for other solvents in the same MOF, our data indicate that benzene can be found at low concentrations only in the smaller tetrahedral pores. With increased concentration, the benzene molecules start to occupy also the larger octahedral pores and later on the inter-grain space. To our knowledge, this is the first time that three different types of solvent species are observed in a MOF material. The findings for benzene are then compared with the results for other benzene derivatives. In particular it was found that 1,3,5-triisopropylbenzole shows at all investigated concentrations only a single species with signals at the same positions as those of the free solvent. This indicates that the size of this solvent molecule is too high and therefore fails to enter the pores of UiO-66.
Atomic-scale structure analysis of a molecule at a (6-nanometer)$^3$ ice crystal

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Water is the most important solvent in nature. It is a crucial issue to study interactions among water molecules and how water structure is on nanoscale. Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful tools to detect magnetic interactions for the structure analysis of a molecule with broad applications. But conventional NMR spectroscopy requires macroscopic sample quantities with hampers in investigating nanoscale structures. Through quantum control of a single spin quantum sensor, NMR spectroscopy of nanoscale organic molecules has been achieved. However, the measurement of the dipolar interaction of nuclear spins within a molecule at nanoscale and the analysis of its structure remain a big challenge. Here we succeed in detecting the NMR spectrum from an ice crystal with (6-nanometer)$^3$ detection volume. More importantly, the magnetic dipolar coupling between two proton nuclear spins of a water molecule was recorded. The resolved intra-molecule magnetic dipolar interactions are about 15 kHz and 33 kHz with spectral resolution at a few kHz. Analysis of the interaction-resolved NMR spectroscopy provides a spatial view of nanoscale ice crystal, from which the orientation of a water-molecule bond is derived and further the length of the bond can be got. This work enables NMR spectroscopy applications in single molecule structure analysis, provides a further tool for nanocrystalline and confined water research.
Complete Structural Assignment of a Pharmaceutical Drug by Combining DNP-Enhanced Solid-State NMR and DFT Calculations

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New developments in NMR crystallography allow for a combined experimental and computational approach for structural characterization and elucidation of powdered crystalline materials [1,2]. Solid-state NMR investigation of pharmaceutical drugs is faced with two main drawbacks: the low sensitivity of NMR experiments involving nuclei such as $^{13}$C, $^{15}$N at natural abundance and long $^1$H $T_1$ relaxation times of many pharmaceuticals. The consequence of these cumulative effects is a very long experimental time for signal averaging required to obtain sufficiently high signal-to-noise ratio in two-dimensional NMR spectra, which are essential for the unambiguous chemical shift assignment of the investigated structure. Advancements in the field of Dynamic Nuclear Polarization (DNP) [3] and its ability to enhance the solid-state NMR signal enable a fast acquisition of NMR experiments at natural abundance and the possibility of structural characterization of organic molecular crystals [4].

In this study we use multinuclear DNP enhanced solid-state NMR in combination with DFT calculations to explore the structure of the sitagliptin phosphate – a pharmaceutical drug used for the treatment of Type 2 diabetes. For this purpose we employ a combination of through-bond $^{13}$C–$^{13}$C J-refocused INADEQUATE and $^{13}$C–$^{13}$C SAR-COSY experiments for the unambiguous assignment of the $^{13}$C resonances. All $^{15}$N chemical shifts are assigned based on the $^{15}$N CP-MAS and $^{13}$C–$^{15}$N TEDOR experiments. $^1$H–$^{13}$C HETCOR experiments are used to identify all the protonated carbons of the molecule and to assign the $^1$H chemical shifts. The $^1$H, $^{13}$C, and $^{15}$N DFT calculated chemical shifts are used as a complementary tool to achieve the full assignment of sitagliptin phosphate.

References
In-Situ Monitoring of Halogen Bond Formation by $^{31}$P Solid-State NMR

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Halogen bonding is a noncovalent interaction between the electrophilic region of a halogen, and an electron donor. This interaction is highly directional and comparable to hydrogen bonding, and therefore it has gained an increasing amount of attention in different fields, such as catalysis, drug design, etc.[1]. The process leading to the formation of halogen bonded adducts and the occurrence of polymorphs is poorly understood due to the fact that many solid-state reactions involve a milling process which is experimentally challenging to study in situ. Fortunately, breakthroughs in the in-situ study of mechanochemical processes using Raman spectroscopy[2] and synchrotron X-ray diffraction[3] have been developed to provide new opportunities to access the mechanisms of milling reactions. However, amorphous or glassy intermediates formed during the reaction are not easily analyzed directly by diffraction methods. This difficulty can be overcome by solid-state nuclear magnetic resonance (SSNMR).

Here we report on two cocrystals featuring halogen bonds between triphenylphosphine oxide and 1,4-diiodotetrafluorobenzene which can be formed under relatively mild conditions.[4] The crystal structures of these two cocrystals have been characterized by single-crystal X-ray diffraction. With the help of increasing temperature and pressure generated by fast sample spinning in MAS NMR experiments, cocrystallization processes can occur, which provides a way to directly track such processes and the interconversion between the two structures using in-situ $^{31}$P MAS NMR. By further varying the temperature inside the probe (from 15 °C to 45 °C), the spinning speed of the sample (from 8 kHz to 12 kHz) and different amounts of solvent added to initiate the reaction (0, 3, and 4 μL acetonitrile), $^{31}$P in-situ SSNMR is able to offer the opportunity to observe the different formation rates of these two co-crystals, to determine the mechanism of halogen bonding formation in real time and even observe the effect from temperature, pressure, and solvent quantities on monitoring the halogen bond formation. Additional kinetic model fittings reveal that the reaction mechanism is predominantly diffusion controlled. Furthermore, the temperature dependence of the reaction rate was used to estimate the activation energy for the formation of one of the cocrystals.

References
High resolution solid state NMR spectroscopy in surface organometallic chemistry: access to molecular understanding of active sites of well-defined heterogeneous catalysts.

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Abstract:
Among various measurements techniques, Nuclear Magnetic Resonance (NMR) is an extremely powerful tool for the identification of chemical composition and the determination of molecular structure. Because of its versatility it is widely applied in chemistry, materials science, and biomedical research. At KAUST, we boast an NMR lab housing 5 Solid State NMR spectrometers from Bruker include one of the world’s first Bruker 900MHz Wide-Bore magnets and 1 DNP spectrometers coupled with a huge range of accessories that would allow break-through research. High resolutions NMR provide a range of exquisitely precise tools to characterize well-defined active sites in heterogeneous catalysis. Implementation of high resolution and 2D solid state NMR techniques helped to identify surface structures at a molecular level, which is a key to implementing structure-reactivity relationships and rational developments in heterogeneous catalysis. It is clear that these methods also have the potential for extensive further developments and applications, for example towards understanding more complex systems (complex oxide materials, active sites with paramagnetic or quadrupolar centres), probing the dynamics of surface species (access to mobility of active sites), and to monitor the active sites as a function of time. 2D high resolution and 2D spectra shows the power of this technique to characterize the well-defined heterogeneous catalysts prepared by surface organometallic chemistry.

Keywords: heterogeneous catalysis, surface organometallic chemistry, solid-state NMR,

Figure 1: Variable-temperature $^{13}$C CP/MAS, $^1$H spin-echo, two-dimensional $^1$H–$^{13}$C dipolar HETCOR NMR spectra MAS solid-state NMR spectra of $\equiv$Si–O–W(Me)$_5$
Multi-spin correlation detection for the central spin problem

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Environmental effects on the evolution of a spin system in the context of the central spin problem, have been studied for more than 60 years. With the growing complexity of quantum information processors there is a need to better understand and control the interactions of qubits with their environment. Decoherence is an apparent loss of quantum coherence of the central spin, which is the result of the coherent evolution of the central spin and its spin environment. This evolution may be understood as the consequence of local field fluctuations induced by heteronuclear dipolar interaction between the central spin and the environment spins and homonuclear dipolar interaction of spins in the environment. A complete theoretical description for the evolution of the central spin does not exist and numerical solutions are restricted to small spin environments.

Using Multiple Quantum Nuclear Magnetic Resonance (MQ NMR) techniques, we have designed experiments for the direct detection of multi-spin correlations between the central spin and environment spins. These experiments are used to observe the progress in production of correlations between the central spin and environment and they reveal the multi-spin dynamics of the decoherence process. Furthermore, these experiments are used to measure the sensitivity of the environment to perturbation. Based on findings of these experiments a comprehensive model for the multi-spin dynamics of the system-environment evolution is suggested that can describe the flow of quantum information from the central spin to the environment and the fragility of the memory functions in the spin environment.
Predicting release profiles of small drug-like molecules from a lipidic cubic phase using translational diffusion coefficients measured by PFG-NMR

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Due to its ability to encapsulate a broad range of molecules, the ordered nanostructured lipidic bicontinuous cubic phase has demonstrated potential to act as a drug-delivering media where drug molecules undergo sustained diffusion-controlled release over time [1,2]. Characterization of molecular translational self-diffusion, which is readily measurable by PFG-NMR, has seen increased application in the field of drug development, including structural studies of drug metabolites in mixtures [3]. The control of drug release from lipid bicontinuous cubic phases has previously been shown to depend on the nanostructural parameters of the lipid mesophase [4]. In this study, the diffusion and release of two amino acids, histidine and phenylalanine, encapsulated within a number of lipidic cubic mesophases (phytantriol, Dimodan, monoolein and monovaccenin) that correspond to different unit cell size and nanoscale water channel diameter, were investigated using PFG-NMR. The translational diffusion coefficients of the encapsulated amino acids, histidine and phenylalanine, were first measured using the standard BPP-STE sequence. A correlation between experimentally measured translational diffusion coefficients and the nanoscale diameter of the water channels within the cubic mesophase was subsequently established. This correlation was then used to predict the release profiles of encapsulated compounds from within the cubic mesophase using the Higuchi equation [5]. Resultant release profiles were then compared with those obtained by directly measuring the release of each amino acid \textit{in vitro}. The predicted release profiles derived from the translational diffusion coefficients measured by PFG-NMR tracked reasonably close to the directly measured release profiles \textit{in vitro} [6].

References
Flavivirus is a medically important genus, which includes Dengue (DENV), Zika, West nile and Yellow fever virus. The mature virion comprises three structural proteins: capsid, membrane protein and the envelope E glycoprotein that contains cell receptor binding site and the fusion peptide. E protein has 3 domains: DI, DII and DIII. DIII domain is highly conserved among flavivirus and is the one related to virus attachment to host cells and host antibody recognition. The exact nature of the cellular receptor has not been fully elucidated but glycosaminoglycans have been reported to play a key role in initial binding and infectivity of DENV over host cells. In the present work, we have mapped GAG binding interaction with DIII domains of all 4 DENV serotypes using NMR and we followed the variation of 1H and 15N chemical shifts (CSP) upon complex formation. Fondaparinux, a synthetic pentasaccharide related to low molecular weight heparin, was used as GAG mimetic. We analyzed CSP perturbation and intensity changes upon GAG complex in DENV1, DENV2 and DENV4 serotypes. Significant changes were observed mainly in the N-terminal residues of serotypes 1, 2 and 4. Nevertheless, in DENV3 serotype we could not observe significant perturbation caused by GAG interaction, probably due to the fact that the construct (recombinant protein) started ahead (302 instead of 295) in the primary sequence and was missing few residues at N-terminal region. These data together report the importance of N-terminal region to GAG and domain III interaction. We intend to use other GAGs mimetic and measure relaxation parameters to further characterize this complex.
Tannins are compounds that naturally occur in plants and notably exist in large amounts in red wine. Wine tannins from grape are polymers consisting of polyphenol subunits belonging to the flavan-3-ol family. These molecules are known both for their sensory and antioxidant properties. Indeed, wine tannins are responsible for wine astringency and bitterness, two feelings resulting from the interaction of tannins with saliva proteins and taste receptors in the mouth, respectively [1]. With their numerous phenolic moieties, they are also good targets prone to counteract the deleterious action of free radicals on human health [2]. Recently, tannins were shown to interact with lipids [3], which might be responsible for their impact on wine taste. Furthermore, these interactions could protect membrane lipids from oxidation, suggesting a health-preserving tannin character.

We have therefore investigated the effect of tannins on the organization and oxidation of lipids within membranes by solution and HR-MAS NMR. In order to achieve this, we have traced the interactions of grape tannin subunits with several defined synthetic membranes as cell-membrane models (isotropic bicelles and multilamellar vesicles). Our work revealed tannins to have a disordering effect on membranes depending on both the initial degree of membrane organization and the specific tannin hydrophobicity [3, 4]. Furthermore, we conceived an innovative NMR method that does not require the use of external probes, to highlight the antioxidant effect of tannins on reconstituted membranes in situ. The method also allowed for demonstrating the synergistic action of tannins for the first time [5]. Whether their effect on membrane organization or lipid oxidation, the interfacial position of the tannins in the membrane seems to be crucial.

In conclusion, we found that the interaction between tannins and oral membrane lipids could influence the taste of wine in two possible ways: by competing with the tannin-protein interactions leading to astringency, and by disturbing the lipid environment of taste receptors within gustarory cells involved in bitterness. Moreover, the interactions of tannins with cell-membrane lipids could explain their antioxidant efficiency, by forming a protective layer at the membrane surface.

References
Comparison of rapid protein structure determination approaches driven by experimental NMR data

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The knowledge of the tridimensional structure of a protein is essential to design drugs, to predict protein function and to study mechanism of protein function. 3D structure can be determined using two experimental techniques: X-Ray and NMR. However, these techniques have limitations: they are time consuming, manually intensive and sometime technically difficult. Due to these limitations, different approaches that combine the strength of computer and sparse experimental NMR data have been proposed to determine rapidly 3D protein structure. Thus different approaches that use sparse NMR experimental NMR data such as backbone chemical shifts, incomplete sets of NOEs distances and residual dipolar couplings of backbone have been designed. To assess whether if these automated methods can indeed produce structures that closely match those manually refined by experts using the same experimental data, Critical Assessment of Automated Structure Determination of Proteins from NMR Data [1] (CASD-NMR) was created. CASD-NMR concept closely resembles to Critical Assessment of Automated Structure Prediction [2] (CASP) that aim to assess performance of protein structure prediction methods from sequence. Accordingly to CASD-NMR 2013 [3] recommandations, we are working on the comparison of different automated protein structure determination methods driven by backbone chemical shifts and homology modeling in terms of the fitness of resulting 3D structures with experimental NMR data. For homology modeling, we have been using two approaches: I-TASSER [4] and MODELLER [5] that are best public CASP-certified protein structure prediction servers. Structure calculation guided by NMR backbone chemical shifts were done using different approaches: (i) ROSETTA method that is widely used by the scientific community. Different rosetta based methods exist that used only backbone chemical shifts as experimental data. Moreover, ROSETTA method is probably the most backbone chemical shifts based structure calculation method used and cited by the community. For our comparison, three ROSETTA-family methods have been used: CS-ROSETTA [6], CS-HM-ROSETTA [7] and RASREC CS-ROSETTA [8]. (ii) Cheshire which is the first backbone chemical shifts based method which performed as well as CS-HM-ROSETTA during CASD-NMR-2013, and (iii) CS23D which is a web server that performed 1000-10,000 times faster than competing methods (Cheshire [9] and CS-Rosetta).

Each of these approaches were used to determine 3D structure of a benchmark of 50 proteins. These 50 proteins were randomly selected within proteins for which NMR backbone chemical shifts are available in the BMRB data bank excluding proteins that have been used during CASD or CASP. In addition, we will applied these methods on cold shock proteins for which we have our own experimental data.

References

Combined use of NMR, Fluorescence and Differential Scanning calorimetry to compare folding, stability and dynamics of three cold shock proteins produced by extremophile microorganisms: A multi-disciplinary approach.

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Extremophile microorganisms are found from about -20°C for psychrophilic up to 122°C for hyperthermophiles [1]. This is not microbial dormancy or survival since these organisms are deeply adapted to these extreme conditions. Psychrophiles do not grow above 20-25°C and hyperthermophiles are unable to divide below 60°C [2]. Life under these extreme conditions requires numerous adaptations at all cellular levels and enzymes play a key role in these adaptations as they drive the cell metabolism and cell cycle. Protein stability and dynamics are two intricately related parameters that have not been analyzed within the whole temperature range where life occurs. Dynamics of these proteins has been studied by H/D exchange and NMR and has revealed a compact structure undergoing restricted internal motions. However, in most studies, the absence of a mesophilic homologue analyzed under strictly identical conditions has precluded a fine analysis [3]. On the other hand, it has been shown that psychrophilic proteins display a metastable state of weak stability. Moreover, this flexibility is required to maintain activity/functionality at low temperatures. Accordingly, two aspects remain to be answered: i) what are the fundamental mechanisms allowing the adaptive adjustments of stability and dynamics of proteins at extreme temperatures, and ii) are there specific mechanisms for high temperature adaptation and other specific to low temperatures? The main goal of this project is to pursue this approach, using small model proteins (simple folders) to provide quantitative results. The selected microorganisms are well characterized gram negative bacteria: the Antarctic psychrophile Pseudoalteromonas haloplanktis, the mesophile Escherichia coli and the hyperthermophile Thermotoga maritima. This series covers nearly all temperatures encountered by living organisms. The model proteins have been selected according to several criterions amount of recombinant production compatible with the project requirement, small size compatible with most biophysical methods, demonstration of a reversible unfolding according to a 2-state model, absence of cysteine residues interfering with refolding. On this basis, the so-called “small cold shock proteins” (Csps, ~7.5 kDa) have been selected for the present project. The three proteins have been produced isotopically labeled (13C/15N). The backbone chemical shifts have been assigned for the three proteins in order to perform spin-relaxation experiments and deuterium/hydrogen exchange experiments to fully characterize the dynamics of these three proteins. Since this work is ongoing, we will present the preliminary results on the comparative dynamics for these three proteins.

References
Blocking the interaction between S100A9 and RAGE V domain using CHAPS molecule: A novel route to drug development against cell proliferation.

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Human S100A9 (Calgranulin B) is a Ca$^{2+}$-binding protein from the S100 family, that often presents as a homodimer in myeloid cells. It becomes an important mediator during inflammation once calcium binds to its EF-hand motifs. Human RAGE protein (receptor for advanced glycation end products) is one of the S100A9’s target-proteins. RAGE binds to a hydrophobic surface on S100A9. Interactions between these proteins trigger signal transduction cascades, promoting cell growth, proliferation, and tumorigenesis. Here, we present the solution structure of mutant S100A9 (C3S) homodimer, determined by multi-dimensional NMR experiments. We further characterize the solution interactions between mS100A9 and the RAGE V domain via NMR spectroscopy. CHAPS is a zwitterionic and non-denaturing molecule widely used for protein solubilizing and stabilization. We found out that CHAPS and RAGE V domain would interact with mS100A9 by using $^{1}$H-$^{15}$N HSQC NMR titrations. Therefore, using the HADDOCK program, we superimpose two binary complex models of mS100A9-RAGE V domain and mS100A9-CHAPS and demonstrate that CHAPS molecules could play a crucial role in blocking the interaction between mS100A9 and the RAGE V domain. WST-1 assay results also support the conclusion that CHAPS inhibits the bioactivity of mS100A9. This report will help to inform new drug development against cell proliferation.
Biophysical characterization of Histone Acetyltransferases Hpa2 of MDR A. baumannii

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ABSTRACT: In the current scenario, widespread multidrug resistivity in ESKAPE pathogens demands identification of novel drug targets to keep their infections at bay. For this purpose, we have identified a novel target Hpa2 of A. baumannii, a member of GNAT superfamily of HATs. But due to sequence identity of equal to or less than 35% with known proteins in PDB, the correct sequence alignment and construction of 3D monomeric and dimeric models of Hpa2 having optimal structural parameters is difficult. To circumvent these problems, we have designed an easy and optimized in silico protocol for Hpa2 modeling. Virtual screening of substrate-derived library and polyamines is performed to achieve a new platform for developing A. baumannii inhibitory molecules. Binding of polyamines with a derived model illustrated the same binding pattern as described experimentally in case of yeast Hpa2. Recombinant protein was expressed; purified and secondary structure was analysed using UV-CD experiment. Binding studies with cofactor acetyl-coA and polyamines indicated that binding of Hpa2 is an enthalpy-driven process. Structural elucidation of Hpa2 enzyme using NMR to understand the molecular recognition interactions involved in enzyme catalysis and its interaction with variety of antibiotics is in progress.
Rabies virus is a non-segmented negative sense RNA virus that causes disease in humans with a 100% case fatality rate, resulting in c. 60,000 deaths/year world-wide. There are currently no treatments for rabies disease, but a number of critical interactions of viral proteins provide potential targets to develop new antiviral compounds. Of particular interest are the interactions of viral nucleocapsid (N), phosphoprotein (P), and large/polymerase (L) wherein the N protein encapsidates genomic RNA to form the helical nucleocapsid (N-RNA) that serves as the template for viral transcription and replication by the RNA-dependent polymerase complex, composed of the enzymatic L-protein and non-catalytic polymerase cofactor P-protein. P-protein is critical in attaching L to the N-RNA template via an interaction between the P-protein C-terminal domain (PCTD) and the C-terminal trypsin-sensitive peptide of N-protein (N-pep). This interdomain interface between N-pep and PCTD provides a potentially valuable target, but the structure of the complex remains only partially resolved.

We have commenced a project to characterize the precise molecular interactions of P- and N-protein. The PCTD and N-pep have been expressed in Escherichia coli, generating protein at high yield and purity. NMR titrations of PCTD with N-pep suggest a single binding mode with a micromolar affinity at the positive patch of PCTD and the flexible loop region of N-pep. The mutagenesis study indicates that the interaction between N-pep and PCTD is mainly mediated through electrostatic and hydrophobic interactions. By introducing mutations and cyclizing N-pep, the binding affinity has been improved more than 100-fold, which enables elucidation of the complex structure and how it forms by X-ray crystallography and NMR.

The ongoing research seeks to investigate the structural and dynamic features of the PCTD-N-pep complex, which will reveal the replication machinery of rabies virus as well as the strategies by which P-protein mediates diverse functions in viral replication and immune evasion by coordinating its multiple interactions with viral protein and host cell proteins. This study will provide valuable information for structure-based drug discovery and anti-viral drug design.

References
Nuclear Magnetic Resonance (NMR)-Based Fragment Screen for Inhibitors of the Clostridium difficile Binary Toxin, CDT

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Clostridium difficile infection (CDI) is one of the most common nosocomial infections in industrialized nations, often prevalent in hospital patients undergoing antibiotic therapy due to the disruption of normal colonic flora caused by antibacterial agents. C. difficile-associated disease (CDAD) includes antibiotic-associated diarrhea, colitis and pseudomembranous colitis (PMC) and in some cases, may progress to toxic megacolon, sepsis and death. Over the last decade, rates and severity of hospital infections have increased dramatically and correlate with the emergence of a hypervirulent strain (NAP1) characterized by the production of a binary toxin, C. difficile transferase (CDT). Due to the opportunistic nature of C. difficile pathogenicity, treatment with broad-spectrum antibiotic therapies often fail to eliminate CDAD or lead to recurrent illness; thus, new methods of prophylactic or therapeutic treatment are needed.

Central to the mechanism of CDT virulence is the coordination of two individual binary toxin components, CDTb (cell binding component) and CDTa (catalytic component) to form a functional toxin. From the perspective of CDTa, complex formation is mediated by catalysis-independent domain 1 (CDTb-interacting domain; CDTaBID), making it an attractive target for pharmacological intervention. Therefore, we have applied protein-observed NMR-based compound fragment screening to the discovery and validation of chemical starting points for inhibition of CDT complex formation. Mapping of ligand-induced chemical shift perturbations of [\(^{1}H-^{15}N\)]TROSY spectra revealed two sites of CDTaBID with high propensity for fragment binding and binary toxin inhibition.

Ligand-induced chemical shift perturbations (A) guide in-silico protein-compound modeling (B). In (C), the location and pose of a Site 1 binder is predicted by CSP-guided docking and confirmed with co-crystallization.
A GENERAL FRAMEWORK FOR STRUCTURAL MODELING WITH PARAMAGNETIC NMR RESTRAINTS IN ROSETTA

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NMR structure determination of proteins with high molecular weight often yields sparse datasets. Recent developments in site-specific labeling techniques with metal ion tags allow for collection of PCSs, RDCs and PREs to supplement sparse data. These effects serve as long-range distance and orientation information that help constrain the global protein fold and restrict the conformational search space. To leverage the limited number of restraints in structure determination, advanced modeling techniques are required. The biomolecular software package ROSETTA provides rich functionality for modeling with sparse NMR data such as chemical shifts, NOEs, RDCs and PCSs [1,2]. However, these applications lack integration and user-friendliness and prohibit the use of several types of restraints simultaneously. Here, we report a new ROSETTA framework for structural modeling with paramagnetic NMR data, which allows modeling with all three restraint types through a unified interface. Previous PCS and RDC energy methods were re-designed, improved for speed, and extended to overcome limitations such as the number of spin label sites or the restriction to selected ROSETTA protocols. A new PRE score term was added to the ROSETTA energy function. To account for internal dynamics of the spin label, it is represented as an ensemble via the ROSETTA rotamer library. The new framework offers a versatile toolbox to leverage paramagnetic NMR data for a diverse set of modeling tasks such as ab initio structure prediction, comparative modeling, protein-protein and protein-ligand docking (Fig.1 and Fig.2).

Fig. 1. Ab initio structure prediction of calmodulin (PDB 2K61) with PCSs and RDCs. (A) Combined ROSETTA and PCS+RDC score vs. RMSD to the crystal structure. (B) RMSD distribution of sampled models. (C) Cartoon representation of the native (grey) and lowest-scoring ROSETTA model (red/green).

Fig.2. PCS-assisted docking of p62 PB1 (PDB 2KTR). (A) Combined ROSETTA interface and PCS score vs. RMSD to the NMR structure. (B) Correlation between experimental and calculated PCSs for the lowest-scoring ROSETTA model. (C) Cartoon representation of the NMR ensemble (grey) and 10 best ROSETTA models.

References
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Antibiotic resistance is rapidly becoming a serious threat to public health. Bacteria have been evolving in ways that have greatly decreased the efficiency of many commonly used antibiotics. One of the ways that bacteria have become resistant to antibiotics is through the expression of a class of proteins called β-lactamases. β-lactamases target antibiotics that contain a β-lactam ring and deactivate them through hydrolysis. A possible method for combating antibiotic resistance would be to develop optimized inhibitors that target these enzymes, allowing current antibiotics to still be viable. KPC-2, Klebsiella pneumoniae carbapenemase-2, is an example of a β-lactamase that exhibits multi-drug resistance and is the enzyme of interest in this study. In order to systematically optimize inhibitors to target this enzyme, additional structural data of KPC-2 is needed. Of particular interest is a sixteen amino acid long loop region, referred to as the Ω-loop. It has been proposed that the Ω-loop may play a key role in antibiotic resistance, and therefore needs to be considered when optimizing KPC-2 inhibitors. Although there are crystal structures of KPC-2, due to the limitations of X-ray crystallography they do not provide insight into the structural dynamics of the Ω-loop. Using various NMR techniques, the structure and dynamics of both apo and inhibitor bound KPC-2 will be studied with the goal of understanding the role of the Ω-loop in antibiotic resistance.
Identification of the presence of Benzoylmethylecgonine in illicit drug samples by Maximum-Quantum NMR spectroscopy

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The most commonly used techniques for the identification of forensic drug samples are GC/MS\textsuperscript{[1]} and HPLC because they can provide specific spectral data on individual compounds in a complex mixture without prior isolation. Recently, NMR spectroscopy has been increasingly applied to the analysis of mixtures of small organic molecules. Specifically, the NMR experiment that carries the closest analogy to chromatography is Diffusion Ordered Spectroscopy (DOSY)\textsuperscript{[2]}.

The usefulness of DOSY NMR has been demonstrated in the analysis of heroin\textsuperscript{[3]} in solution. In parallel, alternative NMR approaches suited for mixture analysis have been sought and developed. Among those, \textsuperscript{1}H Maximum-Quantum NMR has provided the highest resolution, up to tens of molecules in favorable cases\textsuperscript{[4]}.

Herein, we report a simple and fast method to detect the presence of Benzoylmethylecgonine (Fig. 1) in drug samples for a concentration of 80 mM in less than 5 min, by applying 2D Maximum quantum (MaxQ) NMR spectroscopy.

\textbf{Fig. 1.} Chemical structure of Benzoylmethylecgonine (cocaine)

The results highlighted the practical utility of DOSY and MaxQ techniques particularly in forensic identification.

References
NMR spectroscopy is typically applied to solutions for studying the chemical structure, chemical interactions and concentration of various chemical species in mixtures. Such measurements are executed during synthesis for reaction control or after synthesis for product characterization. NMR spectroscopy can also be used for quality control of chemical components during various reaction steps in a large-scale reaction setup where quality and percentage yield of the end and by products depend upon the intermediate steps. Implementation of different analytical methods at the processing site leads to the shortening of the time lags between sample preparation and reception of the measurement results, which becomes significant when the instrument is operated in the separate laboratories away from the chemical workbench. In the case of NMR this results from the large instrument size, maintenance conditions, and operational sophistication. Conventional NMR spectrometers use high-field superconducting magnets. Due to cost and operational complexity these instruments are typically shared among a larger group of users or operated in service mode, so that samples cannot be measured on demand, and on-demand measurements become a luxury commodity for high-field NMR spectroscopy.

On the other hand, compact low-field high-resolution NMR spectrometers can readily be set up on a chemical workbench near the production plant [1]. They are less costly, can be operated in automatic push-button mode by untrained personnel, and are suitable for an increasing variety of NMR experiments including 2D NMR spectroscopy as well as $^{13}$C NMR spectroscopy. We have measured 1D $^1$H NMR spectra and quantified their differences by means of partial least square regression (PLS-R) [2] for 119 raw styrene-butadiene rubber samples. They were obtained from different batches at a manufacturing site and analyzed for their monomer ratios of styrene and butadiene [3]. High-field NMR spectroscopy was used as a reference method for (PLS-R) quantification. Furthermore, the vinylic and allylic monomer components of butadiene were quantified, as they vary depending on the polymerization. Samples were also analyzed with attention to the homogeneity within the same batch. Although the analytical sensitivity of compact NMR systems is low compared to high-field systems, with the aid of chemometrics it is possible to quantify differences within rubber sample from the same batch. Also, with the aid of descriptive analysis, it is possible to characterize the particular repeat unit or its chemical group, which is responsible for the major differences. Differences found in the $^1$H NMR spectra could be detailed in $^{13}$C spectra and assigned to different copolymer sequence statistics, which result from different reaction conditions that give rise to different copolymerization parameters. It is concluded, that desktop NMR spectroscopy in combination with chemometrics provides a way to identify and quantify the differences in raw rubber from different manufacturers and different manufacturing batches.

References

SOLUTION STRUCTURE AND FUNCTIONAL CHARACTERIZATION OF A POTASSIUM CHANNEL BLOCKING PEPTIDE OspTx2b FROM THE SEA ANEMONE *Oulactis* sp.

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Sea anemones are venomous animals that belong to the phylum Cnidaria, and their venom is a rich source of pharmacologically important peptides and proteins. Sea anemone peptide toxins are highly potent and selective against various ion channels \([1]\). ShK, for example, blocks \(K_{V1.3}\) channels that are overexpressed on \(T_{EM}\) lymphocytes in autoimmune diseases, and an analogue of ShK is currently under development for the treatment of autoimmune diseases \([2]\) \([3]\). Peptide toxins are also valuable tools for studying the structure and function of their target channels. Peptide toxins with an ShK-like scaffold are widely distributed in sea anemones and other animals \([4]\). Recent transcriptomic studies on sea anemones have led to the identification of a number of peptide toxins in this family. This work involves the characterization of a potent potassium channel blocking peptide, OspTx2b, which was identified in our transcriptomic study of a sea anemone *Oulactis* sp.

OspTx2b is a 36-residue peptide with an ShK and BgK-like cysteine framework. OspTx2b was chemically synthesized and its solution structure was determined using nuclear magnetic resonance (NMR) spectroscopy. Residue-specific assignments were obtained from two-dimensional (2D) NMR spectra including DQF-COSY and TOCSY. Sequential assignments were obtained from a 2D NOESY spectrum. Distance constraints generated from NOE peak intensities and dihedral angles calculated from \(\text{J}_{\text{HN-Hα}}\) values were used for structure calculation by CYANA. Amides involved in hydrogen bonding were predicted from a TOCSY spectrum recorded in 100% \(^2\)H\(_2\)O. OspTx2b has a BgK-like structure, with two helices spanning residues 9-16 and 24-31. These two helices are separated by a loop between residues 16-24 and the first eight residues are in an extended conformation. The disulfide connectivity are 2-36, 11-29 and 20-33. The orientation of the functional Lys-Tyr dyad in OspTx2b is very similar to that in BgK. In patch-clamp electrophysiology assays, OspTx2b showed high activity against \(K_{V1.3}\) channels. OspTx2b may be a useful new lead in the treatment of autoimmune diseases.

References
Solution NMR structure of RHE_CH02687 from *Rhizobium etli*: a novel flavonoid-binding protein

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*Rhizobium etli*, mainly including the strains *Rhizobium etli* CFN42, *Rhizobium etli* bv. *mimosae* and *Rhizobium etli* bv. *phaseoli*, is one of the most predominant bacteria that can form symbiotic relationship with legumes, and provides nutrients for plants by transforming nitrogen into ammonia[1]. The complete genome of *R. etli* CFN42 contains a circular chromosome and six large plasmids[2]. The gene RHE_CH02687 is located in the circular chromosome of *R. etli* CFN42 with high conservation within rhizobia, and the protein RHE_CH02687 is annotated as a hypothetical conserved protein in the UniProt database (Q2K6S8-RHIEC) with unknown function. In this study, we solved the solution NMR structure of RHE_CH02687, which is comprised by seven β-strands and three α-helices with the secondary structural order of N-β1-α1-α2-β3-β4-β5-β6-β7-α3-C. The strands, β1, β7, β6, β5 and β4 formed a five-stranded antiparallel β-sheet, while β2 and β3 formed another antiparallel β-sheet. Together with three α-helices, these strands form the helix-grip fold, creating one large hydrophobic cavity. Sequence similarity searches revealed that RHE_CH02687 belongs to the activator of Hsp90 ATPase homolog 1-like protein (AHSA1) family, one of the four largest families in the Bet v 1-like superfamily. Further structural comparison suggested that RHE_CH02687 is similar to the eukaryotic protein Aha1 (PDB ID: 1X53), and the prokaryotic protein YndB (PDB ID: 2KTE) from *B. subtilis*, the only two members in the AHSA1 family with known function. Our NMR titration experiments confirmed that RHE_CH02687, similar to YndB, interacts with both flavone and chalcone, which are precursors or intermediates for the production of flavonoid molecules that are used by plants as signals for infection or as phytoalexins for selection of compatible symbionts in their root nodules. Our findings suggest that RHE_CH02687 is involved in the symbiotic relationship between *R. etli* and plants. In addition, no direct evidence was obtained in our study for the interaction between RHE_CH02687 and high temperature protein G (HtpG), the homolog of Hsp90 in *R. etli*.

References


Optimizing Non-Uniform (Sparse) Sampling 3D NMR for Epitope Mapping of Antibody-Antigen Interactions

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The practical applications of high-resolution NMR for antibody epitope mapping are often hindered by the intrinsic dynamics of antibody-antigen complexes exhibiting NMR spectra of very high dynamic range. We aimed to extend and optimize Non-Uniform (Sparse) Sampling (NUS) NMR techniques for the recovery and resonance assignment of low-intensity (epitope) signals amidst the over-whelming intense signals from the non-binding residues. Here, we present a computational scheme for the reconstruction of weak epitope signals and conduct a comparison of 3D HNCA/HNcoCA spectra obtained using different NUS schedules tailored for the detection of broad and low-intensity epitope signals.
Insight into human insulin aggregation using NMR derived translational diffusion parameters

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Intense research is carried out during last decades concerning pathological processes in insulins. The intrinsic topology and environmental factors were considered, among others, as the main factors which influence self association of insulins [1]. These affect the stability of insulin preparations which is a major issue of a wide interest as it directly concerns the diabetics.

Here we present dependence of insulin self association on pH in full accessible range, 1.5-12.0 (Fig. 1). It is worth to highlight the result close to biological pH which for Zn\textsuperscript{2+} ions containing insulin (green triangle) shows Dav value of $0.87 \times 10^{-10} \text{m}^2 \text{s}^{-1}$ rather indicating mixture of lower aggregates but not a hexamer alone. Furthermore, the data also indicate that presence of Zn\textsuperscript{2+} ions in pH range 7.8-12.0 has negligible influence on the insulin association. In contrast to the above, pharmaceutical composition (insulin + Zn + Glycerol + Cresol) results plateau at pH 7.0 - 9.0 with Di value characteristic for single hexameric template. On the other hand, the data acquired at pH 12.0 suggest the monomer structure (Dav, $1.35 \times 10^{-10} \text{m}^2 \text{s}^{-1}$).

The 3D $^{13}$C/$^{15}$N-HSQC-iDOSY experiment [2] performed on fully labeled insulin in pharmaceutical formulation at pH 7.5 allowed to identify single oligomeric component with diffusion coefficient (ca. $0.7 \times 10^{-10} \text{m}^2 \text{s}^{-1}$) corresponding to hexamer. For zinc free insulin at pH 4.4 it was possible to observe two components (panel on the right), which were also confirmed independently by DECRA analysis of PFGSE NMR spectra.

The presented results show the means of precise insight into insulin aggregation in two ranges of pH, i.e at ca. 4 and 7.5, interesting from pharmaceutical point of view, because insulins are secreted to diabetics in these conditions. The results can also aid in understanding various experimental results performed in pursue to assign the insulin misfolding and other conformational processes.


References


Towards the chemical synthesis of native stable isotope modified tRNAs for NMR spectroscopy

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The long-term goal of the presented research project is the chemical synthesis of long stable isotope (SI)-labeled RNA sequences for NMR spectroscopic applications. The main drawback using the ‘classical’ TBDMS or TOM chemical approach to synthesize oligonucleotides is the limitation with respect to size. Currently, ribonucleic acids with approximately 50 nucleotides (nt) can be produced in sufficient amounts and the required quality for NMR spectroscopic investigations. Here, we showcase the high potential of the 2’-cyanoethoxymethyl (CEM) methodology to synthesize RNAs with tailor made stable isotope labeling patterns comprising up to 80 nucleotides. This method offers several advantages, such as higher coupling efficiency and efficient deprotection procedures.

The compatibility of the CEM RNA synthesis method for the incorporation of stable isotope labeled naturally occurring modified RNA building blocks, such as the $^{15}$N$_2$-modified dihydrouridine residue, into a full-length transfer RNA (tRNA) was proven. Furthermore, the method can be combined with standard SI-labeled TBDMS RNA building blocks to give the desired target sequence.

The presented approach gives for the first time the possibility to modify larger RNAs (> 50nt) with atom-specifically $^{13}$C/$^{15}$N-labeled building blocks. The method harbors the very high and unique potential to address structural and also dynamic features of long RNAs with NMR spectroscopy but also using other biophysical methods, such as mass spectrometry (MS) or small angle neutron/x-ray scattering (SANS, SAXS).
THE NMR AND DNP CENTRE AT THE UNIVERSITY OF GUELPH

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The University of Guelph NMR Centre supports 20 local research groups, as well as external academic and industrial researchers, in the disciplines of biophysics, chemistry, food and environmental science, and biology. The NMR Centre comprises six spectrometers spanning 400 MHz to 800 MHz, including a 600 MHz Dynamic Nuclear Polarization spectrometer, to study the structure and function of food, chemicals, proteins, carbohydrates, and biological membranes. The NMR Centre also provides access to a wide range of probes, including 800 MHz bio-MAS probes from 1.3 mm to 4 mm rotor diameter, 5mm solution cryoprobes for enhanced sensitivity, and an F/C/H probe for 19F NMR studies with optional 1H decoupling. An overview of the facility, along with recent experiments of interest to the NMR community in the fields of chemistry, protein biochemistry, and metabolomics, will be presented.

Fig. 1. The 800 MHz and 600 MHz spectrometers.
NMR Experiments for Several Receivers

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NMR experiments involving multiple receivers provide a unique way of increasing the sensitivity and information content of data recorded in a given period of time [1-3]. We present a comprehensive series of such experiments designed for simultaneous detection of abundant nuclei, such as ¹H, ¹⁹F and ³¹P, as well as samples enriched with magnetically active isotopes including ¹³C and ¹⁵N. The multiple receiver experiments are categorized into three main types – (a) parallel acquisition, (b) sequential acquisition and (c) interleaved experiments. The optimum implementation is shown to depend on the relaxation properties of the involved nuclei as well as the intrinsic sensitivity of the directly observed nuclei. We particularly focus on the basic NMR experiments involving the pairs of ¹H / ¹⁹F nuclei and ¹H / ³¹P nuclei not least because of the particularly important role that ¹⁹F and ³¹P NMR plays in drug discovery and pharmaceutical industry [3]. Essentially any of the basic 2D NMR experiments, such as COSY, NOESY, TOCSY, DOSY, HSQC, HMQC, HMBC, HETCOR or relaxation measurements that are routinely used in small molecule NMR can be easily adapted for and more efficiently recorded on systems equipped with multiple receivers.

![Fig. 1. Two-dimensional H-H and H-F COSY experiments recorded in parallel with ¹H (receiver 1) and ¹⁹F (receiver 2) direct detection on a 700 MHz AVIII HD NMR system equipped with a QCIF CryoProbe.](image)

Many of these experiments are amenable to further reduction of experiment time by combining them with other fast NMR techniques, such as Hadamard NMR, non-uniform sampling, spatial encoding or rapid pulsing methods. We believe that the multi-receiver technology will boost the development of new NMR experiments as well as NMR research in general, making the NMR instruments more efficient and making the NMR spectroscopy even more unique in the universe of analytical tools and experimental techniques.

References.
Examining Structural Features of Self Assembling Hydrophobin Proteins  
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Hydrophobins are low molecular weight (5-20 kDa) self-assembling proteins secreted by fungi that accumulate at hydrophobic-hydrophilic interfaces and are extremely surface-active. Hydrophobins may undergo structural rearrangement and oligomerize to form rodlets, which are an insoluble functional amyloid that coats fungal spores to act as a water repellent, facilitate dispersal into the air, and prevent immune recognition. Due to their biochemical properties hydrophobins are a target for commercial applications, where they could be incorporated as biodegradable foam stabilizers or emulsifiers. To better understand what sequence characteristics determine hydrophobin properties, we are characterizing the structure and properties of a class IB hydrophobin from *Serpula lacrymans* (SlaHyd1). SlaHyd1 has only one charged amino acid, meaning it may have unusual properties compared to other hydrophobins. We expressed uniformly $^{13}$C/$^{15}$N-labeled SlaHyd1 in *E. coli* and then purified it to homogeneity using Ni$^{2+}$ affinity and ion exchange chromatography. We then determined the high-resolution structure of SlaHyd1 using NMR spectroscopy. SlaHyd1 contains a four strand anti-parallel $\beta$-sheet that is connected by three loop sequences (L1-L3). The $\beta$-sheet folds upon itself to form a $\beta$-barrel-like structure, L1 contains an $\alpha$-helix, L2 is a dynamic loop, and L3 is a three residue $\beta$-hairpin. Overall, the structure of SlaHyd1 is consistent with SC16, which is another class IB hydrophobin. This data indicates that class IB hydrophobins have a consistent three-dimensional structure despite having a variety of sequence properties and will form the basis of future mutagenesis experiments and examination of rodlet properties to further characterize these proteins.
Better understanding of the mechanism of enzymatic activity of lecithin retinol acyltransferase gained by solution NMR structural studies

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Introduction: The visual pigment of rods, rhodopsin, is composed of a protein and a chromophore, 11-cis retinal. When rhodopsin absorbs light, its chromophore undergoes isomerization, producing all-trans retinal. This retinoid undergoes many enzymatic modifications through a process called the visual cycle, which is responsible for regenerating 11-cis retinal. Lecithin retinol acyltransferase (LRAT) is an important enzyme of the visual cycle, which is responsible for transforming all-trans retinol into all-trans retinyl ester. In fact, LRAT participates in two different enzymatic reactions. Firstly, it hydrolyzes a phospholipid, acylating its C161; secondly, it transfers this acyl group to all-trans retinol, producing an all-trans retinyl ester and deacylating its C161. Mutations found in the LRAT sequence are responsible for eye diseases, such as Retinitis pigmentosa and Leber congenital amaurosis, which cause the degeneration of photoreceptors and lead to severe vision loss. We have previously determined that these mutations cause an almost complete loss of activity in a truncated LRAT without its hydrophobic N-terminal and C-terminal segments (tLRAT). Obtaining information on the mechanism of the enzymatic activity of LRAT will give insight on how these mutations lead to a significant loss in its enzymatic activity, and ultimately explain how they cause severe eye diseases. The research objective is to better understand the mechanism of enzymatic activity of LRAT by gaining structural information of tLRAT using solution NMR.

Materials and Methods: tLRAT was overexpressed in prokaryotic cells and purified using affinity chromatography. Its enzymatic activity is routinely determined using a procedure we have developed. The optimal temperature at which to perform the solution NMR measurements has been determined by measuring the stability of the enzymatic activity of tLRAT as a function of time. Tertiary structural information was obtained using solution NMR with a $^{15}$N,$^{13}$C-labelled tLRAT sample. Structural information was also obtained with C161S/C168S-tLRAT, an LRAT mutant incapable of undergoing acylation.

Results: Using solution NMR, we have succeeded in assigning all backbone atoms of $^{15}$N,$^{13}$C-tLRAT-C161S/C168S. It unambiguously shows that tLRAT is primarily $\alpha$-helical and that there are no well-defined $\beta$-sheets in this protein. This is consistent with our circular dichroism data, but it indicates a different fold from so-called “LRAT-like” proteins. tLRAT-C161S/C168S has been used to avoid the intrinsic heterogeneous acylation of the protein. In addition, the comparison between the HSQC spectra obtained with C161S-C168S-tLRAT and the mutants of tLRAT are showing that these mutations have no significant effect on the global structure of tLRAT. We are currently analyzing NMR spectra obtained with native tLRAT.

Conclusion: The tertiary structural information of tLRAT obtained by solution NMR analysis will lead to a better understanding of the mechanism of the enzymatic activity of LRAT and will give insight into the effect of the mutations on its activity.
Naturally occurring peptides have therapeutic potential due to their properties of being highly selective and efficient in regulating physiological functions. Relaxin is a naturally occurring hormone involved in matrix remodelling and cardioprotective effects during pregnancy. Based on these actions, relaxin is currently being developed as a therapeutic agent for fibrosis and acute cardiac failure, but issues of poor storage stability and rapid clearance from bloodstream hamper development. Thus, a better understanding of the structure-activity relationships of relaxin is essential to improve its pharmacology properties.

Relaxin binds and activates a unique G-protein coupled receptor (GPCR), termed Relaxin Family Peptide 1 (RXFP1) although it can bind to the related receptor RXFP2. Structurally, RXFP1 consists of a 7-transmembrane helical domain and a large ectodomain consisting of a 10 leucine-rich repeat (LRR) domain and an N-terminal low density lipoprotein type A (LDLa) module. Interestingly, the receptor cannot be activated by relaxin without the LDLa module suggesting the LDLa module might be the “true” ligand and acts as a tethered agonist. Our current limited understanding is that relaxin binds to the ectodomain of RXFP1 with high affinity with multiple binding sites and the receptor is activated by an unknown mechanism related to the LDLa module. Thus, the aim of this project is to investigate the binding mechanism of relaxin to RXFP1.

Due to its complexity, it is very challenging to study the system as a whole. Thus a divide and conquer approach has been used. Previous studies show that relaxin has multiple binding sites as follow: the LRR domain, a region between the LRR domain and LDLa module (referred to as the linker) and the extracellular loops in the 7-transmembrane helical domain. Precisely what regions of relaxin interact with the receptor has only partially been assessed by mutagenesis of receptor and ligand. Since isotopically labelled synthetic relaxin is prohibitively expensive, a recombinant relaxin expression and purification protocol was optimized. 15N-labelled relaxin as well as 15N and 13C double labelled relaxin were successfully produced and used to study relaxin and LDLa-linker interaction. Unexpectedly, the relaxin resonances were severely broadened upon addition of LDLa-linker. Thus, by utilizing methyl- and aromatic-13C CT-HSQC, we are able to identify important residues involved in relaxin and LDLa-linker binding. Mapping the binding of relaxin to LDLa-linker relaxin will contribute greatly to the future design of relaxin as a potential therapeutic agent.

References
Monitoring the formation of oil-water emulsions with a fast spatially resolved NMR spectroscopy method

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The knowledge of the formation mechanism of emulsions has great significance in understanding the stability of emulsions and phase separation progress. In present study, a fast chemical shift imaging (CSI) method has been used to dynamically monitoring the formation of oil-water emulsions and the phase separation process of the emulsion phase from the excessive water or oil phase on the molecular level [1]. Compared to the volume-selective NMR spectroscopy [2], with an echo planar spectroscopic imaging (EPSI) detection module and signals from series of small voxels, high resolution ¹H NMR spectra can be simultaneously recorded by 1D PHASICS both with the chemical shift and spatial information within a few seconds (Fig.1).

Fig.1. 1D PHASICS pulse sequence. Fig.2. Experimental results of sesame oil-water emulsion with 0.35 g/L of poloxamer 188. (A) 2D chemical shift contours obtained by 1D PHASICS, (B) sample picture after 120 min, (C) 1D spectra extracted from 2D map, (D) physical appearances, (E) evolutions of sesame oil concentration profiles vs. time. Concentration profiles at different spatial positions are numerically denoted as 1 to 40 from top to bottom. The spatial resolution is 0.40 mm/profile.

2D contour maps not only contain water signals and oil resonances in bottom and upper halves, respectively, but also it can be found that there are both water and oil signals in middle parts (Fig. 2A). On the basis of integrals from these high-resolution ¹H NMR spectra, profiles obtained explicitly demonstrate the spatial and temporal variations of oil concentrations (Fig. 2E). At 40 min, oil concentration differences between upper and lower profiles almost reach the maximum, which indicate that the preliminary phase separation appears. Based on the subsequent distribution of oil concentrations, those overlapped-profiles at the top and bottom are assigned as the same phase, i.e. oil and water phases, respectively. Besides, there are 8 profiles including 4 overlapped-profiles located in the middle part corresponding to the oil-water emulsion phase. According to the spatial resolution 0.40 mm, the length of it takes 3.2 mm. As a complementary proof, the sample picture also exhibit a similar result. Experimental results show that 1D PHASICS provides a quantifiable and promising alternative to research on dynamic processes or chemical reactions.

References
Zinc availability-dependent unfolding of Loz1 zinc finger
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Under conditions of excess zinc, the \textit{Schizosaccharomyces pombe} protein Loz1 (Loss Of Zinc sensing 1), represses expression of \textit{zrt1, adh1AS} and \textit{adh4}, which encode a zinc uptake transporter, alcohol dehydrogenase-1 anti-sense RNA, and a mitochondrial alcohol dehydrogenase, respectively. Loz1 has been proposed to function through zinc-dependent structural changes that alter DNA binding, and is believed to play a central role in maintaining zinc homeostasis.\textsuperscript{1} A 96-residue construct of Loz1 (426-522), consisting of a pair of C\textsubscript{2}H\textsubscript{2}-type zinc fingers (Fig.a) located at the extreme C-terminus plus an adjacent 40 amino acid “accessory domain” are sufficient for zinc modulated function of Loz1.\textsuperscript{2} The accessory domain does not possess identifiable structural activity is lost in constructs lacking a part of this domain.\textsuperscript{2}

The \textsuperscript{1}H-\textsuperscript{15}N HSQC spectrum of Loz1(426-522) prepared with excess Zn\textsuperscript{2+} in purification and NMR buffers has fewer dispersed signals than expected from the amino acid sequence (Fig.b). Backbone resonance assignments reveal that these dispersed signals arise from the zinc finger region, indicating that the adjacent accessory domain is unstructured under these conditions. Further, broadness and doubling of signals from a subset of the residues suggest conformational exchange in the free protein. Removal of free zinc from NMR buffer by dialysis results in unfolding of the first zinc finger (residues 468-490), presumably by loss of its coordinated zinc ion. This is evidenced by disappearance of signals corresponding to residues from this domain, and increased spectral crowding at the center of the spectrum. On-going experiments aim to elucidate the function of the zinc finger domain as a zinc sensor and it’s role in regulating DNA binding.

References
DETECTION OF CHOLINE CONTAINING COMPOUNDS IN DAIRY PRODUCTS USING $^1$H-$^{14}$N SOFAST-HMQC NMR EXDPERIMENT

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Choline containing compounds (CCCs) play important roles in biology. Glycerolphosphocholine is a major component of eukarytic cell membrane. Choline and phosphocholine are precursor and intermediate of phosphatidylcholine, respectively. Part of the CCCs can be synthesized by human body, but most of them come from daily diet. CCCs intake has been found to be related to growth retardation, renal dysfunction and heart diseases, abnormal CCCs content is considered to be a marker for cancer diagnosis. Therefore, the investigation of CCCs in food and human body may relate choline intake to risk for diseases.

NMR spectroscopy is a non-destructive method suitable for the investigation of CCCs in food. However, its application is impeded by the broad line width and serious overlap in the $^1$H NMR spectroscopy. $^1$H-$^{14}$N HSQC NMR spectroscopy has been proved to be a sensitive method for CCCs selective detection, but it required relative long experiment time. Herein, to further shorten the experiment time, and enhance the sensitivity of CCCs detection, $^1$H-$^{14}$N SOFAST-HMQC has been applied to investigate the CCCs in dairy products. It was found that CCCs can be detected selectively using the experiment, the unit time sensitivity of which is 1.5-2.0 times higher than that of the previous developed selective HSQC method.

![Fig. 1. Relative signal intensities of CCCs in $^1$H-$^{14}$N NMR experiment as a function of prescan delay.](image)

References
13C DETECTED 15N-13C COUPLING MEASUREMENTS AT THE NATURAL ISOTOPIC ABUNDANCE

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We describe a 13C detected experiment, the (H)CNMQC pulse sequence for measuring one-bond and long-range 15N-13C scalar coupling constants in small organic molecules at the natural isotopic abundance[1,2]. The previously proposed proton detected H(C)NMBC experiment [3,4] performs poorly in situations where the carbon atom of interest has no attached protons, in the presence of H-H splittings (homonuclear couplings), unwanted coherence leaks and considerable t1-noise due to instrumental instabilities (see Fig. 1). We introduce a new parameter, SRF (sensitivity reduction factor) for quantifying the sensitivity loss due to the t1-noise and describe a way to measure the SRF experimentally. To a large extent the aforementioned problems are avoided in the new (H)CNMQC experiment that is based on direct 13C detection allowing also one-dimensional 15N-13C coupling measurements. In many cases the new experiment improves the measurement sensitivity and accuracy, not least because the 15N-13C couplings are measured in the directly detected dimension enabling one-dimensional measurements.

Fig. 1. Comparison of 13C detected and 1H detected measurements in one and two dimensional experiments.

We show examples of both one-bond and long-range 15N-13C coupling measurements in small organic molecules with the coupling constants ranging between 1.3 and 27.0 Hz. It is worth noting that the commercially available TXO CryoProbes that are optimized for direct 13C detection and are equipped with cold 13C preamplifier dramatically reduce the measurement time and increase the sensitivity of the measurements.

References
THE ROLE OF TANNIN-LIPID INTERACTIONS IN WINE GUSTATIVE PROPERTIES: A BIOPHYSICAL STUDY

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Tannins are polyphenol polymers present in significant amounts in red wine responsible for astringency and bitterness. The former is a tactile perception involving dryness and roughness in the mouth due to the interaction between tannins and saliva proteins [1] and the latter is a primary taste due to the interaction between tannins and taste receptors in taste buds [2]. Tannins are now known to also interact with lipids [3]. Although not present in wine, lipids are yet present during tasting in the oral membranes of tasters and in fatty foods when wine is consumed during a meal. However, although the influence of lipids is well known to wine tasters through food pairing, there is no scientific evidence to support this hedonic feeling.

The aim of the topic is to study tannin-lipid interactions at molecular level in order to better understand their implication in wine gustative properties. The present work describes the effect of the main representative grape tannin subunits, the catechin monomer and the B₁ dimer, both on a model of oral membranes and food fat globules. They are represented by a dispersion of POPC/cholesterol multilamellar vesicles and a olive oil in water emulsion stabilized by DMPC as emulsifier, respectively. The organization and dynamics of the lipids composing these two models were investigated by solid-state NMR spectroscopy (¹H, ²H, and ¹³C) in the absence and the presence of the two tannin subunits. The affinity of tannins for lipids was also explored by the determination of the thermodynamic association constant.

The results pointed out a fluidizing effect of tannins both on the membrane model, as previously shown on a simpler membrane model [3, 4], and on the emulsion lipid droplets. The disorder caused by tannins was shown to be related to their location in the lipid structure depending on the tannin chemical nature. Moreover, the strength of the interaction between tannins and membrane lipids was revealed to be in the same order of magnitude of that between tannins and saliva proteins using plasmon waveguide resonance (PWR). In addition, the biophysical results were in accordance with those of a sensory analysis led in parallel that revealed that fatty foods are prone to decrease wine astringency [5].

These pioneering works in the field of oenology highlight for the first time the potential role of the tannin-lipid interactions on wine taste. On the one hand, by disrupting the lipid environment of taste receptors embedded in oral membranes, tannin-lipid interactions could affect the receptor functionnality and therefore the interaction with tannin molecules, so bitterness. On the other hand, the existence of a possible competition between lipids and saliva proteins for interacting with tannins during tasting could reduce astringency.

References

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Oleogels\textsuperscript{1} are multi-phase solid networks composed of a liquid oil contained in a porous solid matrix. The commercial application of new oleogels is often complicated by the poor stability of the gel, leading to oil separation and short shelf-life. From percolation theory\textsuperscript{2} we know that the oil leakage rate should be related to the size and nature of the pores, (open or closed cell), as well as the tortuosities of the network in the case of open cell pores. Unfortunately this information is difficult to obtain due to the opaque nature of most oleogel systems. Two common examples of oleogels, Vaseline and Crisco shortening, illustrate the challenges faced in the measurement of oleogel pore-size. Both are opaque and fragile, limiting the application of optical methods due to a lack of contrast between the solid and liquid phases.

High resolution Pulsed Field Gradient NMR diffusion (PFG-NMR)\textsuperscript{2} is well suited to pore-size studies of oleogels, the solid domain is essentially invisible to high-resolution PFG-NMR diffusion methods, and the pores tend to be in a size range easily measured by NMR diffusion methods\textsuperscript{3,4}.

![Fig. 1. Apparent diffusion vs. mean diffusion distance, measured as $(\Delta D_{\text{self}})^{1/2}$, of the oil phase in Vaseline at 25°C. The blue data points have been fit using a simple model which will be discussed in the presentation. The model is extrapolated to the origin to obtain the pore radius using the Mitra equation as indicated by the red line. The inverse tortuosity is one of the fitting parameters of the simple model.](image)

The present work describes the application of PFG-NMR diffusion methods for the study of opaque oleogels. This method has been used to characterize the porosity of oleogel networks in the size range of ~1 µm to 75 µm, a size regime in which the mean diffusion distance of the oil is comparable to the radius of the pores in the oleogel.

References


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wxProdOp: A Product Operator Calculator

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When analyzing nmr pulse sequences via density matrices and their corresponding transformation matrices, the calculations become very cumbersome. This is especially so for multi-spin systems with the matrices generally being quite large. For a spin-1/2 system, the matrix size is $2^n \times 2^n$ where $n$ is the number of spins.

The product operator formalism, as used in nmr spectroscopy, was introduced to reduce the visual and computational complexity of the matrix analysis method[1]. Used in conjunction with a visual method of doing rotational transformations, it is an extremely powerful tool for the nmr spectroscopist[2]. However, even the product operator method can result in a large number of terms such that one sometimes cannot “see the tree for the forest”.

wxProdop was developed to do product operator calculations with a view towards simplification of the results as much as possible. The program applies trigonometric identities and other methods to achieve this goal and handles spin-1/2 systems from one to four spins.

Unlike all other product operator calculation systems, wxProdOp does not rely on a proprietary, underlying symbolic algebra program. The program is fully standalone and is freely available under the GPL license for MS Windows and Linux.

![wxProdOp preferences dialog.](image)

References

Witnessing nonclassical correlations via a single-shot experiment on an ensemble of spins using nuclear magnetic resonance

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A bipartite quantum system in a mixed state can exhibit nonclassical correlations, which can go beyond quantum entanglement. While quantum discord is the standard measure of quantifying such general quantum correlations, the nonclassicality can be determined by simpler means via the measurement of witness operators. We experimentally construct a positive map [1] to witness nonclassicality of two qubits in an NMR system. The map can be decomposed in terms of measurable spin magnetizations so that a single run of an experiment on an ensemble of spins suffices to detect the nonclassicality in the state, if present. We let the state evolve in time and use the map to detect nonclassicality as a function of time. To evaluate the efficacy of the witness operator as a means to detect nonclassicality, we measure quantum discord by performing full quantum state tomography at each time instant and obtain a fairly good match between the two methods.

References

Three-dimensional localization spectroscopy of individual nuclear spins with a diamond quantum sensor

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One of the visionary goals of nanoscale quantum sensing with nitrogen-vacancy (NV) centers in diamond is the structural imaging of individual molecules attached to the surface of the diamond chip. The long-term perspective is to reconstruct the species and three-dimensional location of the constituting nuclear spins with sub-nanometer resolution by transferring, adapting and extending measurement techniques known from nuclear and electron spin resonance [1].

Here, we report on the three-dimensional localization spectroscopy of individual $^{13}$C nuclear spins in the vicinity of the NV center based quantum sensor (see Fig. 1). Due to the azimuthal symmetry of the hyperfine interaction between the electron spin of the NV center and the nuclear spins conventional spectroscopy experiments only allow for the determination of two spatial coordinates. We combine high-resolution correlation spectroscopy [2] with a rapidly switchable external magnetic field source, which allows us to dynamically break the azimuthal symmetry of the hyperfine interactions, thus making the third spatial coordinate accessible.

Fig. 1. Three-dimensional location of two $^{13}$C nuclear spins ($I_1$, $I_2$, blue) detected and localized using a single NV center as the quantum sensor (red).

References

Intact Single Seed Fatty Acid Profiling by $^1$H HRMAS

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The fatty acid profiles of single intact flax seeds have been determined using $^1$H HRMAS NMR. A germination test was performed after spinning the seeds which showed that the viability of the seeds was the same as untested seeds. An average of the individual seed results was compared to a rotor with 8 random seeds and there was no significant difference in the fatty acid profile determination. These results will be compared to the usual solution $^1$H method following oil extraction from seeds.
Optical dependence of electrically-detected magnetic resonance in lightly-doped Si:P devices

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Electrically-detected magnetic resonance (EDMR) provides a highly sensitive method for reading out the state of donor spins in silicon [1]. The technique relies on a spin-dependent recombination (SDR) process involving dopant spins that are coupled to interfacial defect spins near the Si/SiO₂ interface.

At cryogenic temperatures and low-doping concentrations, optical excitation is used to generate the free carriers necessary for EDMR. The influence of this optical excitation on the SDR rates and the observed EDMR signal is still not well understood.

Here, we investigate the wavelength dependence of the EDMR signal in a Si:P device, using three different optical sources: a 980 nm laser whose energy is just above the band edge of silicon at cryogenic temperatures, a 405 nm laser to generate hot surface-carriers, and a broadband tungsten-halogen lamp white light source. With near-infrared excitation, we find that the EDMR signal primarily arises from donor-defect pairs, while at higher photon energies there are significant additional contributions from defect-defect pairs. Using frequency modulated (FM) continuous-wave (CW) EDMR we measure the modulation frequency and microwave power dependence of the EDMR signal for each optical excitation and show that the optical excitation energy can strongly modulate the kinetics of the SDR process. Careful tuning of the optical photon energy could therefore be used to control both the subset of spin pairs contributing to the EDMR signal as well as the dynamics of the SDR process.

Fig. 1. FM EDMR spectrum measured under optical excitation at a) 405 nm, b) white light and c) 980 nm. In each panel, the colored line shows the recorded spectrum while the black line shows a spectral fit. The 117.4 MHz hyperfine split phosphorus peaks were used to calibrate the field, with the g-factor of the phosphorus peak set to g=1.9985. The center defect peak was fit to the sum of two Lorentzian lines, one with a g-factor of 2.0058 (assigned to Pb0) and the other with a g-factor of 2.0002 (assigned to E'). The peak with g-factor 2.0058 has a mix of dispersive and absorptive lineshapes.

Here, we investigate the wavelength dependence of the EDMR signal in a Si:P device, using three different optical sources: a 980 nm laser whose energy is just above the band edge of silicon at cryogenic temperatures, a 405 nm laser to generate hot surface-carriers, and a broadband tungsten-halogen lamp white light source. With near-infrared excitation, we find that the EDMR signal primarily arises from donor-defect pairs, while at higher photon energies there are significant additional contributions from defect-defect pairs. Using frequency modulated (FM) continuous-wave (CW) EDMR we measure the modulation frequency and microwave power dependence of the EDMR signal for each optical excitation and show that the optical excitation energy can strongly modulate the kinetics of the SDR process. Careful tuning of the optical photon energy could therefore be used to control both the subset of spin pairs contributing to the EDMR signal as well as the dynamics of the SDR process.

References
POSTER SESSION 2

Tuesday, July 25
16:30-18:30

Janet E. Lovett
Benjamin Nforneh
Adelheid Godt
Tatyana I. Smirnova
Peter Martin
Arina Dalaloyan
Aidin R. Balo
Mizue Asada
Mohammad Hadi Timachi
Timothy Keller
A. V. Lalithaphani
Toshikazu Nakamura
Kouichi Nakagawa
Lihuang Zhu
Anh Phuc Duc Nguyen
Kurt Warncke
Marek Oja
Kouichi Nakagawa
Boris Dzikovski
Boris Rakvin
Madhur Srivastava
Boris Epel
Ryan P. Barnes
William Lindemann
Yuya Ishikawa
Yilin Shi
Pragy R. Shrestha
Thomas H. Edwards
Pierrick Berruyer
Meghan E. Halse
Aurélien Bornet
Silvia Cavagnero
Anne M. Carroll
Magnus Karlsson
Ashok Ajoy
Juan Lopez
Morteza Esmaeili
Morteza Esmaeili
Gigi Galiana
Riqiang Fu
Akira Naito
Andrea Bertarello
Yongae Kim
Peter Gor’kov
Myriam L. Cotten
Pascal Fricke
Daryl Good
Lauren Klein
Ghada Khouqer
Arisu Shigeta
Marcus Tuttle
Elliott E. Burnell
Miwa Murakami
Igor Moudrakovski
Kazuma Gotoh
Bing Wu
Chris J. Franko
Ryohei Morita
Valérie Montouillout
Abhoy Karmakar
Pu Duan
Robin S. Stein
Janelle G. Vander Hout
Cesar Leroy
Carlos Pacheco
Pierre-alexandre Martin
Hai-Young Kim
Markus Orbkircher
Liping Yu
Rodolfo Rasia
Sunjay Sunjay
Andrea Bodor
Ling Jiang
Raphael Plangger
Takuro Wakamoto
Kanakaraju Marumudi
Gyula Batta
Daiwen Yang
Bastian Kohl
Heike Hofstetter
Lech Kozerski
Luciana Coutinho de Oliveira
Ping Wang
Shubhadra Pillay
Michael Juen
Calem Kenward
Takuma Kasai
Arndt Wallmann
Sonja A. Dames
Julia M. Würz
Qinyan Song
Yong-hui Zhang
Brendon W. Lovett
Aharon Blank
Marie-Rose Van Calsteren
Mark S. Conradi
Michael C. Boucher
Chandrasekhar Ramanathan
2’-AlkynylNucleotides: A Sequence- and Spin Label-Flexible Strategy for EPR Spectroscopy in DNA

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EPR’s array of dipolar spectroscopy methods provide distance information in the nanometre range and this is often complementary to other structural techniques. EPR could be ideally suited for exploring and mapping the tertiary folds of nucleic acids such as RNA, particularly as the sensitivity of the spectrometers and methods increases. To improve the current scope of labelling we synthesized 2’-alkynylnucleosides of C, U and A to demonstrate that these bases can be interchangeably utilized for sequence flexible labelling using solid-phase methods. We then incorporated these into a family of 16-mer DNA duplexes with a modification on each strand either internally or at the 3’ terminus (Fig. 1) [1].

![Fig. 1. A representation of the 16-mer duplex with the different distance measurements shown by red lines.](image)

Six nitroxide-containing azido spin labels were synthesized. These included spirocyclohexyl and tetraalkylisoindoline frameworks [2]. The labels were attached to the oligonucleotides using a copper-catalyzed click cycloaddition reaction.

CW EPR and DEER experiments were performed, including a W-band orientation-selective DEER using spirocyclohexyl labels at liquid nitrogen temperatures. We will also present results from full atom molecular modelling for two of the labelled oligonucleotides. We show that through this systematic approach we can site-selectively label nucleic acids and that the spin label design allows for different environments to be measured.

References


The effect of dimethyl sulfoxide (DMSO) on the dynamical properties of the solvent around the B$_{12}$-dependent ethanolamine ammonia-lyase (EAL) from *Salmonella typhimurium* was studied over the temperature ($T$) range of 190-265 K in frozen aqueous solutions. Spin probe electron paramagnetic resonance (EPR) was used with 4-hydroxy-TEMPO (TEMPOL) and the added DMSO concentration was 1% v/v. The aim is to identify and characterize the behavior of the protein hydration and bulk components of the solvent around EAL. The rotational dynamics of the TEMPOL as a function of $T$ were revealed by the EPR line shape and quantified by the rotational correlation time ($\tau_c$) obtained from EPR simulations. Single, rigid component spectra are obtained at $T<200$ K. At $210<T\leq245$ K, two mobile spin fractions both with $\tau_{c,f}, \tau_{c,s} \leq 10^{-7.0}$ s, are obtained with normalized weights, $W_f=60\pm1$ %, $W_s=40\pm1$ %. At $T>245$ K, $W_f$ increases relative to $W_s$ to a value of 69 % at 265 K. Pure 1% v/v DMSO-water solution EPR spectra, with no EAL, over the same $T$ range were collected and simulated by using a single component simulation. The $\tau_c$ values for these samples were the same as the $\tau_{c,f}$ values at the same $T$. In order to further address the origin of the components corresponding to $W_f$ and $W_s$, the protein concentration dependence (12−120 μM EAL) of the components was determined. The $W_s$ fraction shrinks to 8 % at 12 μM EAL. The results indicate that TEMPOL occupies two phases in the EAL samples, and that the $W_s$ and $W_f$ components correspond to protein hydration layer$^1$ and the “bulk” solvent mesodomain.$^2$ The DMSO addition and $T$ systematically controls solvent dynamics around the protein, and provides an approach for characterizing the influence of solvent-protein dynamical coupling on catalysis in EAL.

Supported by NIH R01DK054514.

References


Ligands for Gd\textsuperscript{III}- and Mn\textsuperscript{II}-based spin labels

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With in-depth studies on complexes of Gd\textsuperscript{III} and Mn\textsuperscript{II} as spin labels for distance determination between two sites of a (bio)macromolecule through DEER [1], RIDME [2], and line broadening analysis [3], a data basis was acquired for selecting a concrete complex based on its spectroscopic properties. A most recent comparative study on complexes of Gd\textsuperscript{III} even provided detailed insight into the structure-property relationship. An additional important selection criterion, when it comes to application, is the attachment of the spin label to the molecule of interest in respect to geometry, reaction conditions, and selectivity, and, very simply, the accessibility of the chelating ligand with a functional group enabling bioconjugation. In the poster we will present our strategy to evaluate spin labels with the help of model compounds [4] and our work on making complexes differing strongly in their spectroscopic properties (Figure 1) ready for bioconjugation.

![Structural formulae of complexes with PyDTTA, PyMTA, and NO3Pic as ligands. R stands for the functional group that enables bioconjugation.](image)

**Fig. 1.** Structural formulae of complexes with PyDTTA, PyMTA, and NO3Pic as ligands. R stands for the functional group that enables bioconjugation.

**References**


EFFECT OF SILICA SUPPORT ON MEMBRANE SURFACE POTENTIAL, DYNAMICS OF TRANSMEMBRANE PEPTIDE, AND EFFECTIVE $pK_a$ OF IONISABLE SIDECHAINS

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Interfacing biological and artificial systems at the nano-scale level is essential for developing novel hybrid living-nonliving biotechnology platforms for applications in biology and medicine as well as in design of biosensors. Although an impressive progress has been already achieved in creating new bio-nano hybrid systems, there is a high need to understand the influence of nanostructured support and nanoscale confinement on structure and properties of the lipid bilayers and membrane-protein interface.

In this work we report on spin-labeling EPR studies to 1) evaluate the effect of anionic lipid surface charge density on effective $pK_a$ of membrane-burried ionisable sidechains and 2) assess effects of a solid inorganic interface and, particularly, silica support, on the phospholipid membrane surface potential and effective $pK_a$ of the membrane-burried ionisable sidechains of transmembrane protein domains. The ionizable sidechains were modelled by a site-directed incorporation of pH-sensitive ionisable nitroxides into the transmembrane $\alpha$-helical WALP peptide. The change in the protonation state of an ionisable nitroxide was directly observed by CW EPR allowing for determination of an effective $pK_a$ of the probe. We have shown that the effective $pK_a$ of such a nitroxide positioned along the interface of the transmembrane $\alpha$-helix and the phospholipid bilayer increases by more than 2 pH units in depth-dependent manner upon replacing zwitterionic PC with anionic PG lipids. Almost 80% of that $pK_a$ shift was observed upon replacing only half of the PC with PG lipids.

We have also investigated effects of nanostructured support on local peptide dynamics and the effective $pK_a$ of the probe by forming substrate-supported lipid bilayers containing an integrated transmembrane $\alpha$-helical WALP peptide on the surface of silica nanoparticles of different diemters. Novel EPR-active pH-sensitive lipids IMTSL-PE and IKMTSL-PE [1-3] were then employed to assess the surface electrostatic potential of lipid bilayers encapsulating silica nanoparticles. We have shown that placing bilayers composed of POPC or mixed POPC/POPG lipids on silica nanoparticles increases the negative electric potential at the membrane surface with the potential of the mixed bilayer being more sensitive to the silica support. Supported by NSF 1508607 to TIS.

References
SIMULATING ELECTRON PARAMAGNETIC RESONANCE SPECTRA OF SLOW-MOTION SYSTEMS IN THE TIME DOMAIN

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We have used computational molecular and stochastic dynamics trajectories to simulate electron paramagnetic resonance (EPR) spectra of spin-labeled biomolecules. EPR spectroscopy is a powerful tool for probing local structure and dynamics in biological systems, but the corresponding spectra can be complex and difficult to interpret. To accurately model experimental data, user-friendly computational tools have been developed to simulate spectra, especially for continuous-wave (CW) EPR in the slow-motion regime (dynamical time scales of $\approx 10$-$100$ ns for nitroxides at 9-10 GHz). The standard method is to simulate spectra in the frequency domain, e.g. by numerically solving the stochastic Liouville equation\cite{1,2}. Such programs are very fast, but are often restricted to specific models, e.g. only including one diffusion frame, restricted to electron spin-$\frac{1}{2}$, etc. When more complex models are needed, trajectory-based time domain simulation methods provide a promising alternative, but there are varying techniques in the literature\cite{3-8}. Here we demonstrate our implementation of time domain methods in EasySpin\cite{9}, which allows us to simulate spectra using these different methods for direct comparison. As a starting point, the program can use trajectories that are calculated either internally using stochastic dynamics with an arbitrary orienting potential or externally by molecular dynamics. The latter feature will allow for detailed studies of how both spin label and protein dynamics contribute to EPR spectra, and the program will be made publicly available.

References
In-vitro and in-cell EPR distance measurements for studying structural dynamics of Calmodulin

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The use of Gd³⁺ labeling for distance measurements by pulsed double electron-electron resonance (DEER) at Q- and W-band frequencies (about 34 and 95 Ghz) offers several advantages for DEER distance measurements, including high sensitivity and good chemical stability of the label towards reducing agents [1]. To develop the technique towards in-cell distance measurements, we applied it to the calcium-binding messenger protein Calmodulin (CaM, 16.7 kDa). CaM has two calcium-binding domains and can bind up to 4 Ca²⁺ ions. Upon Ca²⁺ binding the protein undergoes a conformational change from a relatively closed form [2] to a more extended (open) conformation [3]. This enables it to bind target proteins and as a result CaM assumes a collapsed conformation [4].

Site-directed spin labelling of CaM was performed using the Gd³⁺-DOTA-maleimide tag, which is reactive towards cysteines and forms a stable in-cell C-S bond with the protein. Two mutants with a pair of site-directed cysteine mutations were introduced into the lobes at the N- and C- termini of CaM and labelled to follow the conformational rearrangements of CaM via changes in the Gd³⁺-Gd³⁺ distance. In vitro DEER measurements were performed on CaM in its Ca³⁺-free (apo), Ca³⁺-bound (holo) and Ca²⁺, peptide-bound states. Changes in the Gd³⁺-Gd³⁺ distance distribution could be observed between the three different conformations of the protein, closed, open and collapsed, respectively. This approach was extended to incubation with cell extracts, where interaction of CaM with proteins from the cytoplasm of HeLa human cancer cells led to the observation of the collapsed structure in the presence of Ca²⁺ and of the closed conformation in the absence of Ca²⁺. This behaviour, as well as the Gd³⁺-Gd³⁺ distance probability distributions, were similar to what observed in the in vitro studies.

CaM was successfully delivered into HeLa cells using the electroporation technique. Internalization of the apo- and holo- forms of CaM generated a distance distribution similar to the one observed for in vitro apo and holo CaM. Measurements were also carried out in the presence of ionomycin, which as an effective Ca²⁺ ionophore raises the intracellular level of Ca²⁺. The collapsed conformation could not be detected in the cell.

References
Modelling discrete rotamers of the internally anchored V1 spin-labelled side chain

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Pulsed electron paramagnetic resonance experiments can measure individual distances between spin-labelled side chains in proteins in the range of ~1.5–8 nm. However, the flexibility of traditional spin-labelled side chains leads to diffuse and often unpredictable spin density loci, resulting in broad peaks of distance distributions with seemingly arbitrary maxima. Here, we present the most commonly observed rotamers of the internally anchored spin-labelled side chain V1 as determined by crystallization of V1-labeled T4 lysozyme constructs carrying the V1 side chain on α-helical segments.¹,² The resulting model can be applied to other V1-labelled proteins for higher-precision structural model building (Figure 1).

**Fig. 1.** V1-labelled side chains observed by crystallography on a generic α-helical peptide.

The $C_2$ symmetry element in the homodimer fluoroacetate dehalogenase from *R. palustris* simplifies the side chain-modelling process from DEER spectroscopy measurements by reducing the number of rotamer variables from two to one per measurement. Such a protein is therefore a useful model system for establishing and evaluating the approach of modelling the rotamers observed by crystallography on V1-labelled T4 lysozyme. Comparisons between modelling the V1 side chain onto the protein and measured distance distributions to validate the approach will be presented (Figure 2).

**Fig. 2.** Modelling two rotamers at a particular site on homodimeric fluoroacetate dehalogenase.

References

Detection of the structural changes of MurD enzyme by distance measurement between rigidly labelled Gd$^{3+}$ tags

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ATP-driven Mur ligases perform addition of amino acids to UDP-N-acetylmuramoyl-L-alanine (UMA), and are necessary for building bacterial cell walls. MurD is an enzyme for formation of the bond between UMA and D-glutamic acid. The reaction is regulated by a series of dynamic conformational changes of the three domains. Since crystallization of proteins in transition state is difficult, the intermediate state of the catalytic process of MurD has not been detected by X-ray crystallography. Recent NMR analysis with lanthanide probe mapped the chemical shift perturbations by binding of the ligands, and suggested the formation of the structure between open and closed state [1]. However, the detailed mechanism of the regulation has not been clarified. In this report, we applied the distance measurement between lanthanide Gd$^{3+}$ tags induced into different domains of MurD by pulsed electron-electron double resonance (PELDOR). We used CLaNP-5 lanthanide chelating tags which have two arms and rigidly associate with the Cys-induced residues in domain 2 and domain 3 [2]. The obtained distance distributions were assigned to the conformational changes from open to closed structure.

References
DEER and ODNP: complementary tools to study conformational transitions of a heterodimeric transporter

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ABC exporters pump substrates across the membrane by coupling ATP-driven movements of nucleotide binding domains (NBDs) to the transmembrane domains (TMDs), which switch between inward- and outward-facing (IF and OF) orientations. Double Electron-Electron Resonance (DEER) distance measurements on the heterodimeric ABC exporter TM287/288 from the hyperthermophile T. maritima reveal that with nucleotides the transporter exists in an equilibrium between the IF and OF states. ATP binding without hydrolysis is sufficient to partially populate the OF state, and an almost complete conformational shift is observed when nucleotides are trapped in a pre- or post-hydrolytic state. At physiological temperature and without nucleotides, the NBDs disengage asymmetrically while the conformation of the TMDs remains unchanged. The conformational changes associated with the transition from the IF to the OF states are intimately associated with rearrangement in the hydration properties of the protein.

Liquid state Overhauser effect Dynamic Nuclear Polarization (ODNP) is emerging as a strategic tool to measure at room temperature the water accessibility of spin-labeled macromolecules and was already applied to an ABC transporter protein containing spin probes at the sites of interest [1]. Here we address challenges associated to X-band ODNP data acquisition and evaluation for ABC transporters and present some preliminary ODNP results on TM287/288. Comparison with MD simulation on the transporter embedded in lipid bilayers will also be presented. This transporter, for which X-ray structures exist in two IF states [2],[3] and an extensive set of DEER data in several conformational states was obtained in solution [4], is an appropriate candidate to address the complementarity between DEER and ODNP.

References

STUDY OF AGGREGATION OF INTRINSICALLY DISORDERED PROTEINS BY PULSED DIPOLAR EPR SPECTROSCOPY

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While intrinsically disordered proteins (IDPs) lack a regular 3D structure, certain conformers are thermodynamically preferred and can be probed by pulsed dipolar EPR spectroscopy. For example, tau protein is an IDP which has a propensity to aggregate into beta-sheet fibrils. These fibrils are associated with the formation of amyloid plaques and are a characteristic of certain neurodegenerative diseases such as Alzheimer’s disease. Unfortunately, IDPs and, in particular, aggregating IDPs, are a challenging system to study by pulsed EPR distance measurements. Upon aggregation, spin labelled proteins are clustered together and interactions between electron spins lead to a dramatic reduction in phase memory time, $T_M$. Such reduction in relaxation times yields significantly diminished dipolar evolution periods and therefore decreases the range of resolvable distances for pulsed dipolar EPR measurements. In addition to relaxation, for aggregating systems, dilution with unlabeled “analog” protein is necessary to prevent contribution from inter-protein distances in the distance distribution. Due to limits on protein solubility, dilution will limit signal by limiting the concentration of spin-labelled protein. We therefore investigate techniques for improving the reliability and sensitivity of pulsed dipolar EPR spectroscopy for the model aggregating system, tau protein. Previously, it has been shown that the introduction of an arbitrary waveform generated (AWG) pump pulse results in roughly double modulation depth for nitroxide labels and enhanced signal to noise ratio (SNR) for double electron electron resonance (DEER) distance measurements. Other pulsed EPR techniques such as SIFTER and DQC offer the advantage of observing the entire 200 MHz nitroxide linewidth. SIFTER has the distinct advantage of less contribution of instantaneous diffusion to $T_M$. We find that SIFTER offers an improvement in SNR over DEER by roughly a factor of 4, but at the expense of increasing the number of artifacts. Lastly, full deuteration of tau protein is used to extend the $T_M$ and increase the maximum dipolar evolution time for distance measurements.
Electron Paramagnetic Resonance Spectra of CdO-Al₂O₃-Bi₂O₃-B₂O₃ Quaternary Glasses Containing VO²⁺ ions


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Borate glasses containing different concentrations of heavy metal oxide (CdO) with 2mol% of V₂O₅ as the paramagnetic probe were prepared by the conventional melt quenching technique. The prepared glasses were characterized by XRD to confirm the amorphous nature. EPR and Optical absorption studies were carried out at room temperature. EPR spectra of these glass samples were recorded at X-band frequency with 100 kHz field modulation at room temperature. From the EPR spectra the spin Hamiltonian parameters were evaluated. The spin-Hamiltonian parameter values indicated that $g_{||} < g_{\perp} < g_e [=2.0023]$ and $A_{||} > A_{\perp}$. This suggests that VO²⁺ ions are present in octahedral sites with tetragonal compression and belong to $C_{4v}$ symmetry with $d_{xy}$ being the ground state. The measure of tetragonal distortion ($\Delta g_{||}/\Delta g_{\perp}$) varies non-linearly with glass composition indicating change in tetragonal distortion. The covalency rates were estimated. The number of spins participating in the resonance $[N]$ and susceptibility $(\chi)$ values were also evaluated. Using the Optical absorption studies the refractive indices, optical band gap and Urbach energies were calculated.
Pulsed ESR Investigation for Electric Conducting Molecular Material
And Biological System in IMS

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So far, we have investigated several kinds of systems such as a) organic conductors, b) photoconductive materials, c) functional polymers, and d) biological systems using multi-frequency and pulsed ESR measurements [1-3]. In this talk, we introduce a few topics of ESR investigation in Institute for Molecular Science.

COF (Covalent-Organic-Framework) materials are porous crystalline materials, which attract much attention because of their functionalities. COF materials based on the donor(D)-acceptor(A) molecules are unique systems: DA molecules connected by tight covalent bonds within the two-dimensional layers stack to form one-dimensional columns perpendicular to the planes. After photo-excitation to D-A type COFs, an ESR signal originated from the charge-separated state was observed, which could not be observed in isolated molecules. Surprisingly, the lifetime of the photo-excited carriers was extremely long even at R.T. [2]

Recently, it was found that several chemically modified COF systems show thermally equivalent paramagnetic state. Theses molecules forms two-dimensional porous network with covalent bond. Aromatic carbon molecules are stacking along perpendicular to the slide. The frontier orbitals are elongated along this direction. The pristine COF system is closed shell. However, applying iodine doping for this system, the color of the sample drastically changes. On the same time, ESR signals suddenly appeared and became larger as the doping time increases. The electronic state of the Py-sp2 may changes with iodine doping. To understand the low-temperature electronic state, the detailed magnetic investigation was carried out. In the second part, we discuss the itinerant nature of a series of COF materials.

MurD is one of the ATP-driven muramyl ligases that are responsible for peptidoglycan biosynthesis. The crystal structures of MurD indicate that one of the three domains of MurD, domain 3, undergoes drastic conformational change from open to closed state, which controls the process of the reaction. However the detailed mechanism of its regulation has not been clarified. How the binding of the ligands triggers the open-close transition of domain 3 is an open question. To investigate the dynamic structural changes of domain 3 coupled with the enzymatic process, Hokkaido Univ. group exploited paramagnetic lanthanide probe, where paramagnetic lanthanide ion is fixed in a protein frame. ESR is advantageous because we can get long-distance information and high sensitivity. To understand the steric structure and mechanism of function especially for MurD, we investigate the MurD tagged Gd³⁺ ligand by ESR spectroscopy.

References
HEAVY-ION INDUCED SUCROSE RADICALS INVESTIGATED BY 9 GHZ EPR IMAGING

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We investigated stable radical distribution and particle tracks in sucrose irradiated by C-ion irradiation with CW electron paramagnetic resonance (EPR) and 9 GHz EPR imaging. Although EPR detects stable organic radicals, it cannot verify the location of the radicals in a sample. In addition, most stable organic radicals appear around the \( g = 2.002 \) region of the spectrum. The 9 GHz EPR imaging can nondestructively verify the stable radicals in a sample. We investigated the stable radicals of crystalline sucrose radicals produced by C-ion and X-ray irradiation at the same dose. The radicals were measured using electron paramagnetic resonance (EPR, a JEOL RE-3X) [1, 2] and 9 GHz EPR imaging (a Bruker E500 ELESYS system) [3]. Both irradiations of sucrose produce stable free radicals. EPR of sucrose radicals showed a linear increase of the signal intensity as well as accumulation of the dose. EPR imaging suggests that the radical distribution can vary with changes in LET and absorbed dose. The EPR and 2D EPR imaging results suggested that radical species were mostly located inside the sucrose crystal. Fewer radicals were found at surface region of the sucrose crystal. Therefore, we confirmed that the CW EPR and 9 GHz EPR imaging were sufficient for the determination of the spatial distribution of paramagnetic species in C-ion irradiated sucrose.

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References

Optical dependence of electrically-detected magnetic resonance in lightly-doped Si:P devices

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Electrically-detected magnetic resonance (EDMR) provides a highly sensitive method for reading out the state of donor spins in silicon [1]. The technique relies on a spin-dependent recombination (SDR) process involving dopant spins that are coupled to interfacial defect spins near the Si/SiO$_2$ interface.

At cryogenic temperatures and low-doping concentrations, optical excitation is used to generate the free carriers necessary for EDMR. The influence of this optical excitation on the SDR rates and the observed EDMR signal is still not well understood.

![Fig. 1. FM EDMR spectrum measured under optical excitation at a) 405 nm, b) white light and c) 980 nm. In each panel, the colored line shows the recorded spectrum while the black line shows a spectral fit. The 117.4 MHz hyperfine split phosphorus peaks were used to calibrate the field, with the g-factor of the phosphorus peak set to $g=1.9985$. The center defect peak was fit to the sum of two Lorentzian lines, one with a g-factor of 2.0058 (assigned to Pb$_0$) and the other with a g-factor of 2.0002 (assigned to E'). The peak with g-factor 2.0058 has a mix of dispersive and absorptive lineshapes.](image)

Here, we investigate the wavelength dependence of the EDMR signal in a Si:P device, using three different optical sources: a 980 nm laser whose energy is just above the band edge of silicon at cryogenic temperatures, a 405 nm laser to generate hot surface-carriers, and a broadband tungsten-halogen lamp white light source. With near-infrared excitation, we find that the EDMR signal primarily arises from donor-defect pairs, while at higher photon energies there are significant additional contributions from defect-defect pairs. Using frequency modulated (FM) continuous-wave (CW) EDMR we measure the modulation frequency and microwave power dependence of the EDMR signal for each optical excitation and show that the optical excitation energy can strongly modulate the kinetics of the SDR process. Careful tuning of the optical photon energy could therefore be used to control both the subset of spin pairs contributing to the EDMR signal as well as the dynamics of the SDR process.

References

INTERACTIONS OF GOLD NANOPARTICLES WITH FUNCTIONAL BIO-MATERIALS – AN EPR STUDY

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Gold nanoparticles (AuNPs) can be coated with a wide variety of self-assembled monolayers (SAMs). Such SAMs form the nanoparticles’ ligand shell and are the key component of the nanoparticle to interact with external molecules, e.g. biomolecular materials. Recently, we found that AuNPs, when coated with 11-mercaptoundecane sulfonic acid ligands, interact in specific ways with many biological materials, such as cell membranes or proteins [1,2]. In the current study, we aimed at designing a special class of monodispersed AuNPs whose ligand shell would be composed of a mixture of two thiolated ligands, i.e. an EPR active thiolated molecule (TEMPO-derivative) and a sulfonate-terminated thiol. This design allowed us to take advantage of both the environmentally-responsive EPR signal of TEMPO and the aqueous stability of the nanoparticle dispersion due to the presence of the sulfonate groups in the ligand shell. We used cw X-band EPR to explore the interaction of the thus prepared AuNPs with several functional bio-materials, such as proteins, vesicles and vitamins. The mechanisms of these interactions were derived from various parameters of the observed EPR signals, i.e. the ratios of peak amplitudes, g-factor values, hyperfine constants, and peak intensities. The influence of the size of AuNPs in the range of 2 to 6 nm on such bio-nano-interactions was also addressed.

References
COPPER(II) COORDINATION SITE STRUCTURE AND ARRANGEMENT IN SELF-ASSEMBLING Aβ-PEPTIDE RIBBON ARRAYS REVEALED BY EPR and ESEEM SPECTROSCOPIES

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The amyloid-β peptide (Aβ) is a 38-42 amino acid peptide associated with Alzheimer’s disease (AD). Full-length Aβ can form extended β-sheet fibrillar structures, which can coordinate metal ions in uniform, periodic array(s) along the long-axis, as shown for Cu(II) binding in Aβ40 fibrils [1]. Our aim is to use the self-assembling and Cu(II)-binding properties of designed, truncated Aβ peptides to create materials with unique energy storage, electron-transfer and redox catalysis properties.

HHQALVFFA-NH₂ (K16A) is a 9 amino-acid truncation of Aβ that contains its nucleating core (LVFFA), critical to self-assembly, and the HH-dyad, as a potential metal ion binding site [imidazole (Im) in histidine (H) side chains avidly coordinates Cu(II)] when assembled. In the presence of Cu(II) ions, K16A forms extended (µm scale) twisted ribbon ultrasutures. The Cu(II) site arrangement and metal ion-guided peptide assembly properties of the K16A ribbons were addressed by using CW-EPR spectroscopy. Changes in the Cu(II) hyperfine and Im 14N superhyperfine features of the EPR line shape as a function of Cu(II) concentration (0.1–2.0 mM) at uniform peptide concentration (1.0 mM), and EPR simulation analysis, resolve three species of peptide-bound Cu(II): (1) 3-Im coordination at magnetically isolated sites, (2) 3-Im coordination at weakly-coupled ($R_{CuCu}$≥10 Å) sites, (3) A broad-line species that represents a relatively strongly dipolar-coupled Cu pair ($R_{CuCu}$~6 Å). Saturation of binding occurs at 0.6<Cu(II)/peptide<0.8, which informs the Cu/Im-ligand ratio. The EPR results agree with results from UV/visible and solid state NMR spectroscopies, and lead to a model of a four-Cu(II) repeat unit along the ribbon long-axis: three in-line Cu(II), with one Cu(II) extended from the center at the relatively short $R_{CuCu}$. Multiple-quantum modulation in the 14N ESEEM from the Cu(II)-Im interactions is consistent with 3-Im coordination at lower Cu(II)/peptide values, and is being used to determine the local Im-Cu(II) coordination geometry [2], to provide constraints for modeling peptide organization in the vicinity of the metal ion site. The local site structure and global arrangement of Cu(II) arrayed in K16A ribbons provide a foundation for interpretation of Cu(II)-ribbon redox functions and design iteration.

References
EPR Spectroscopy of Mn Doped Garnets for LED Applications

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There is a tremendous demand for a new generation of white LEDs, which are capable of emitting warm white light similar to thermal light sources and are perceived as cozy by humans. Usually, blue or near UV radiation of LEDs based on an (In,Ga)N semiconductor are combined with one or more down-converting phosphors, mostly Ce3+ doped garnets, to additively produce white light. The quality of the white light is classified by the color rendering index and by the correlated color temperature (CCT). The main problem with the applied phosphors is the deficiency of red light, yielding a high CCT.

In recent years, this problem has been continuously addressed by searching for new Mn4+ based phosphors [1, 2], which have the potential to fulfill the requirements set. In principle, these materials can provide narrow-band red emission suitable for next generation warm w-LEDs. The emission wavelength of the Mn4+ ion is strongly influenced by its local geometry and chemical environment. Therefore, electron paramagnetic resonance spectroscopy is an excellent tool to reveal peculiarities in properties of such centers. The aim of this study was to characterize the paramagnetic Mn centers in different oxide materials under investigation for LED applications.

In this work, Mn4+ doped silicate garnet ceramic phosphors CaY2MgM2Si2O12 (M = Al, Ga, Sc) were synthesized using a high temperature solid state reaction. Additionally, Y2Mg2.97Li0.03Ge3O12 doped with 0.5% Mn4+ synthesized by sol-gel method was studied. The EPR experiments were carried out at a Bruker CW X-band spectrometer in the temperature region of 4 – 300 K. The results were interpreted using the Xepr software provided by Bruker and the EasySpin analysis package [3]. It is known that the Mn4+ ions substitute octahedrally coordinated cations in most crystalline materials. The influence of variation of cation (Al, Ga, Sc) substitution in the studied host materials on local center geometry will be discussed based on changes of the g-factor, hyperfine splitting, and the zero field splitting of the manganese center in silicate garnet ceramic phosphors. A complicated EPR spectrum pointing to the presence of multiple different manganese centers was observed for Y2Mg2.97Li0.03Ge3O12. Structure details and the values of the parameters obtained for these manganese centers will be discussed. Finally, the correlation of the structural properties of Mn4+ centers and their EPR parameters with determined optical properties will be discussed.

References
SKIN SURFACE IMAGE OF PSORIASIS VULGARIS INVESTIGATED BY X-BAND EPR IMAGING

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We investigated locations and identification of structural abnormality of the stratum corneum (SC) in patients with psoriasis vulgaris (PV) by using X-band EPR imaging. The X-band EPR spin-probe imaging of PV-SC samples provided a useful image concerning the status of the SC.

The Hirosaki University Internal Review Board approved all protocols used in this study. For EPR imaging, a Bruker E500 ELESYS system was used. Spin probe (5-doxylstearic acid, 5-DSA) solution was used to investigate structural aspects of PV-SC [1, 2]. We measured the thick SC, the outermost layer for PV. The control SC was taken from skin lesions on the forearms.

A small, broad three-line pattern of 5-DSA in PV-SC was observed. The spectral pattern of PV-SC was quite different from those of the control SC. Two-dimensional (2D) imaging using X-band EPR imager showed that radical locations vary between control and PV skin [3]. The results showed that the intense red signal was due to probe penetration into the PV skin. No red lesion region was observed in the control. The EPR images showed various sizes and number distribution concerning the disordered states in the SC. Thus, X-band EPR imaging can be useful for detecting and identifying the location of abnormality of the SC states. In addition, EPR imaging can potentially offer further quantitative insights into skin-lipid states.

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References

Nitroxides in phospholipid membranes: Where are they located, how fast do they exchange, and what do they actually report?

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We review our recent studies on the location and dynamic behavior of some nitroxide probes, e.g., TEMPO, acyl-chain, and headgroup spin labeled lipids, in different phases of model phospholipid membranes. We used various ESR techniques including high-field cw ESR, high field 2D-ELDOR, and cw saturation ESR.

Similar to the exclusion of solutes from crystallizing solvents, the gel phase excludes nitroxides. This exclusion manifests itself for TEMPO in DMPC in a sharp decrease in the partition into the lipid phase both upon the main phase transition (Lα-Pβ) and pre-transition(Pβ-Lβ) by lowering the temperature. For PC spin labels this exclusion forces acyl chains to take predominantly bent conformations with the nitroxide moiety located near membrane interface. Upon further cooling into low-temperature gel and subgel phases, PC spin labels become further excluded from the bilayer and form separate aggregated domains. We conclude that the ESR parameters of PC spin labels in frozen membranes do not represent the membrane polarity or water penetration profile. Instead, they show a distribution between hydrogen-bonded and non-hydrogen bonded states of the nitroxide, which is affected by a number of factors in the membrane composition.

In the fluid phase lipid membranes these bent conformations are not predominant, but are always present in a dynamic equilibrium. Their presence explains the large values of spin relaxation enhancement (RE) for PC spin labels induced by paramagnetic metal salts dissolved in the aqueous phase: the bent conformations allow for close proximity of nitroxides to the paramagnetic ions, resulting in spin exchange between them. We discuss also the observed strong effect of the counter anion on the RE of PC labels by metal ions and the effects of membrane composition, especially the role of cholesterol. For lipid labels, acyl chain as well as head-group labeled, we compare our experimental results with recent MD simulations.

For our TEMPO partitioning study by 95GHz 2D-ELDOR, we achieved complete separation of signals corresponding to the location of the nitroxide in the water and the lipid phases by combining the advantages of 2D ESR and High Field ESR. This separation not only allowed for precise measurements of T1 and T2 relaxation times for each signal but also for observation of cross-peaks between them due to exchange resulting from partitioning between phases – the first direct observation of exchange of this kind by ESR. The exchange rate constant estimated from this cross-peak development is 0.52 µs⁻¹.
ENHANCED ACCURACY OF THE METHOD; MEASUREMENT OF MICROWAVE FIELD STRENGTH IN CW-EPR BY A PULSED MODULATION TECHNIQUE

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The microwave magnetic field strength, $B_{mw}$, is essential parameter for all quantitative measurements in EPR spectroscopy. It can be also noted that $B_{mw}$ in the frequency units $(2\omega_1=\gamma_z B_{mw})$ corresponds to Rabi frequency of a spin system in the microwave field (MW). In the present consideration among the various different methods for estimation $B_{mw}$ the experimental method with pulsed modulation detection proposed several decades ago [1] will be discussed and revised by employing recently suggested multiple photon description of CW-EPR spectra [2, 3]. The method is based on measurements of the splitting, $d$, between the first sideband signals under the small modulation index, $\varepsilon=2\omega_2/\omega_{rf}$, ($2\omega_2$ corresponds to modulation amplitude and $\omega_{rf}$ to modulation frequency of radiofrequency, RF, field). There are also some limitations for this method due to approximation in theoretical description which was based on modified Bloch equations. It can be noted that the experimental method of the pulsed modulation detection (zeroth-harmonic of modulation signal) employed in this method [1] coincides with more recently suggested method of amplitude-modulated CW-EPR, AM-CW-EPR [4, 5]. Thus, in order to increase accuracy of the corresponding $B_{mw}$ and to simulate recorded experimental modulation spectra [1] theoretical description of the AM-CW-EPR spectrum for an individual homogeneous line will be employed [5]. Moreover, this description will be additionally improved by introducing Bloch-Siegert-like shift, $\Delta_k$, obtained from the expanded Hamiltonian for resonant multiple photon transitions [2, 3]. Fig. 1 shows simulated AM-CW-EPR spectra by applying improved model description ($\Delta_k$ is included) and the same value of parameters as was used in ref. [1]. The simulated spectra well describe experimental spectra and corresponding $d$ values. These splitting values exhibit nonlinear behavior in the plot $d$ versus $\omega_{rf}$ and it can be used for estimation $B_{mw}$ value with higher accuracy than the $B_{mw}$ value obtained in ref. [1]. The detailed calculation procedure and discussion will be presented.

References

Regularization-free and Model-free Determination of Distance Distributions for Pulsed Dipolar Spectroscopy

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Pulsed Dipolar Spectroscopy (PDS) is a powerful method for studying the structure and function of biological systems. In PDS, a dipolar signal is acquired from the interaction between a pair of spin labels, from which the distance distribution between them, $P(r)$ may be obtained. The $P(r)$ can be in the range of 1 to 10 nm. However, due to the ill-posed nature of the inversion of the dipolar signal to yield the $P(r)$, one must resort to regularization or model fitting methods to obtain reasonable results. The method of Tikhonov regularization (TIKR) is commonly used, but it relies heavily on the choice of regularization parameter ($\lambda$) that yields a compromise between good resolution and stability of the $P(r)$. However, this procedure is still vulnerable to the appearance of spurious peaks and negative values for $P(r)$ as well as effects of noise in the dipolar signal. Model fitting methods, on the other hand, require \textit{a priori} model functions to estimate $P(r)$, which may not accurately represent the actual distance distributions. This is especially true if the $P(r)$ is multimodal. We developed a new and objective approach to this matter which yields accurate distance distributions with high resolution and without any spurious peaks or negative $P(r)$s. The dipolar signal is first denoised using our recently developed WavPDS method\textsuperscript{1} based on wavelet transforms\textsuperscript{2}. This denoised signal is then directly converted into the $P(r)$ by our new Singular Value Decomposition (SVD) based method. Its effectiveness is illustrated below and compared with TIKR plus the Maximum Entropy Method (MEM), also on the denoised dipolar signal.

**Unimodal Sample:**
Initial SNR 3.8
Denoised SNR 488

**Bimodal Sample:**
Initial SNR 11
Denoised SNR 1046

\textbf{*Blue:* References for TIKR+MEM and for New SVD Method}

SpecMan4EPR ver. 2.5: next generation AWG engine

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SpecMan4EPR is a software platform for the construction of EPR instruments.\textsuperscript{1} The customizable structure of SpecMan4EPR allows the use of the same software for a variety of instruments - from low-frequency EPR imagers to high-frequency DNP spectrometers.

Arbitrary waveform generators (AWGs) are commonly used for a pulsed EPR instruments. High flexibility of these devices allows to expand instruments capabilities and improve their performance. At the same time, this flexibility creates a challenge for generalized control software to maintain the simplicity of the user interface and unrestricted capabilities. A new generation of SpecMan4EPR AWG engine features new pulse programming language (PPL) for representation of the microwave pulse sequences and detection events; and multiple tools such as pulse resynchronization and pattern libraries. These innovations allowed extending the capabilities of the software without compromising its usability. AWG-generated two-pulse echo sequence of chirp pulses can still be programmed with three parameters and in just seven lines:

\begin{verbatim}
\% tp, frequency, bandwidth are parameters \\
set1 = [0.5, frequency, bandwidth, 0deg] \\
set2 = [1.0, frequency, bandwidth, 0deg] \\

chirp tp,set1      % the first pulse %
wait tau - tp \\
chirp tp/2,set2    % the second pulse %
wait tau - tp/4 - 50ns \\
detect 'I','Q'
\end{verbatim}

This presentation details the features of the new AWG engine and describes instruments constructed using the new technology. All projects use different AWGs have different designs and utilize the same software.

References
Uniform phase excitation via optimal control for 200 GHz EPR.

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The application of amplitude and phase modulated microwave pulses, produced by an arbitrary waveform generator (AWG), has seen many successful implementations in pulse EPR experiments. However, the application of AWG pulses to high frequency EPR spectrometers has only recently been achieved [1]. The authors achieve broadband spin manipulation over 10 MHz but find that spin manipulation is hindered by the low power microwave source, resulting in low $\omega_1$. To improve the control over the spin system and utilize all the available microwave power we employ an optimization scheme based on optimal control that produces phase modulated constant amplitude microwave pulses. We employ the optimal control scheme to generate a series of broadband excitation pulses - up to 10 MHz - that produces uniform phase magnetization over the bandwidth of the pulse. To extend this approach to such applications as DEER spectroscopy we apply this broadband excitation pulse to the three-pulse echo sequence that is commonly employed in the DEER experiment. The broadband excitation pulse is the first pulse of the sequence followed by two chirp refocusing pulses that are set to the same bandwidth of the excitation pulse. The broadband excitation pulse improves the signal to noise of the echo compared to a standard rectangular excitation pulse. We also discuss that application of phase modulated pulses at X-band.


We thank Prof. Mark Sherwin for stimulating discussions. Daniel Sank is acknowledged for his help with DAQ programming. This work was supported by the National Science Foundation (NSF) (CHE 1505038) and NSF IDBR grant through the DBI division (1152244). IK acknowledges the support of the long-term postdoctoral fellowship by the Human Frontier Science Foundation.
A tool for Bootstrap and Monte Carlo analysis of ESR in the slow-motion regime

William Lindemann, Olivia Saouaf, Julia Ortony

Electron spin resonance (ESR) experiments can provide important information about the conformational dynamics of spin-labeled molecules. Typically, spectra are fit in the slow-motion regime, using nonlinear approaches pioneered by the Freed group, and implemented in the NLSL, EasySpin, and other software packages. Desirable developments to the current fitting capabilities would include features such as the ability to achieve high-throughput spectral analysis and implementation of Bootstrap and Monte Carlo methods for error analysis. We have implemented these changes, and present them here in an open-source MatLab package to be used in conjunction with NLSL. Further, we have demonstrated the use of this software tool to achieve high quality fits with reliable confidence intervals to spin labeled liposomes over a range of temperatures. To the best of our knowledge, this software tool represents the first implementation of these methods for the fitting of slow-motion spectra, and we expect it to enhance the reliability of ESR fits for quantitative dynamics analyses.
DEVELOPMENT OF MM-WAVE ESR/NMR DOUBLE MAGNETIC RESONANCE MEASUREMENT SYSTEM AT VERY LOW TEMPERATURES


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We report the development of mm-wave ESR and NMR systems on a 3He/4He dilution refrigerator (DR) to reach below 1 K. This system potentially makes us possible to perform ESR/NMR double magnetic resonance (DoMR) measurements such as ENDOR and dynamic nuclear polarization (DNP)-NMR which are powerful methods to study electron-nuclear coupled spin systems.

One of our targets to perform DoMR is lightly phosphorus-doped silicon (Si:P). Since a quantum computer (QC) design using Si:P was proposed by Kane [1], which is one of the best practical QC designs, requires high magnetic fields (B > 3 T) and low temperatures in the range of 100 mK in order to study spin dynamics including DNP phenomena of Si:P at very low temperatures. However, 31P-NMR signal from Si:P has not been observed directly because of the low concentration of P atoms. We have been studying on Si:P with ESR and ESR/NMR double resonance [2].

We report the following subjects: (1) We have developed a homodyne ESR measurement system. The InSb detector is cooled below 2 K in the DR. (2) We have developed a Fabry-Perot type resonator for DoMR with a Helmholts-type NMR coil and with a flat mirror made of thin gold layer. (3) We have observed 31P nuclear polarization in Si:P up to approximately 50 % due to DNP effect. NMR measurement for 1H contained in the resonator body was also successful. (4) We have also performed ESR measurements of a single crystal of Cu(C6H3N2)(NO3)2 to confirm that the sample temperature is cooled, a spin-1/2 quasi one-dimensional antiferromagnet [3], at 128.9 GHz. The ESR spectrum consisted of two lines at low temperatures and the split width of the two lines increased continuously as the temperature decreased down at least above 1 K.

Fig. 1. Temperature dependence of the split width of Cu(C6H3N2)(NO3)2 at 128.9 GHz.

References
Field Stepped Rapid-Scan EPR Imaging of CTPO

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Abstract
Small molecule probes with unpaired electrons are being developed to monitor properties of living systems. This can be achieved by performing EPR imaging of the spatial variation of the spectral properties of these probes. Because the large water content of living systems can be problematic, these experiments are performed in relatively low magnetic fields. This makes the signal weaker than would be observed at higher magnetic fields. Our research group is developing an improved detection method, called rapid-scan EPR, where the magnetic field is scanned through the signal much faster than in conventional spectroscopy [1]. The goal is to design an instrument that can be used in clinical studies of small animal models of human disease.

To decrease the impact of interfering signals that are generated by the rapid field scans, methods are being developed to acquire the spectrum of the probe molecules in segments and combine the segments to construct the complete spectrum [2]. Field stepped EPR spectra of CTPO solutions with a series of gradients were taken at room temperature at VHF. Images were reconstructed by a new spectral-spatial imaging algorithm developed in our lab [3]. Several parameters, including field sweep width, stepping size, number of averages, rapid scan frequency, rapid scan width, maximum gradient, and gradient step size, were varied in order to optimize acquisition conditions and improve image quality. The linewidths and resolutions were calculated and compared.

A larger gradient and a smaller gradient step size give better image quality, as well as better resolution. To minimize the rapid scan background, 5 kHz rapid scan frequency and 10 G rapid scan width were selected. Field sweep width of 80 G was needed to encompass the whole spectrum at high gradients.

Reference
Transmission Mode Non-Resonant Detection for Radiation Dosimetry

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We perform electron spin resonance measurements employing a custom non-resonant transmission line structure for microwave excitation \textit{and detection}. In this structure, the radiation sensitive sample is located between the top signal line and the bottom ground plane of a transmission line. Location of the sample between the signal line and ground plane (rather than above the signal line) maximizes the amplitude and uniformity of $B_1$. This “ESR probe head” is constructed such that the planar radiation sensitive sample can be readily inserted or removed without any experimental difficulty ($\approx$ 1 s). The probe head also includes a single modulation coil mounted beneath the ground plane. This unique arrangement presents a remarkably simple user interaction without sacrificing sensitivity. The ESR probe head is complemented by a custom-designed permanent magnet and sweep coil, as well as a compact custom-built continuous wave X-band microwave bridge [1].

We utilize this unique measurement capability to measure planar poly-crystaline L-alanine samples exposed to gamma radiation. Gamma radiation is known to deaminate the L-alanine radical [2-3] and form a remarkably stable structure that lends itself quite nicely to transfer dosimetry [4-5]. Measurements of the central peak of $< 40$ mg of L-alanine is achieved at the sub 1-Gy dose level. As composed, this system allows for highly-sensitive ESR dosimetry of radiation sensitive materials in a compact spectrometer package.

References

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Abstract: The most common method for the inference of spin-spin distance distributions, \( P(r) \), from Double Electron-Electron Resonance (DEER) data is a penalized least-squares approach called Tikhonov regularization. Although the method typically provides reliable estimates of \( P(r) \), it requires the input of a regularization parameter, \( \alpha \), which scales the magnitude of the penalty term with respect to the fitting error. This nuisance regularization parameter significantly influences the solution to the Tikhonov functional, so selecting its optimal value is crucial for accurate analysis of DEER data. The most popular method for selecting \( \alpha \) in DEER data analysis is a graphical method called the L-curve criterion, which has empirical justification. However, to date, a systematic exploration of popular extant regularization parameter selection methods has not been carried out in the context of DEER data analysis. Here, we evaluate 16 methods, including the L-curve criterion, against a large, physically-derived test set of simulated DEER data.
Frozen Acrylamide Gels as DNP Matrices.

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Dynamic Nuclear Polarization (DNP) is a hyperpolarization approach providing an increase in NMR signal intensity by up to 2 orders of magnitude. It allows unprecedented atomic-level characterization of systems that were previously inaccessible.[1]

In DNP experiments, the high polarization of the electron spins is transferred to nuclei. Samples are doped with a stable radical (TEKPol,[2] AMUPol,[3]…) to provide a source of unpaired electron in the presence of a propagation medium to relay hyperpolarization through spin diffusion. Aggregation of free radicals can have disastrous effects on DNP, so that typical matrices for DNP are formulated to ensure good glass formation. Popular matrices include glycerol/water (so-called DNP juice), 1,1,2,2-tetrachloroethane or orthoterephenyl. These systems are compatible with many substrates. However, there is still today only one water based formulation. Even the organic solvents encounter problem with systems prone to aggregation, with nanoparticles being a prime example that have not so far been amenable to study in any ordinary solvents. [1b] Here, we introduce a new water-based DNP matrix using acrylamide gels, coined DNP Jelly. We demonstrate that optimized gel formulation can provide enhancements of over 200 on 13C of the gel at 9.4 T and 100K. We characterize CdTe-COOH quantum dots dispersed in DNP Jelly. The gels introduced here prevent aggregation while maintaining high enhancements on 115Cd.

Fig. 1. (a) Structure of DNP Jelly: Polyacrylamide gel with the polarizing agent AMUPOL (b)115Cd DNP CP-CPMG with microwaves (black) and without microwaves (dashed) of CdTe-COOH NPs dispersed in DNP Jelly. The MAS rate was 10 kHz.

References
The development of robust, high-resolution benchtop NMR spectrometers based on permanent magnets presents the opportunity for exciting new applications of NMR spectroscopy outside of the typical laboratory environment. One of the significant barriers to progress in this field is the relatively low sensitivity of these devices, which is a direct consequence of the lower magnetic fields (1 – 2 T) when compared to standard NMR spectrometers (7 - 23.5 T). Therefore benchtop NMR is an area where hyperpolarisation techniques, which can boost NMR sensitivity by several orders of magnitude, can have a transformative impact. In this work we focus on the use of the parahydrogen ($p$-$H_2$) induced polarisation (PHIP) approach [1] because $p$-$H_2$ is relatively cheap and easy to produce and so does not significantly compromise the portability and affordability of the benchtop NMR device when compared to other hyperpolarisation methods. PHIP can be achieved either using a hydrogenation reaction or through catalytic transfer of polarisation at a metal centre, the so-called SABRE approach.[2] In this work, we demonstrate that high levels of hyperpolarisation can be observed on a benchtop NMR spectrometer using both hydrogenative PHIP and SABRE and show how a fully integrated flow system (see Fig. 1) can be used to generate reproducible and renewable SABRE hyperpolarisation. In addition, we explore how these methods can be applied to species identification and reaction monitoring in a process control environment.

Fig. 1. (a) Photo of the benchtop NMR spectrometer with flow system for SABRE hyperpolarization and (b) single-scan SABRE hyperpolarised $^1$H NMR spectrum of 50 mM 4-amino-pyridine (bottom) with the equivalent thermally polarised NMR spectrum (top).

References

Microwave-Gated Dissolution Dynamic Nuclear Polarization

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Dynamic Nuclear Polarization (DNP) aims at transferring the large electron spin polarization to surrounding nuclear spins via microwave irradiation. Dissolution-DNP (d-DNP) experiments are usually performed in frozen samples doped with paramagnetic polarizing agents (PAs) where $^{13}$C polarization enhancements factors as high as 10’000 are possible with respect to thermal polarization in the liquid state [1]. We have recently implemented $^1$H→$^{13}$C cross-polarization (CP) during d-DNP experiment to further boost $^{13}$C enhancements to factors of about 50’000 [2].

However, $^1$H→$^{13}$C CP has so far been suboptimal because of the rapid proton relaxation in the rotating frame arising from the presence of PAs. We show in this work that $T_{1ρ}(^1$H) can be significantly extended, and therefore CP greatly improved, by switching off the microwave irradiation briefly prior to CP. During this interruption, the electron spins relax from their partially saturated state to their highly polarized state ($P_e = 99.9\%$ at $B_0 = 6.7$ T and $T = 1.2$ K), so that paramagnetic relaxation becomes ineffective. As a result, $T_{1ρ}(^1$H) is extended by several orders of magnitude and CP contact times can be extended to achieve optimum transfer.

The use of microwave gating in this context has two favourable effects; (i) preventing losses of proton magnetization during spin-locking and (ii) improving the CP transfer efficiency. Altogether, the efficiency of multiple contacts CP is greatly improved by microwave gating; polarizations as high as $P(^{13}$C) = 65\% can be achieved in acetate with an overall polarization build-up time constant as short as $τ_{bup} = 3$ min. A record polarization $P(^{13}$C) = 78\% was even achieved in $^{13}$C labelled urea [3].

EFFICIENT NUCLEAR HYPERPOLARIZATION OF BIOMOLECULES IN SOLUTION IN THE PRESENCE OF A CRYOGENIC PROBE

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The low sensitivity of NMR hampers its applicability to many problems of biological significance. To address this limitation, we employed photochemically induced dynamic nuclear polarization (photo-CIDNP) at high field in the presence of novel photosensitizers and a cryogenic probe in liquids. Hypersensitive data collection within the nanomolar concentration range was efficiently achieved within only a few seconds. The challenges posed by cryogenic probes in the presence of intra-probe laser irradiation were overcome. The observed intensity-enhanced NMR resonances include the backbone (Cα) and aromatic side chain of amino acids, peptides and proteins (Fig. 1). Pump-probe transient absorption experiments were exploited to assess the dependence of photoexcited triplet lifetimes on the extent of nuclear hyperpolarization. Applications of this technology to the 1D and 2D heteronuclear-correlation NMR analysis of proteins in buffered solutions and more complex milieux, including cell-like media, will also be presented.

References
Optimization of EPR and DNP for the Investigation of Small Samples

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Investigating small sample volumes is an attractive direction for DNP NMR of biosolids. Small samples can be manufactured more easily, and spun at faster speeds. In addition, less total microwave power is required to obtain the required saturation, provided the microwaves can be sufficiently focused. As power needs decrease, compact microwaves sources become a competitive alternative to gyrotrons in DNP instrumentation. Focusing the microwaves does demand careful management of the microwave path. Toward this end, we have been using EPR and DNP in the same probe to diagnose the transmission of microwaves into the sample. In our 7T system we use a diode based microwave source which produces 180mW at 198 GHz and primarily observe the EPR and $^{13}$C NMR spectra in diamond. We are investigating the saturation behavior of the room-temperature EPR as a means of determining the microwave $B_1$ field strength and correlating this to DNP enhancement. We are also studying the interaction between MAS rotors and microwaves; particularly how different rotor orientations, coil geometries and materials affect mm-wave sample penetration. Conclusions from these experiments have informed the construction of a triple resonance MAS and EPR probe in which we have achieved DNP enhancements over 450-fold at room temperature in $^{13}$C MAS spectra of diamonds. In this probe we demonstrate the utility of a dielectric antenna as an alternative to a microwave horn for delivering microwaves into MAS samples. Support for this work by the NSF Chemical Measurement and Imaging program under grant CHE-1413096 is gratefully acknowledged.

Fig 1. Waveguide horn and attachment for the study of rotor orientation. Dielectric lens antenna is also shown.
Dissolution DNP of $^{133}$Cesium ions

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Introduction
In this work we present dissolution DNP experiments with the 100% natural abundance $^{133}$Cesium ($^{133}$Cs) ions. $^{133}$Cs is a quadrupolar nucleus (spin 7/2) with a gyromagnetic ratio of approximately half that of $^{13}$C. Its quadrupolar moment is low, however, leading to narrow NMR line widths and moderately long relaxation time constants in solution. $^{133}$Cs is the largest of the stable alkali metal ions and its electron cloud is highly polarizable, which makes its solution NMR shift very sensitive to its chemical environment. This latter feature has been employed in biological NMR experiments where $^{133}$Cs was employed as a potassium (K⁺) analogue in cell uptake experiments where intra- and extra-cellular populations show different chemical shifts.¹

Scope
We have investigated the solid state DNP properties of $^{133}$Cs in various sample preparations comprising trityl radicals, and with and without the addition of a gadolinium complex to boost the polarization. In most of the preparations we used cesium [1-$^{13}$C]-acetate to make a direct comparison between $^{133}$Cs and $^{13}$C possible. We have also investigated the samples after dissolution in aqueous buffers by monitoring the decay of the hyperpolarized signal and its changing shift when temperature and chemical environment change. Finally, we have employed hyperpolarized $^{133}$Cs ions in buffer to investigate impaired cell membrane in a yeast model system.

Results
Data from the investigations shows a strong gadolinium effect for $^{133}$Cs resulting in almost 3 times higher polarization (Figure 1, left). The polarization buildup curves of $^{133}$Cs differ from $^{13}$C in being clearly bi-exponential with a very fast and a slow component. The absolute solid state polarization of $^{133}$Cs was determined by a direct comparison with a recorded thermal spectrum of the same sample. More than 50% solid state polarization was obtained for the gadolinium containing samples. A large but reproducible fraction of the solid state polarization was lost in dissolution of the samples leading to a liquid state polarization of approximately 15% (max). The chemical shift of the signal from the hyperpolarized $^{133}$Cs ions was shown to be highly sensitive to solution ion strength and temperature, but less so to changes in pH in the biologically interesting range around neutral. In experiments with yeast cells with compromised cell membranes the $^{133}$Cs ions partitioning could be investigated due to a large chemical shift difference between the extra- and intracellular environments (Figure 1, right).

Figure 1, left. Solid-state polarization build-up of $^{133}$Cs $^{13}$C-Ac signal at 3.35 T and 1.4 K followed by dissolution and 10 s transfer to liquid-state polarization decay monitored at 9.4 T magnet and 318 K. A) Solid-state $^{133}$Cs polarization build-up as a function of time. Filled triangles (with Gd'⁺) and filled squares (without Gd’⁺). B) Liquid-state $^{133}$Cs polarization as it decays over time with a $T_1$ of 16 s. Figure 1, right. Uptake of $^{133}$Cs in membrane impaired yeast cells. Chemical shift difference between the extracellular and intracellular $^{133}$Cs signal is approx. 7.7 ppm.

Optically hyperpolarized nuclear spins in diamond powder

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Nitrogen Vacancy (NV) centers in diamond are an attractive platform for dynamic nuclear polarization of nuclear spins, particularly because they are electronic spins that can be optically polarized at room temperature with modest laser powers. In the quest towards NV driven DNP, nanodiamond powder is particularly attractive: they have huge surface areas, and one could arrange for a close physical contact between the polarized NVs and external nuclear spins.

However, the strong orientation dependence of the NV center spectra have made this extremely challenging. This manifests as a broadening of the NV electron spectrum by around 6GHz which makes DNP transfer process inefficient. On the other hand, working at low magnetic fields (<10mT) allow one to overcome this challenge, since the powder pattern is narrow enough to excite in a coherent fashion.

We demonstrate optically polarized $^{13}$C nuclear spins in diamond powder. The DNP is performed in a novel experimental platform where DNP excitation occurs at low fields (8mT) and enhancements are characterized at high fields (7T). Currently we have polarized $^{13}$C in diamond powders at the 0.1% level.

**Fig. 1.** Figure caption: (a) Optically polarized $^{13}$C spins in diamond. (b) Experimental platform involving field cycling by sample shuttling that allows DNP excitation at low fields and detection at high field.

**References**


STUDYING INTRINSICALLY DISORDERED PROTEINS UNDER TRUE IN VIVO CONDITIONS BY COMBINED CROSS POLARIZATION AND CARBONYL DETECTION

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Under physiological conditions, studies of intrinsically disordered proteins (IDPs) by conventional proton-detected NMR methods are severely limited by fast amide proton exchange with water. 13C detection has been proposed as a solution to the exchange problem, but is hampered by low sensitivity. We propose a new pulse sequence combining proton-nitrogen cross polarization and carbonyl detection to record high-resolution, high-sensitivity NMR spectra of IDPs at physiological conditions. We demonstrate the efficacy of this approach by recording a high-quality N-CO spectrum of alpha-synuclein in bacterial cells at 37°C [1].

Fig. 1. 1D versions of CT CP-H$_N$CO (constant-time), non-CT CP-H$_N$CO (non constant-time), BEST-H$_N$CO, and CON experiments recorded on an alpha-synuclein in-cell sample [1].

References

The Direction of Glioblastoma Tumor Growth

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INTRODUCTION: Derived from glial cells, glioblastoma multiforme (GBM) is an extremely aggressive malignant brain tumor in adults. Generating MR-derived growth pattern models for GBM has been an attractive approach in neuro-oncology, suggesting a distinct pattern of lesion spread with tendency in growing along the white fiber direction for the invasive component [1, 2]. Here we report results from a retrospective analysis, designed to provide a brain atlas predicting the dominant directions of tumor expansion/shrinkage prior to surgery.

METHOD: We examined Fifty-six adult GBMs at two time-points; diagnostic and pre-operation, with contrast-enhanced anatomical MRI. The study was approved by the regional ethics committee and adhered to the Helsinki Declaration. The tumor volumes were segmented semi-automatically, and with the resulted tumor masks we performed a non-linear registration to generate deformation fields of tumor lesions. Angles between the vector fields of DTI WM and tumor growth directions were calculated (within sub-regions with prominent lesion overlaps) to estimate an angle map of lesion growth to that of white matter tracts.

RESULTS: The generated angle maps were color-coded to visualize the alignment agreements between two dataset, in which the maximum (parallel/anti-parallel) and the minimum (perpendicular) alignments were determined as $\theta < 20^\circ$ or $\theta > 160^\circ$ and $70^\circ < |\theta| < 110^\circ$ respectively. Tumor displacement measured in this study showed a tendency of moving along the white matter tracts, as evidenced by the dominant vector population with maximum alignments towards the tensor direction of the WM atlas (Fig. 1). Growth parallel to the white matter tracts was statistically more common than perpendicular growth (Mann-Whitney t-test, $P < 0.01$).

CONCLUSION: Our findings represent a step forward in investigating the hypothesis that tumor cells tend to grow preferentially along the white matter. The resulting mean vector field of GBM tumor movements may provide useful information on tumor growth pattern prior to surgery.

REFERENCES


Figure 1. Angle map. The degree of alignment between the 3-dimensional mean vector field and a DTI-WM atlas (white matter fibers) is demonstrated as an angle map. Selected axial slices are depicted, demonstrates the predominant alignment of the mean deformation field vectors with WM fibers. The color-coded map indicates the tendency of tumor growth direction along white matter fibers with red (parallel and anti-parallel). Vectors with perpendicular direction to the white matter fibers are determined by blue color.
Robust detection of 2-HG at 7T high field with a fully adiabatic LASER sequence

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INTRODUCTION: Accumulation of 2-hydroxyglutarate (2-HG) metabolite in glioma patients that harbor isocitrate dehydrogenase (IDH) mutation is associated with improved clinical outcome and response to treatment. Therefore, unambiguous and robust detection of this oncometabolite can benefit accurate gliomas stratification and targeted therapy. Despite recent development of MRS sequences, improving sensitivity and specificity of 2-HG detection is still an area under investigation. Previous work using PRESS and semi-LASER sequences at 7T have shown that an inverted 2.25 peak can be obtained for 2-HG which is distinguishable from modulation of glutamate (Glu) and glutamine (Gln). However, spectral modulations are very sensitive to inaccuracies of flip angles which are exacerbated at 7T due to B1 inhomogeneity. In this work we aimed to optimize a fully adiabatic LASER sequence that can compensate better the B1 inhomogeneity for robust detection of 2-HG at 7T.

METHODS: 2-HG, Glu, and Gln spectra were simulated for different echo timings of a LASER sequence at 7T field strength using GAMMA library. The LASER sequence consists of three pairs of adiabatic refocusing pulses, which can be separated in three sub-echo times TE1, TE2, and TE3, respectively. The best timing combinations for adiabatic refocusing RF pulses in the LASER sequence, were simulated (Fig. 1A) to provide a unique behavior of 2-HG resonances compared with other resonances. In addition, we searched for the combinations that achieved these goals for the minimum total echo time. The candidate timing intervals in our LASER sequence were verified experimentally on phantoms containing 10mM 2-HG and identical concentration of Glu and Gln, and 20mM of glycine.

RESULTS: A LASER sequence with TE=90 ms (TE1/TE2/TE3=15/45/30 ms) provided a large negative 2-HG resonance at 2.25 ppm well separated from those of overlapped resonances. The same 2-HG resonance pattern was observed in MR spectra obtained from phantoms applying the same timing intervals, accompanied with reduced levels of overlapping peaks Glu and Gln (Fig. 1B).

CONCLUSION: The proposed sequence timing of our LASER sequence may provide unambiguous detection of 2-HG resonances on a 7T clinical scanner with high specificity and sensitivity. Our preliminary results from simulations and phantoms are currently under investigation in patients. Further, this technique provides a great opportunity to non-invasively monitor the effect of new anti-cancer drugs that target mutant IDH enzymes.

REFERENCES
The HOT sequence is an iMQC method that isolates zero quantum couplings from fat-water spin pairs. The resulting indirectly detected frequency reflects \((\omega_{\text{water}}-\omega_{\text{fat}})\) between two spins approximately 100\(\mu\)m apart, so chemical shift information is preserved while most inhomogeneous broadening is removed. The method has been used to monitor the proton resonance frequency of water and thus track temperature in adipose and in bone marrow, despite the presence of inhomogeneous broadening. This abstract presents results from using a version of the HOT sequence in patients undergoing breast MRI to do spectroscopy of the lipid region of the spectrum.

Imaging parameters for each timepoint were FOV=250mm, matrix 128x32, slice thickness 10mm, RARE ETL=4, Initial t1=4ms, \(\tau=5.2\)ms, \(G1=32\)mT/m*ms, \(G2=50\)mT/m*ms. Sixty three subsequent images were taken incrementing t1 by 300us to generate the HOT spectrum of each voxel, including subtraction of low frequency components of the spectrum. T1w 3D imaging was also performed across the entire volume with a matrix size of 896x357x128 also with an in plane FOV of 250mm.

In appropriate voxels, HOT spectra reveal strong components at 0.8, 1.5, and 1.9-2.2. However, contamination from DC water signal still presents a significant challenge to quantification and identification.

References

Probing Specific Water-Protein Chemical Exchange in a Membrane Protein via Indirect Observation of Dipolar-Dephased $^{15}$N signals in Solid-State MAS NMR

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Nowadays, it has become clear that the membrane lipids have profound impacts on the structure, dynamics and function of membrane-bound proteins and peptides$^{1,2}$. The implications of the lipid-protein interactions have been increasingly recognized in past decades and many solid-state NMR techniques have been developed to study such lipid-protein interactions. Of these interactions, water-protein chemical exchange in membrane-bound proteins is another important parameter in the dynamical relationship between the proteins and their surrounding environments that has been investigated in the past primarily through $^1$H-$^{15}$N heteronuclear correlation (HETCOR) experiments$^{3-5}$. Direct observation of water-protein exchange is challenging because of strong $^1$H spin diffusion associated with the relatively rigid membrane-bound protein environments. Here, we propose one-dimensional pure chemical exchange measurements via indirect dipolar-dephased $^{15}$N signals for probing the specific water-protein chemical exchange kinetics of membrane-bound proteins$^6$. In the example of the Influenza A full length M2 (M2FL) protein, the buildup of dipolar-dephased $^{15}$N signals from the tetrad of His37 sidechains has been observed as a function of spin-lock time. This confirms that hydronium ions are in exchange with protons in the His37 NH bonds at the heart of the M2 proton conduction mechanism, with an exchange rate constant of $\sim$1750 s$^{-1}$ for pH 6.2 at -10ºC.

ACKNOWLEDGEMENTS
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References
Melittin is a bee venom peptide that disrupts lipid bilayer at temperature below the liquid-crystal to gel phase transition temperature. Notably, the ability of melittin to disrupt acidic dimyristoylphosphatidylglycerol (DMPG) bilayers was weaker than its ability to disrupt neutral dimyristoylphosphatidylcholine bilayers. The structure and chemical shift anisotropy of [1-13C]-labeled melittin obtained from solid-state 13C NMR spectra. 13C chemical shift anisotropy showed oscillatory shifts with the index number of residues. Analysis of the chemical shift oscillation properties indicated that melittin bound to a DMPG membrane adopts a bent α-helical structure with tilt angles for the N- and C-terminal helices of -32 and +30º, respectively. The transmembrane melittin in DMPG bilayers indicates that the peptide protrudes toward the C-terminal direction from the core region of the lipid bilayer to show a pseudotransmembrane bent α-helix structure [1]. MD simulation indicate that N-terminal K7 interacts with one DMPG molecule (Fig. 1).

The structure topology and orientation of membrane-bound antibiotic alamethicin were studied using solid state NMR spectroscopy. 13C chemical shift interaction was observed in [1-13C]-labeled alamethicin. The isotropic chemical shift values indicated that alamethicin forms a helical structure in the entire region. The chemical anisotropy of the carbonyl carbon of isotopically labeled alamethicin was also analyzed with the assumption that the adjacent peptide planes form an angle of 100º or 120º when it forms α-helix or 310-helix, respectively [2]. These properties lead to an oscillation of the chemical shift anisotropy with respect to the phase angle of the peptide plane. The chemical shift oscillation curves indicate that the N- and C-termini form α-helix and 310-helix, and the N- and C-termini are tilted 17º and 32º to the bilayer normal, respectively.

References
PARAMAGNETIC METALLOPROTEINS AND FAST MAGIC-ANGLE SPINNING: THEORY AND EXPERIMENTS MEET AT THE METAL CENTER

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We show how the application of a new set of NMR experiments, recently developed for the study of complex paramagnetic inorganic battery materials [1], can be adapted to the solid-state NMR analysis of paramagnetic metalloproteins, and can be used to improve the information obtainable from these systems. These experiments combine ultra-fast (60-111 kHz) magic-angle spinning frequencies and short high-powered adiabatic pulses (SHAPs) [2], and are applied to the 13C,15N-labelled microcrystalline metalloenzyme superoxide dismutase (SOD), which has two high-affinity binding sites for metal cations [3]. Here, by the use of the aforementioned experimental setup and first-principles paramagnetic NMR calculations [4], we are able to detect, characterize, and assign 1H, 13C and 15N signals from residues directly coordinating the metal centers (Fig. 1). Furthermore, we investigate the use of paramagnetic NMR shifts of atoms in the contact regime as well as pseudo-contact shifts of distant atoms [5] for assessing the structure of the metal center. The present work represents a robust approach to the NMR study of paramagnetic metalloproteins, opening a new avenue for the study of the structure and the reactivity of metal centers in complex insoluble systems.

Fig. 1. A) 1H spin-echo spectrum of E, Co2+ - SOD at 500 MHz, 100 kHz MAS rate. B) Active site of E, Co2+ - SOD. C) 1H – 13C – 1H TEDOR spectrum of E, Co2+ - SOD at 500 MHz, 100 kHz MAS rate.

References
3D topological studies of antimicrobial peptides with enhanced activities by using solid-state NMR spectroscopy

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Since antibiotic resistance has been prevalent and the development of new antibiotic agents is delayed, it is important to investigate antimicrobial peptides (AMP) known as cationic peptides from the nature as its alternative. More than 600 cationic peptides have been discovered in virtually all organisms from microbe to man. AMP is short positively charged polypeptides and exhibiting amphipathic characteristics. Despite significant differences in their structures, all AMPs discovered share the ability to interact with cellular membranes, thereby disrupting membrane organization.

Lactophoricin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, was currently utilized as the framework to design the novel analogs and study the effect of peptide hydrophobicity/hydrophilicity, amphipathicity on antimicrobial activities. Eleven LPcin analogs were designed and modified to enhance antibacterial activity using conservative sequence change. Three analog peptides were selected among LPcin analogs via results of bacterial killing and growth inhibition assays against Gram-negative and Gram-positive bacteria. We successfully overexpressed LPcin analogs in the form of fusion protein in E. coli and purified them from the cell extracts with many biophysical techniques.

In order to elucidate the structure-antibiotic activity relationships, we studied the 3D conformation and antimicrobial activity using various spectroscopic methods like MALDI-TOF MS spectroscopy and CD spectrometry, as well as 1D/2D solution NMR spectroscopy and 1D/2D solid-state NMR spectroscopy in membrane environments. The structural calculations of LPcin analogs using Discovery Studio 2016 were also used to refine the orientational information of 3D structure and topology based on 2D SAMPI4 solid-state NMR spectra. And we will also present the optimized design, construction, and good efficiency of a home-built 800 MHz (NB) narrow-bore $^1$H-$^{15}$N solid-state NMR probe for solid-state NMR studies of antimicrobial peptides.
Solid State NMR Probes for 1.5 GHz Spectrometer

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We report three solid-state NMR probes constructed for 1.5 GHz NMR magnet that reached its field in late 2016. Drastic increase in resolution and sensitivity opens new possibilities for half-integer quadrupolar nuclei and for “unreceptive” low-γ nuclei. The first probes built for 1.5 GHz Bruker Avance spectrometer include 2.0-mm triple-resonance ¹HXY CPMAS probe, a ¹HX static probe for materials and aligned samples, and 3.2-mm MAS probe for low-γ and quadrupolar nuclei. We will report NMR spectra, B₁ homogeneity and power efficiencies of NMR probes.

Both 2.0 mm ¹HXY MAS and static probes are based on Low-E coils design.²,³ Separating high- and low-frequency circuits made it easier to tune both probes to high ¹H frequency while affording larger sample size. The triple-resonance ¹HXY probe is designed around tune cards⁴ – the two vertical boards shown in left photo that slide out sideways – for quick change of X and Y channel nuclei. The static probehead, in the middle, features modular slide-in RF coils to accomodate different sample shapes and sizes.

The 1.5 GHz magnet pairs superconducting outsert with resistive DC insert in series.¹ Active field-regulation is used to compensate B₀ fluctuations and enables signal average and 2D NMR experiments. Each probe has an inductive sensor tracking B₀ field and driving outer correction coil to cancel 60 Hz fluctuation and its harmonics. An external ⁷Li NMR frequency lock is provided to correct slower B₀ drift. A 750mM MnCl₂-doped LiCl solution capillary placed in the vicinity of main rf coil is used for the lock. Combination of active shims and thin passive ferroshims achieves spatial B₀ inhomogeneity of <1 ppm over 1 cm³ DSV.

References

HOMOLOGOUS BUT MECHANISTICALLY DIFFERENT: HOST DEFENSE PEPTIDES PISCIDIN 1 AND PISCIDIN 3 HAVE CONTRASTED DISRUPTIVE EFFECTS ON MEMBRANES AND DNA

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Piscidin 1 (P1) and piscidin 3 (P3) are efficacious host-defense peptides (HDPs) active against antibiotic-resistant bacteria, and thus important for designing novel antimicrobial therapeutics. Both peptides are positively charged and attracted to the anionic surface of bacterial cell membranes. Both peptides also have cell penetrating properties and co-localize with intracellular DNA following translocation. While they are both highly potent and share structures bound to model membranes, only P1 is strongly membrane active. We thus postulated that P3 acts through an alternative mechanism that involves DNA disruption. Here, we report on comparative studies of P1 and P3 on lipid bilayers and DNA, and demonstrate that their Amino Terminal Cu and Ni (ATCUN) binding motif is implicated in covalently damaging their targets through oxidative stress.

We employ a range of biophysical and biochemical methods, including solid-state NMR, oriented CD, solution NMR, neutron diffraction with deuterium labeling, X-ray diffraction, surface plasmon resonance, microscopy, and activity assays. Through biological assays, we demonstrate that P3 has stronger neutralizing and condensing (aggregating) effects on DNA than P1. Remarkably, only P3 cleaves the DNA of bacteria in a Cu²⁺-dependent manner and achieves fast nuclease activity rate on isolated DNA. Furthermore, while P1 is generally more active than P1 on planktonic bacteria, the nuclease activity phenotype of P3 gives it an edge on biofilms and persister cells. Using solid-state NMR, we investigate the structures of the peptides in complex with aggregated DNA. We demonstrate that both peptides adopt an α-helical structure in the presence of DNA and metal ions, and molecular dynamics show that P3 experiences more extensive intermolecular bonding than P3 (Figure 1). Bound to bacterial model membranes at a peptide-to-lipid (P/L) of 1:25, both P1 and P3 are α-helical as demonstrated by solid-state NMR. P1 induces maximum thinning of the membrane and significant permeabilization, as measured by X-ray diffraction and calcein release assays, respectively. Using neutron diffraction, we show that it inserts through two-thirds of the membrane and achieves a major structural rearrangement of the phospholipid headgroups by dragging them inside the bilayer. In contrast, P3 is surface bound at the same P/L. Overall, these findings demonstrate that homologous peptides with similar structures can have contrasted mechanistic strategies and nuclease activity is a highly effective way to attack modes of bacterial growth. The new knowledge gained from this research could be useful to the design of novel therapeutics to fight drug resistant bacteria, including resilient biofilms and persisters.

**Figure 1.** REDOR solid-state NMR ¹³C/¹⁵N distance measurements in the ATCUN motif of P1 (left) and P3 (right) bound to condensed DNA (gray; orange) or to DNA and Ni²⁺ (blue; red). Distances were measured between the ¹³C of the carbonyl at position 2 and the ¹⁵N of the amide at position 6 of P1 and P3 bound to duplex DNA (AAATACACTTTTGGT). The distances of about 4 Å in the peptide-DNA complexes are consistent with folding of the ATCUN motif into a helical structure and conservation of this structure when the metal is present. Hydrated samples were prepared in buffer at pH 7.4 and studied at 280 K on the NHMFL 800 MHz Bruker spectrometer.
In light of the antibiotics crisis caused by the rapid development of bacterial resistance to available medication, non-soluble proteins attached to, or part of membranes are emerging as promising drug targets. Knowing their atomic structure allows for the design of specifically tailored drugs that promise fewer side effects and longer perseverance in terms of the development of new resistance.

Unfortunately, there are very few methods that can study unsoluble proteins at atomic detail. Among them is solid-state NMR spectroscopy. Its major limitations are its inherently low sensitivity and the spectral crowding that occurs for proteins with a large number of amino acids. Both shortcomings have recently been mitigated by the introduction of proton detection in conjunction with ultra-fast Magic Angle Spinning (MAS). This technique has higher sensitivity and provides another chemical shift dimension (protons) facilitating the analysis of larger protein (sub)units (see Fig. 1 for an example of spectral quality).

![Fig. 1. 2D projection along the proton dimension of a 3D (H)CACO(N)H NMR spectrum of $^{2}H,^{13}C,^{15}N$-labeled MxiH needles. Acquired at 40 kHz MAS in a 900 MHz NMR spectrometer. Note the single-residue resolution.](image)

Until now, this methodology is only available in a few laboratories worldwide, as it requires spectroscopists already familiar with the technique because the corresponding literature is widely scattered and not intended for non-specialists. In this work, we present a detailed step-by-step protocol [1] that explains the set-up and acquisition of five proton-detected three-dimensional NMR experiments needed for the chemical shift assignment of the backbone of a deuterated protein. It was specifically written to be easy to use and is abundantly illustrated. Parameters for all experiments and corresponding pulse sequences are delivered with guidelines concerning common problems and troubleshooting. We believe this will make state-of-the-art solid-state NMR spectroscopy available to a broader audience.

References

Solid-state NMR provides evidence for small-amplitude slow domain motions in a multi-spanning transmembrane α-helical protein

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We use solid-state NMR relaxation rate and dipolar recoupling measurements to probe the backbone motions of the membrane protein Anabaena Sensory Rhodopsin (ASR). ASR is a seven transmembrane (7TM) alpha helical photosensor which interacts with its cytoplasmic transducer (ASRT) in a light-dependent manner. An absorpion of light results in the release of ASRT, which interacts with DNA, and likely regulates the expression of genes of several light-harvesting proteins[1]. Here we report $^{15}$N $R_1$ and $R_{1ρ}$ relaxation rates measured at two temperatures (7 °C and 30 °C) and at two magnetic fields (600MHz and 800MHz $^1$H frequency). We observed an order of magnitude variation in the $R_1$ and $R_{1ρ}$ relaxation rates between the transmembrane (TM) regions and the interhelical loop regions of ASR. Qualitative analysis of the field and temperature dependences of the $R_1$ and $R_{1ρ}$ relaxation times shows evidence of motions on two timescales, which we modeled as fast local motions and slower (nanosecond) collective motions. Combining relaxation data sets with the order parameters from dipolar recoupling experiments [2] we estimated the amplitudes and time scales of the two types of motions. Fast local motions occurring on the picosecond timescale were found to be of similar amplitude throughout the protein. In contrast, slow nanosecond collective motions were found to be of small amplitude in the center of the TM helices, and increasing towards the cytoplasmic end of the TM helices and in the interhelical loop regions. Larger amplitudes of motions on the cytoplasmic side of helices correlates with the ability of ASR to undergo large conformational changes in the process of binding/unbinding ASRT.

Fig. 1. Top: Structure of ASR monomer (PDB ID: _2M3G). Bottom: $^{15}$N $R_1$ relaxation erates, measured at 7 °C and 30 °C as a function of residue number. The secondary structure of ASR is shown on top. The regions of higher $R_1$ relaxation rate are indicated by the arrows.

References
Solid-State NMR mobility studies of Cellular Prion Protein in complex with Amyloid-B Oligomers

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With the emergence of a large aging population, it is becoming critical that we understand the pathology of neurodegenerative diseases on a structural and functional basis. Many of these neurodegenerative diseases, such as Alzheimer’s disease, have significant societal impacts. For example, AD is the only top ten leading cause of death in the United States that cannot be prevented, cured, slowed down, or diagnosed before symptoms are present. However, despite intense research efforts into AD, the mechanism by which the disease proliferates is still poorly understood, hindering the efforts to find preventative treatments or cures.

Recently, research has indicated that amyloid-B (AB) oligomers have a specific binding affinity to cellular prion protein (PrP⁰)¹. Consequently, we have conducted several studies into the toxicity of this interaction and have found that these proteins form an insoluble complex in vitro which leads to cell death and cognitive impairment in AD mice models²,³. It is therefore critical that we gain an understanding of the mechanism that drives the formation of this complex.

In this study, we apply solid-state NMR techniques to examine the mobility of the proteins in the complex. The goal of the study is twofold: (1) to gain insight into how the complex may be formed in vivo and (2) to determine if mobility or structural perturbations are important for toxicity. Here, we show solid-state NMR experiments performed on both a sample of N-terminal PrP construct with residues 23-111 (which have been shown to be critical in binding) complexed with ABo, and a sample of full-length PrP⁰ complexed with Abo. Comparisons of the cross-polarization based experiments to directly polarized experiments conducted on both samples, show that the two variations of PrP have differing levels of mobility upon complexation with Abo. These experiments, in combination with multidimensional experiments, may provide key insight towards understanding the importance of the PrP interaction with Abo in AD.

References

**In Vitro NMR Study of Proteoglycan Depletion in Articular Cartilage**

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Articular cartilage (AC) tissue is a smooth, connective tissue made up mainly of collagen, proteoglycans and water. AC is a complex tissue that works as a shock absorber and provides smooth surfaces for articulating joints. Unlike most tissues, AC has limited ability to repair itself due to the presence of only a relatively small number of chondrocytes (~5% by volume). Chondrocytes are the only cells found in cartilage and are responsible for maintaining the cartilage matrix (collagen fibers and proteoglycans (PG)). With a view towards development of effective treatment protocols for osteoarthritis (OA), schemes for the early detection of OA are being sought after. Ideally such schemes should be noninvasive. Considerable literature exists on the development of early OA detection schemes using noninvasive Magnetic Resonance Imaging (MRI) techniques.

Loss of PG is an important indicator of early OA. An ideal MRI scheme for OA detection can be envisioned to involve the direct detection of the degradation of PG with progression of the disease, while at the same time monitoring the collagen content, which is not significantly affected by early OA and can be used as internal reference. In this study, first, high field (Larmor frequency of 500 MHz) Magic Angle Spinning (MAS) NMR was used to monitor the changes in the PG spectra of the articular cartilage with dehydration, and with PG-degradation (using trypsin). Second, articular cartilage samples were modified enzymatically to achieve different PG-depletion levels using the trypsin enzyme and then chemical shift imaging (CSI) was used to obtain the PG spectra and a static spectral experiment was performed to measure the broad collagen spectra. The ratio of the PG spectral area to that of the collagen spectral area defines PG content relative to collagen content. As the collagen content does not change appreciably for early OA, this approach provides a relative PG content, which is expected to be useful for in vivo OA detection.

A key aspect of early OA detection is sensitivity. Typical relaxation approaches do not monitor PG directly, but rather monitor changes in PG (degradation), with OA progression, indirectly by measuring changes in relaxation parameters of water protons. Here a technique that determines the PG/collagen ratio from a direct measurement of PG in cartilage and relating the PG content to collagen content as an internal reference is proposed [1].

**References**

STRUCTURAL ANALYSIS OF RETINAL-BINDING POCKET OF KR2 UNDER NEUTRAL AND ACIDIC PH BY SOLID-STATE NMR

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Retinal-binding pocket of rhodopsin protein holds key residues and interactions for its function. Krokinobacter rhodopsin 2 (KR2) is one of the attracting target since this is the first example of sodium ion pumping rhodopsin (NaR) which crystal structure is resolved. [1, 2]. NaRs have counterion of protonated Schiff base (SB) at unique but conserved position. Crystallography showed two conformations of counterion, Asp116, at neutral and acidic pH suggesting that protonated and deprotonated counterion changes the interacting pair. This result indicates how the electrostatic barrier is removed around protonated Schiff base to transport the cation. Thus, observation of NMR signals of retinal-binding pocket deepen the understanding of interaction and functional correlation.

Here, we present solid-state NMR results of [14, 20-13C]Retinal, [Phenyl-4-13C]Tyr, [6-13C]/[7-15N]Lys-labeled wild-type (WT) KR2 and D116N mutant samples in POPE/POPG membrane at pH 8.0, 6.0, and 4.0 to understand the structural difference of retinal-binding pocket in acidic and neutral condition. We applied 13C and 15N CP-MAS and 13C-13C DARR 2D experiments [3]. Deprotonation and structural information of two Asp residues near SB were indirectly observed by the signal change of SB and Tyr218 interacting with Asp116 and Asp251, respectively.

From 13C-13C DARR and 13C and 15N CP-MAS spectra, we assigned the chemical shifts of Retinal C20, Retinal C14, Tyr218Cζ, Lys255Ce, Lys255Nζ at each pH. Firstly, 15N NMR signal of protonated SB in WT KR2 at pH 8.0 showed sharp peak at 150.2 ppm and correlation between 15N SB chemical shift and maximum absorption wavelength indicated the existence of torsion around N-Cε at neutral condition compared to BR. This is caused by the unique location of counterion, which is one helical pitch away towards the cytoplasmic side. Change in chemical shift of Lys255Nζ at pH 8.0, 6.0, 5.0, 4.0, and 3.0 was largest between pH 6.0 and 5.0, thus, it is presumed that Asp116 at pH 4.0 sufficiently forms a protonated state and this was confirmed with D116N mutant. This data indicated that there is a change in Asp116-SB interaction and reorientation at lower pH on WT KR2. Secondly, 13C-13C DARR revealed the chemical shift of Tyr218Cζ which is sensitive to the hydrogen bonding strength. 13Cζ NMR signals of Tyr218 in WT and D116N mutant are at 155.8 ppm showing that Tyr218 forms a weak hydrogen bond with Asp251 at dark state compared to BR. This supports the Asp251 function as a temporal binding site of Na+ during Na+ pumping process. The chemical shift value did not change between neutral and acidic pH, therefore, suggesting that Tyr218 is interacting with Asp251 at both pH and pKa of Asp251 is lower than 4.0.

References
Solution and Solid-State NMR Investigations into the Complexation of Cellular Prion Protein and Amyloid-β Oligomers

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Protein aggregation is implicated across a wide spectrum of neurodegenerative diseases, including Alzheimer’s (AD)(1), Parkinson’s(2), and Prion diseases(3) (such as Creutzfeldt-Jakob (CJD) and bovine spongiform encephalopathy (BSE)). AD, in particular, is the only top 10 cause of death in America that cannot be prevented, cured, or slowed and can typically only be diagnosed based on patient symptoms(4). It has been shown that the associated protein aggregates in neurodegenerative diseases have specific structures that has been hypothesized to be important for their pathogenicity and for their proliferation via a poorly understood prion-like mechanism(5). These insoluble aggregates have proven difficult to study structurally, and solid-state NMR (ssNMR) spectroscopy has proven to be the most reliable method to give atomic-level structural information of these protein aggregates(6-9). These ssNMR studies have shown that conformational changes in these aggregates correlate directly with changes in the pathology of the disease.

In this study we show solution and ssNMR spectra acquired on soluble and insoluble complexes of PrPC and Aβ0 where, using the 13C chemical shifts for PrPC(23-230) from solution NMR studies(10) and previous chemical shift assignments from solution NMR studies of PrPC, we were able to predict the expected cross peaks for PrP(23-111). We then compare these shifts with ssNMR spectra and, with several important exceptions, nearly every residue in the PrP(23-111) Aβ0 globuomer complex is nearly indistinguishable by 13C NMR when compared to the expected solution shifts, suggesting only local changes in PrP to residues proximal to the interaction interface. We also compare the chemical shifts of full length PrPC in the complex to those from solution NMR. Of particular interest are several chemical perturbations suggest a binding interface between the Aβ0-PrPC complex that is consistent with mutagenesis studies, suggesting interactions in this aggregate. We envision that these results combined with future experiments will lead to a greater understanding of the structure and formation of this complex and aid in the design of compounds that could be used to rescue cognitive performance in AD.

References

MOLECULAR GEARS -- HEXAMETHYLBENZENE

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The 1H NMR spectrum of hexamethylbenzene (Fig. 1) orientationally ordered in the nematic liquid crystal ZLI-1132 is analysed using covariance matrix adaptation evolution strategy (CMA-ES). The spectrum contains over 350,000 lines with many overlapping transitions, from which four independent direct dipolar couplings are obtained. The rotations of the six methyl groups appear to be correlated due to mutual steric hindrance. Adjacent methyl groups show counter-rotating or geared motion. Hexamethylbenzene thus behaves as a molecular hexagonal gear [1].

Fig. 1. Experimental and fitted NMR spectra of hmb in 1132 at 298 K. a: Experimental spectrum (1742 scans). The line broadening applied before Fourier transform is 0.1 Hz. The experimental line full width at half height is of order 6 Hz. The program LEQUOR was used to calculate the simulated NMR spectra. In order to deal with the broad underlying liquid-crystal NMR signal in the experimental spectrum and to improve the dynamics in the spectra, a background signal averaged over 274 Hz is subtracted from both the experimental and calculated spectra depicted in b through e [2]. Here b and d are the full and zoomed in experimental spectra, while c and e are the corresponding calculated ones. The blowups d and e show the excellent fit obtained. The arrow in d points to the central line of the tcb triplet. Reproduced with permission from reference [1].

References


In and ex situ $^7$Li NMR observation of plating and relaxation of metallic lithium in graphite electrodes at 0 °C

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Graphite is one of the most widely used materials for the negative electrode in lithium-ion batteries. By charging, in addition to lithium intercalation into graphite, metallic lithium deposition (plating) can occur when the amount of reduced lithium exceeds the limit of LiC$_6$. Moreover, lithium plating can also occur in a non-equilibrium manner when there is reaction distribution in the graphite electrode. $^7$Li NMR is well suited for in situ examination of metal plating as the metallic $^7$Li NMR signal appearing at ca. 265 ppm is isolated from the other $^7$Li signals of electrodes/electrolytes. In this study, lithium plating in LiCoO$_2$/graphite cells is studied at 0 °C by direct in situ observation of $^7$Li NMR. Examination of the $^7$Li NMR signals of metallic lithium, the graphite intercalation compounds (LiC$_x$, $x = 6$ and 12), the electrolyte (LiPF$_6$), and LiCoO$_2$ reveals that, at high charging rates over 0.5 C, the amount of the lithium metal signal increases with the charging rate. We show that lithium metal deposition slows down or almost ceases at the last stage of the constant-voltage charging with slow relaxation of lithium inhomogeneous distribution among different stages. During the rest period, the observed $^7$Li NMR spectral change indicates that the deposited metallic lithium is used for lithiation of the low-stage compounds. At the end of discharging, a small metallic signal still remains. Further, ex situ examination of the Li re-distribution among the LiC$_x$ structures (structural relaxation) by using 2D-exchange NMR is presented.

Fig. 1. In situ $^7$Li NMR spectra observed at 0 °C with the charging rate of 1 C during the charging process. Red and blue lines are corresponding to first and last spectrum respectively. The other spectra are drawn in gray lines.

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PROBING IONS DYNAMICS IN METHYLAMMONIUM LEAD IODIDE BY MULTINUCLEAR SOLID STATE NMR AND $^{127}$I NQR.

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Hybrid organic-inorganic perovskites such as methylammonium lead halides demonstrate great potential as an effective and inexpensive material for solar cells, resulting in an exponential rise of their studies in recent years. Even though the original reports on the synthesis and first NMR studies go back well over 30 years, characterization of their semiconducting properties and application in optoelectronic devices is much more modern[4-6]. Despite the great effort made in characterization of these compounds, however, a significant portion of the physics and chemistry underlying these materials properties is still not well understood.

In this contribution we analyze methylammonium lead iodide (CH$_3$NH$_3$PbI$_3$), the most studied compound of the halide perovskite family, by means of $^1$H, $^{13}$C, $^{14,15}$N, $^{127}$I and $^{207}$Pb solid state NMR, complemented with the results of $^{127}$I NQR spectroscopy. The goal of our study is to probe the short-range ion dynamics in MAPbI$_3$, with a particular attention to the ions mobility and their potential as possible charge carriers.

Our $^1$H, $^{14}$N, $^{13}$C spectroscopic and relaxation time measurements focus on the rotational motion of the MA cation in different crystal phases, and have conclusively demonstrated several rotational modes of the cation depending on the in the crystal phase in a complete absence of its translational motion. $^{207}$Pb solid state NMR spectra and relaxation time measurements indicate a very low ions’ mobility and a strong spin-coupling to the neighboring $^{127}$I ions. The detection of $^{127}$I NMR is the most challenging, mainly due to a very high quadrupolar coupling constant of 550 MHz and a very short spin-spin relaxation time on an order of 10 $\mu$s. In this situation the $^{127}$I NQR becomes a more suitable technique for studying this system. Variable temperature NQR spectra allow for accurate detection of the phase transitions, while the NQR spin-lattice relaxation time measurements together with the resonance frequency dependence offer an additional insight into the dynamics of iodine ions in the lattice.

Together with the results from the tracer diffusion and doping experiments, stoichiometric variation, conductivity and polarization experiments, iodine ions are shown to be the mobile species in CH$_3$NH$_3$PbI$_3$, with iodine vacancies to represent the mechanistic centers under equilibrium conditions.

References
Graphite intercalation compounds (GICs) form when graphite is reduced or oxidized, and cations or anions, respectively, intercalate between the graphene layers. This intercalation chemistry is important for electrochemical energy storage in lithium ion batteries (LIBs) and sodium ion batteries (NIBs). Although most of LIBs include graphite as active material in negative electrode, graphite had not been considered as anodes of sodium ion battery because low-stage secondary GIC with Na (NaC x) don't form electrochemically [1]. However, Jache et al. [2] and Kim et al. [3] recently reported superior cycle life for NIBs by making use of co-intercalation of sodium and diethylene glycol dimethyl ether (diglyme) into graphite. The possibility of the use of graphite to NIB's electrode is largely expected, but the structure of the ternary GICs has not been understood. Our group synthesized ternary GICs consisting of sodium, deuterated monoglyme(d10) or diglyme(d14), and graphite by a solution reaction, and investigated the dynamics and the coordination structure of molecules using 2H solid state NMR. We also inspected the dynamics of crown ether (15-crown-5) in Na-15C5-GIC by 1H NMR.

Na-diglyme(d14)-GIC and Na-monoglyme(d10)-GIC samples were prepared referring our previous 2H NMR research of Na-ethylenediamine(d4)-GIC and Na-tetrahydrofuran (d8)-GIC [4][5]. Na-15C5-GIC was prepared by the similar procedure to the previous report [6]. Li-diglyme(d14)-GIC was also synthesized for comparison. 2H NMR spectra (45.3 MHz) were obtained from 123 K to ambient temperature using a quadrupole echo with 2.8 μs pulse. 1H NMR (200 MHz) was taken from 140 K to ambient temperature using a single pulse sequence.

2H NMR spectra obtained for the Na-diglyme (d14)-GIC include two different broad powder patterns with qcc =165 kHz and 50 kHz under 233 K. Since the former corresponds to rigid deuterated methylene groups (-CD2-, 167 kHz) and the latter is assignable to rotating methyl groups (-CD3, 50 kHz), diglyme molecules should coordinate rigidly to sodium ions at low temperature. At ambient temperature, at least three components with different qcc were observed. Diglyme's fluctuation motions are activated, which enable sodium-diglyme complexes to diffuse between carbon layers. Monoglyme in GIC showed similar behavior at 93 K, and two components of qcc = 17 kHz and 0 kHz appeared at ambient temperature. Detailed simulation of 2H NMR spectra and probable structures of monoglyme molecules and crown-ether molecules in the GICs will be discussed in the presentation.

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References
Spectroscopic Analyses of Nano-meter Scale Heterogeneity in PEG-based Polyacrylate Gel

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Due to its biocompatibility, PEG-based polyacrylate material has been widely used in the field like biomedical coating and tissue engineering. On the other hand, UV-curing has been extensively implemented to produce these materials in large scale in industry partially because of its easy applicability. However, the rather uncontrollable radical polymerization often results in a heterogeneous product with complicated morphological property. These distributed microstructures traditionally are probed by atomic force microscopy (AFM), electron microscopy (EM) and X-ray and neutron diffraction. However, solid state NMR spin diffusion experiments open up a new possibility in this area with its unique feature which does not require any sample preparation like staining.

In this study, we focused on using various spectroscopic techniques to probe the ‘nanogel’ formation during the curing procedure (see Fig. 1). As suggested by previous AFM¹ and SAXS analyses², a two–phase-system was formed during the curing stage, and this two-phase system would gradually disappear when the curing reaction completed.

![Fig. 2](image)

Through analyzing the domain size with three different techniques (AFM, SAXS and NMR spin diffusion analyses), the ‘nanogel’ formation is discussed under the context of changing swelling degree and sample curing degree. We found a gradually growing ‘nanogel’ structure in dry sample with comparable domain size from all the three techniques when the degree of double bond conversion is increasing in the sample.

References

Solid State NMR for Investigating the Sodium-Air Battery

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Metal-air batteries are currently being investigated as the most promising replacement for the Lithium-Ion (Li-Ion) cell. Sodium-air (Na-air) cells in particular are examined due to high coulombic efficiency stemming from a low over-potential on charge when sodium superoxide (NaO₂) is selectively formed on discharge.¹ However, NaO₂ is thermally unstable and requires a precise environment to form, and may degrade parasitically in reactions that hinder recyclability.¹ Examination of electrochemical products and their mechanism of formation is imperative to qualify improvements to the Na-air system.

Solid State ²³Na magic angle spinning (MAS) NMR is shown to be an effective tool in the investigation of the Na-air system at fields as high as 19.9 T and at spinning rates up to 40 kHz. Reference NaO₂ is synthesized and a slurry of each of the expected electrochemical products [NaO₂, sodium peroxide (Na₂O₂), and parasitic sodium carbonate (Na₂CO₃)] is examined. Each ²³Na environment is separated by high resolution 2-D multiple quantum MAS (MQMAS) experiments, the technique is then applied to a discharged carbon cathode (figure 1b).²

Figure 1: a) ²³Na spectrum of cathodes discharged to different capacities. D100 represents a cell Discharged to a capacity of 100 mAh/g. b) ²³Na 3QMAS spectrum of D750 cathode. Spectra collected at 11.7 T with 20 kHz MAS.

Studies of cycled carbon-based cathodes show that the formed NaO₂ reacts with polyvinylidene fluoride (PVDF), a universally used cathode binding material, to produce sodium fluoride (NaF). It is also found that NaO₂ will react with the backbone carbon of the cathode to form Na₂CO₃ (figure 1a).²

Binder free carbon cathodes are examined in an attempt to improve the stability of NaO₂. It is found through time dependant NMR that formation of NaO₂ is improved but will still degrade to Na₂CO₃ over time. Oxidized analogs of these cathodes show a new electrochemical product believed to be sodium peroxide dihydrate (NaO₂*2H₂O).³ NaO₂*2H₂O is synthesized and characterized by 2-D solid state HMQC, and this technique is then applied to the cycled cathodes of the Na-air cell.

References
23Na solid state NMR analysis of sodium inserted in nanopore of hard carbon anode for sodium ion battery

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Lithium ion batteries (LIBs) are used in various portable electric device owing to their large power and energy density. However, lithium resource is limited and locally distributed. Sodium ion batteries (NIBs), which sodium is used instead of lithium, are studied for the alternative. Hard carbon is one of the most promising materials for active material of negative electrodes because it stably works and keeps large capacity. It is proposed that sodium is adsorbed in the defective site or is intercalated between graphene layers. Recently, we reported the state of sodium in hard carbon prepared at 700~2000 °C using 23Na NMR, and discussed why the adequate heat treat temperature of hard carbon for high capacity sodium storage is higher than the temperature for lithium storage [1]. In this study, we focused on the correlation between the pore size in hard carbon and the state of the inserted sodium.

Hard carbon was prepared at 1300 and 1600 °C from sucrose. Pore size was investigated by small angle X-ray scattering (SAXS). Sodium was inserted into hard carbon electrochemically using two electrode type cell (Hard carbon / Na metal / 1 M NaPF6 in EC : DEC 1:1 + 2 % FEC). The state of sodium in sodiated hard carbon sample was investigated by solid state 23Na MAS NMR using 3.2 mmφ sample rotor.

SAXS patterns indicated that hard carbon carbonized at 1600 °C includes closed pores larger than those of the hard carbon carbonized at 1300 °C. In 23Na MAS NMR of each sodiated sample was observed quasi-metallic sodium signal, which was recently reported by Stratford et al.[2]. NMR signals showed that the state of sodium in hard carbon treated at 1600 °C was more metallic than at 1300 °C. It was revealed that the larger pore can include the more metallic sodium cluster in hard carbon.

References
Follow-up of the hydration of a ternary binder based on Portland cement, Calcium aluminate cement and calcium sulfate using High Resolution $^{27}$Al Solid-State NMR

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The most widely used hydraulic binder in industrial mortars is Portland cement. However, the current trend is to replace it, at least in part, with other binders with less environmental impact as for example calcium aluminate cement. During hydration, these high alumina systems offer short- and long-term properties that are largely governed by the evolution of the hydrated or anhydrous aluminate phases. The resolution and the sensibility obtained by combination of high magnetic field strength (20 T) and high MAS spinning speeds provide unique opportunities to monitor the structural changes occurring during the main periods of hydration.

In this work, we present a quantitative analyses of the aluminum environments modification during the hydration of a ternary binder based on Portland aluminate cement (PAC), Calcium aluminate cement (CAC) and calcium sulfate. High Resolution $^{27}$Al Solid-State MAS and MQ-MAS NMR spectra have been acquired at significant hydration milestones, from the early stage (few hours) to the long ages (few months). This characterization has been completed by $^{29}$Si and $^1$H-$^{29}$Si CP-MAS analyses and correlated with DRX measurements.
Solid-State NMR Investigation of Next-Generation Energy Materials

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Organic-inorganic lead halide perovskite materials have drawn a great interest over the last decade due to their low-cost applications in solar cells and in optoelectronic devices. Here we present the results of a solid-state nuclear magnetic resonance (NMR) investigation of organic-inorganic formamidinium lead halide (FAPbX3, X = Cl, Br, I) materials and on mixed-halide analogues; the latter are well known for moisture stability and band gap tenability. In addition, we show that 207Pb NMR can distinguish between the α- and δ-phase of FAPbI3. The chemical shifts depend directly on the local Pb environment and on halogen electronegativity. We observed that 207Pb NMR peaks are shifted towards the higher resonance frequency and are broader on halide substitution from X = Cl to Br to I in FAPbX3. The 207Pb T1 values increase with increasing size of the halides from chloride (T1 = 1.53 s) to bromide (T1 = 1.85 s) to iodide (T1 = 1.94 s for the α-phase, T1 = 1.88 s for δ-phase), suggesting that the lead-halogen orbital interaction is greater in heavier halogens. A chemical shift anisotropy (CSA) powder pattern is observed for the 207Pb spectrum of the δ-Phase of FAPbI3 acquired at 11.75 T. Properties of mixed halide perovskites, of homogeneous and heterogeneous substitutions will also be shown.

References
Optimizing $^{13}$C and $^{31}$P Spin Diffusion in Materials: R$^2$ in Compressed Benzene and MOFs

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Spin diffusion among low-$\gamma$ spin-1/2 nuclei ($^{13}$C, $^{31}$P, etc.), based on rotational resonance (R$^2$) [1], dipolar-assisted rotational resonance (DARR), etc., has experienced an enormous development in the last two decades, in particular in biomolecular NMR. We present two interesting applications of R$^2$ to complex materials. In the first example, R$^2$ was applied to a polymer synthesized from $^{13}$C-enriched compressed benzene. Spin diffusion rates were optimized by heteronuclear dipolar decoupling (Fig. 1B), so that different structural models could be differentiated (Fig. 1C). In metal-organic frameworks (MOFs) containing multiple $^{31}$P sites, R$^2$ accelerated the spin diffusion between $^{31}$P sites 50-fold and made long-range (inter-linker) exchange detectable (Fig. 2).

Fig. 1. A) $^{13}$C-13C 2D NMR of compressed $^{13}$C-benzene, at the R$^2$ condition marked by dashed lines; B) Experimental data and simulations of the decrease of the alkyl diagonal peak; C) Experimental data and simulations of the ratio of the alkyl cross peak to the residual aromatic diagonal peak.

Fig. 2. $^{31}$P-$^{31}$P spin exchange in MOF at 12.5 kHz (left, 1 s), and 8.2 kHz MAS (middle, 10 ms, and right, 3 s), with TOSS-deTOSS/TOSS (“TOSS”)[2,3] sideband suppression. The R$^2$ condition is marked by dashed lines. R$^2$ accelerated spin diffusion by at least 50 times (left vs. middle), and made long-range exchange detectable (right).

References
Using MultiCP to Determine the Purity of Chitin with Solid-State NMR

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Chitin is a versatile biopolymer which can be extracted directly from biomass, and, when pure, used for the preparation of high value materials. Establishing that proteinaceous material has been completely removed from chitin obtained from biomass sources is traditionally difficult. We report a quick, nondestructive method to measure chitin content using solid state multiCP ¹³C NMR and a calibration curve constructed from spectra of mass-based mixtures of commercial chitin and bovine serum albumin protein. The solid-state NMR method agrees with measurements made by the chemical pulping method of Black and Schwartz, within the error of each of the methods.
Zeolite Structure Determination Using Solid-State NMR Experiments through the Application of Combinatorial Tiling Theory

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Zeolites are 3D network structures which are widely used in many industrial and commercial applications because of their high porosity and channels of molecular dimensions. A subset of zeolite materials that has been increasingly investigated for their diverse functionality are layered silicates, or 2D layered zeolites, which consist of discrete zeolite-like layers. If these layers align in an ordered and periodic fashion, their crystal structure can be elucidated from powder x-ray diffraction experiments in a relatively straightforward manner. However, structure determination is often much more difficult for 2D materials in which the layers do not align in a regular ordered fashion resulting in broadened x-ray diffraction peaks [1].

We are developing an approach to solve the structures of difficult-to-characterize network materials such as layered silicates that combines solid-state NMR (ssNMR) experiments with combinatorial tiling theory. We have applied combinatorial tiling theory, which involves tiling a 2D plane or 3D space with geometric shapes, to explore zeolite-like structures in the 2D plane to gain insights to its eventual application in 3D space. The relative intensities and connectivities of atoms in these network materials determined by 1D and 2D ssNMR experiments can be used to systematically generate possible “D-symbols” which describe the connections between tiles, and thus atoms, in the plane. Each D-symbol, shown in Figure 1, uniquely encodes for a possible crystal structure and contains all the information needed to completely tile the 2D plane. After systematically determining all possible crystal structures, the most likely structure can then be identified from additional information provided by x-ray diffraction, quantum chemical calculations, and energy optimization calculations. This combinatorial tiling approach to structure determination of network materials holds promise for solving crystal structures from ssNMR experiments directly in cases where diffraction data is quite limited.

Fig. 1. A D-symbol (left) and its corresponding crystal structure (right)

References
Linear dicoordinate beryllium environments: a $^9$Be solid-state NMR study

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Beryllium-9 is a very suitable isotope for solid-state NMR studies. It is a quadrupolar nucleus ($I = 3/2$) with a relatively small quadrupole moment ($Q = 5.288 \times 10^{-30} \text{ m}^2$) and a natural abundance of 100%. However, only a few solid-state NMR analyses can be found in the literature due to the fact that beryllium is a highly toxic element, and many of its compounds are moisture sensitive and/or require very careful handling. From a spectroscopic point of view, it exhibits a small chemical shift range (< 50 ppm) and small quadrupolar coupling constants which are nevertheless strongly dependent on the local environment.

Here, we present a $^9$Be, $^{11}$B, and $^{13}$C SSNMR study of two recently reported compounds featuring linear geometry at Be: beryllium dicyclo(alkyl(amino)carbene) [Be(CAAC)$_2$, 1] [1] and a beryllium bis(diazaborolyl) compound (2) [2]. Experiments have been performed in magnetic fields of 9.4 and 21.1 T under both static and MAS conditions. Compound 1 features a closed-shell singlet configuration with the Be(0) metal center involved in strong beryllium-carbon bonds (1.664(2) Å and 1.659(5) Å), whereas the second compound features the first example of a non-cluster Be-B bond. The corresponding $^9$Be SSNMR spectra are expected to be much broader than those for tetrahedrally-coordinated $^9$Be reported in previous literature [3] since these compounds consist of a linear coordination geometry centered on the beryllium atom. For compound 2, dipolar and $J$ coupling to the boron atoms (Be-B distances of 1.870(3) Å and 1.873(3) Å) provide additional broadening mechanisms which complicate the spectral analysis.

Preliminary computational results support the above notions. For 1, DFT results lead to a $C_Q$ of 2.68 MHz, whereas for 2 we obtain a $C_Q$ of -3.66 MHz and $\eta_Q = 0.05$ ($\Omega = 122.8$ ppm and $\kappa = 0.76$). These quadrupolar constants are up to an order of magnitude larger than previously reported values for $^9$Be. Such a trend is consistent with a recent report on $^{11}$B SSNMR spectroscopy of linear two-coordinate boron compounds [4]. The quadrupolar and CSA parameters extracted from the experiments are discussed in the context of the results obtained from the DFT calculations. Despite structural similarities between the two compounds, the directly-bonded functional groups highly affect the $^9$Be signal as expected. Additional natural localized molecular orbital analyses have also been conducted to determine the main contributions from the bonding orbitals between the $s$-block element Be and the surrounding atoms to the $J$ couplings and quadrupolar coupling constants.

References


Origin of High Selectivity of P-loaded Na-ZSM-5 during the Dehydration of Lactic Acid to Acrylic Acid probed by Solid-State NMR

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The dehydration of lactic acid in the presence of water a potentially green, synthetic approach to produce acrylic acid.¹-³ We developed a highly selective dehydration catalyst based on MFI-zeolite modified with potassium phosphates. Through rigorous optimization of catalyst formulation and reaction parameters, we identified that KH₂PO₄ loaded Na-exchanged ZSM-5 catalyst can achieve >90 mol% conversion of lactic acid and ~80 mol% selectivity for acrylic acid at 330°C, which is among the best yields reported in literature. The modification with phosphates was crucial to realize a delicate balance between acidic and basic properties of successful catalysts, which minimized the otherwise dominant decarbonylation pathway on the parent acidic zeolite. The selectivity of the modified catalyst is greatly influenced by various factors including the types of zeolite and phosphate species, calcination temperature, and the loading amount of the salt. Interestingly, we made the first observation of a significant influence of SiO₂/Al₂O₃ ratio of the parent zeolite (ZSM-5) on the selectivity, which may serve as a guide to better design of zeolite-based dehydration catalyst for lactic acid. ³¹P and ⁷⁷Al solid-state NMR were utilized to understand the origin of the initial activity of constant P-loaded Na-ZSM-5 zeolites calcined at different temperatures. Additionally, ex-situ ³¹P NMR after lactic acid dehydration demonstrated that polymerization of dispersed surface phosphate species led increased acrylic acid selectivity with time. Catalytic data demonstrated that selectivity to acrylic acid increased with decreasing Si/Al ratio in Na-ZSM-5 at constant P loading, suggesting that Al-P connectivity is important. This observation prompts ⁷⁷Al-³¹P cross-polarization NMR studies in the future. NMR has been instrumental in detailing the origin of activity and deactivation in these catalytic systems since most other characterization techniques appear insensitive to the activation and deactivation causes.

Experimental

Catalyst preparation. 4.0 mmol/g_zeolite K₂HPO₄ was loaded on Na-ZSM-5 zeolite by a wet impregnation method.⁴ A required amount of the K₂HPO₄ precursor salt was dissolved in DI water, and the solution was slowly added to the zeolite drop by drop in a mortar with ample mixing. The sample was dried at RT for 2 h, followed by 393 K for 10 h, then calcined at various temperatures for 3 h under a flow (50 mL/min) of air.
Catalyst Characterization (including NMR). The surface acidity of the catalysts was quantified with NH$_3$ temperature programmed desorption (TPD) using a Micromeritics Autochem 2910 instrument. X-ray powder diffractograms were collected using a PANalytical Empyrean X-ray diffractometer (Cu K$_\alpha$ radiation). Nitrogen adsorption-desorption measurement was performed using Micromeritics Gemini V at 77 K after degassing the sample at 200°C for 8 h under vacuum ($\sim$10$^{-2}$ Torr), and Brunauer–Emmett–Teller (BET) surface areas were calculated utilizing a relative pressure (P/P$_0$) of 0.05 – 0.25.

Direct polarization (DP) magic angle spinning (MAS) spectra were acquired with a Bruker AVANCE™ 300 (Bruker Biospin, Billerica, MA) with 1H operational frequency of 300.43 MHz, 121.60 MHz for $^{31}$P, 78.28 MHz for $^{27}$Al, and equipped with a 4-mm wide-bore H/X CPMAS probe. Samples were packed into 4-mm zirconia rotors with Kel-F caps and spun at 10 kHz. $^{31}$P spectra were reference to Na$_2$HPO$_4$ at 5.5 ppm (Conte et al. Eur. J. Soil Sci. 2008, 59, 584) and $^{27}$Al to AlCl$_3$$\cdot$6H$_2$O at 0 ppm. Typical acquisition parameters for $^{31}$P MAS spectra were: Bruker’s pulse program “hpdec”, 4k points with acquisition time of 20.5 ms and spectral width of 60 kHz, relaxation delay of 10 sec., 100-500 scans, and $^{31}$P 90° pulse of 35 μs at an attenuation level of 4 dB. Typical acquisition parameters for $^{27}$Al MAS spectra were: Bruker’s pulse program “hpdec”, 2k points with acquisition time of 20.5 ms and spectral width of 50 kHz, relaxation delay of 1 sec., 2k-3k scans, and $^{27}$Al pulse of 1 μs at an attenuation level of -3 dB. All $^{31}$P and $^{27}$Al NMR data were acquired at RT with $^1$H decoupling (RF field of 62.5 kHz, SPINAL128). Bruker Topspin 1.3 software was used to acquire the spectra. MNova 10.0 software (http://mestrelab.com/) was used for processing. The NMR data (FID) were zero-filled to 128k points and multiplied by the exponential window function with a line broadening (LB) of 30 Hz prior to FT.

Catalytic testing. LA dehydration reactions were conducted in a trickle-bed Titanium tubular reactor (1/2” diameter) feeding the nominal 20 wt. % LA aqueous solution over a catalyst (1.0-1.1 grams) at a constant rate of 0.1 mL/min (0.22 mmol LA/min) with a syringe pump in a carrier flow (55 mL/min) of He at a reaction temperature of 603 K. Product analysis was performed with both gas chromatography (GC) and high-pressure liquid chromatography (HPLC). Gaseous products (i.e., CO and CO$_2$) were separated with Supelco 60/80 Carboxen-1000 packed column and analyzed on-line with an Agilent 7890A GC. Condensable products were collected with in-line condenser held at 277 K located at the bottom of the reactor after each hour of reaction, and analyzed off-line using the same GC system on an Agilent HP-FFAP column with a FID detector (more details in Supplemental Information). For accurate determination of LA and L$_2$A, a Shimadzu 10A HPLC with SPD-10A UV-Vis detector at 210 nm was employed with a Phenomenex Synergi 4 Hydro-RP column.

Results and Discussion

After impregnation, the P-loaded Na-ZSM-5 materials are calcined in air at various temperatures (473 – 673 K) for 3 h prior to catalysis. For a constant loading of P (4 mmol/g), the selectivity to the desired product, acrylic acid is optimized at a calcination temperature of 473 K (Table 1). None of the standard zeolite characterization – acid-base titration, x-ray diffraction or elemental analysis – provided any concrete insight into the origin of the optimum calcination temperature. Table 1
demonstrates that calcination at 473 K produced a catalyst that was significantly more AA selective than any other catalyst.

Table 1. Conversion of LA and molar selectivity of major products over different catalysts.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>K/P loading (mmol/g)</th>
<th>Calc. Temp. (K)</th>
<th>C-Balance (%)</th>
<th>LA Conv. (%)</th>
<th>Selectivity (%) of AD</th>
<th>23P</th>
<th>HyAce</th>
<th>PropA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 K1 Na-ZSM-5</td>
<td>4.0</td>
<td>373</td>
<td>70.7</td>
<td>83.0</td>
<td>22.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>4.0 K1 Na-ZSM-5</td>
<td>4.0</td>
<td>473</td>
<td>89.3</td>
<td>93.6</td>
<td>11.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>80.8</td>
</tr>
<tr>
<td>4.0 K1 Na-ZSM-5</td>
<td>4.0</td>
<td>573</td>
<td>89.3</td>
<td>65.8</td>
<td>14.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>74.4</td>
</tr>
<tr>
<td>4.0 K1 Na-ZSM-5</td>
<td>4.0</td>
<td>673</td>
<td>89.1</td>
<td>66.1</td>
<td>13.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>74.7</td>
</tr>
<tr>
<td>4.0 K1 Na-ZSM-5</td>
<td>4.0</td>
<td>773</td>
<td>90.8</td>
<td>46.0</td>
<td>13.7</td>
<td>1.7</td>
<td>0.6</td>
<td>0.6</td>
<td>68.1</td>
</tr>
</tbody>
</table>

AD: Acetaldehyde; 23P: 2,3-pentanedione; HyAce: Hydroxyacetone; PropA: Propionic acid; AA: Acrylic acid; Temp. 603 K.

$^{31}$P and $^{27}$Al solid-state NMR were utilized to investigate the differences between P-loaded Na-ZSM-5 calcined at different temperatures in air. Figure 1 is a stacked plot of 1D spectra for a 4.0 mmol P/g catalysts calcined at different temperatures in air for 3 h. Figure 1 demonstrates that the P precursor (KH$_2$PO$_4$) has a single resonance at 3.75 ppm and upon impregnation on Na-ZSM-5, this resonance is found downfield at 2.39 ppm with the appearance of weaker features further downfield. Upon impregnation, all peaks shift much further downfield. Only at calcination temperatures less than approximately 473 K is the original feature of the precursor retained to any significant fraction.) There are three groups of $^{31}$P signals in the calcined K1 catalysts, which were named as Q$^0$, Q$^1$ and Q$^2$. The presence of the most upfield resonance (Q$_2$, ~ -19 ppm) is indicative of a polymerized P species. As the calcination temperature increases, this resonance persists while the other resonances (Q$^0$ and Q$^1$) decrease in intensity with calcination temperature. It has been proposed the relative intensity of the Q$^0$ and Q$^1$ peaks can be used to estimate the relative length of the polyphosphate; it appears a critical length of the polyphosphate may be responsible for the high AA selectivity.

We’ve also determined that while selectivity isn’t negatively impacted with time-on-stream, the activity (i.e., lactic acid conversion) decreases with time on stream. The increasing followed invariant change in acrylic acid with time-on-stream can be rationalized by comparison of the $^{31}$P NMR spectra of the used catalyst after 4 h time-on-stream. It appears that the polyphosphate chains decrease in overall length (attenuation of Q$^2$) which leads to a greater fraction of phosphide chains.
with an optimal internal to end group ratio (i.e., $Q^1/Q^0$). $^{31}$P solid-state NMR data of the fresh and spent catalyst suggest that smaller fragments of polyphosphides are responsible for higher AA selectivity. Therefore, the optimal catalyst is formed by a mild calcination treatment which most likely leads to less rigid (lesser extent of polymerization) polyphosphate framework that can evolve during catalysis to maximize selectivity to acrylic acid.

References

STUDIES OF MOLECULAR MOTIONS AND INTERACTIONS IN IONIC LIQUIDS ELECTROLYTES BY NUCLEAR MAGNETIC RESONANCE

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Ionic liquids (ILs) are room temperature molten salts, typically with asymmetric organic cation and fluorinated anion. Thanks to their unique properties, ILs are currently used for a wide range of applications, for example their conductivity and Li diffusion properties make them very good candidates for applications linked to electrochemistry, such as electrolytes in batteries. However, the molecular interactions and motions in ILs are still not fully understood. The main goal of this research project is to study the molecular dynamics and ionic interactions in a series of Li salt-containing ammonium/phosphonium TFSI and FSI based ionic liquids by NMR, primarily using pulsed field gradient diffusion measurements and $T_1$ measurements but also using heteronuclear Overhauser effect spectroscopy (HOESY).

One goal of these studies is to improve the HOESY pulse sequence to provide gains in sensitivity and in resolution, and also to improve the way that the HOESY data are extracted and analyzed. In order to achieve this, we have written a home-made Maple code which allows consistent and automated fitting. We have also developed the fitting equation presented in Giernoth et al paper[1], by including a term accounting for the decay of the signal at long mixing times due to the diffusion of ions, caused by the gradients used during the experiments. Secondly we normalize the signals to different variables allowing different samples or concentrations to be compared. All these developments can permit us to extract more quantitative cross relaxation rates which can be combined with Molecular Dynamics simulations to correlate them with distances.

Observation of untoward $^2J_{cc}$ and $^3J_{cc}$ correlations in 1,1-ADEQUATE spectra of pyrimidine analogs: potential interpretation pitfalls

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1,1-ADEQUATE is a powerful and robust NMR experiment used to establish carbon-carbon connectivities with modest sample quantities when cryogenic probe technology is available $^{[1,2]}$. However, potential pitfalls, such as unusually large multi-bond ($^aJ_{cc}$) correlations for particular functional groups and/or weak or missing $^1J_{cc}$ correlations in strongly coupled $^{13}$C-$^{13}$C AB spin systems $^{[3]}$ are not widely appreciated. These unexpectedly large $^aJ_{cc}$ correlations can sometimes be observed in 1,1-ADEQUATE spectra with rather strong intensity leading to a structural misassignment $^{[3]}$. Responses due to anomalous $^aJ_{cc}$ correlations are intensified when the homodecoupled version of the experiment (HD-ADEQUATE $^{[1]}$) is utilized. Pyrimidines and pyridines exhibiting three-bond carbon-carbon couplings greater than 10 Hz in 1,1-ADEQUATE spectra are illustrated, with the results supported by both the experimental measurement of $^3J_{cc}$ coupling constants in question using $J$-modulated-ADEQUATE $^{[4]}$ and theoretical DFT calculations.

References

Quantitative NMR (qNMR) spectroscopy has become an important tool for the content determination of organic substances and the quantitative evaluation of impurities. Since the signal intensity is directly proportional to the number of protons contributing to the resonance, qNMR is considered as a relative primary method [1-3]. Sigma-Aldrich R&D demonstrated the validity, robustness and precision of the $^1$H qNMR technique through the optimization of High-Performance qNMR (HP-qNMR®) to its maximum level of accuracy using metrological weighing equipment and a specially designed experimental setup for the certification of organic compounds with combined, expanded uncertainties down to 0.1% [4].

The implementation of qNMR in new application fields (e.g. metabolomics, biomarker discovery, physiological pathways) brings along more complex molecules and systems, thus making the usage of $^1$H qNMR challenging. The use of other NMR active nuclei provides an elegant solution for which new qNMR standards are required. Therefore, we developed two additional classes of qNMR Certified Reference Materials (CRM), based on different NMR active nuclei. Figure 1 shows the certification concepts for $^{31}$P and $^{19}$F qNMR CRM to achieve traceability to the SI by using primary Reference Materials from the National Institute of Standards and Technology (NIST) and the National Metrology Institute of Japan (NMIJ) [5-6].

Fig. 1. Traceability schemes of newly developed $^{31}$P and $^{19}$F qNMR standards.

References
NMR Studies of Structure and Function of AIPL1 FKBP Domain

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Aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) is a specialized chaperone of phosphodiesterase 6 (PDE6), a key effector enzyme in the phototransduction cascade. AIPL1 is composed of a N-terminal FKBP-like domain and a C-terminal tetratricopeptide repeat (TPR) domain. The AIPL1 FKBP domain binds the farnesyl moiety of PDE6, while the TPR domain binds to chaperone Hsp90. Mutations in AIPL1, including many missense mutations in the FKBP domain, have been associated with Leber congenital amaurosis (LCA), an early-onset inherited retinopathy that causes severe blindness in children.

The AIPL1 FKBP domain shares a high sequence homology with the FKBP domain of aryl hydrocarbon receptor-interacting protein (AIP). Both AIP FKBP and AIPL1 FKBP do not bind FK506, lack PPIase activity, and have a long insert sequence (57 aa) replacing the hairpin loop of classical FKBP5s. However, AIPL1 FKBP binds isoprenyl moieties, while AIP FKBP does not. Moreover, AIPL1 is expressed selectively in the retina and the pineal gland. But, AIP is ubiquitously expressed and acts as a co-chaperone with Hsp90 in the maturation of the aryl hydrocarbon receptor and other nuclear receptors. Furthermore, the AIPL1 FKBP structure is not reported presently.

Here we report the NMR studies and resonance assignments of the N-terminal FKBP domain of AIPL1 in apo and in complexes with isoprenyl ligands. These results revealed the mechanism of isoprenyl binding and the function of AIPL1 FKBP.
Plant development is a process that is finely regulated by the action of various molecular mechanisms. One of these mechanisms comprises the activation or repression of the expression of certain genes, through the action of specific proteins. The GRF-GIF system is formed by two families of activators and transcriptional coactivators conserved in terrestrial plants. A range of studies have demonstrated the function of the combined action of these proteins on plant development, but there is no biophysical evidence of such interaction so far.

Here we present the first structural study of Arabidopsis thaliana GIF1. The protein shows a high degree of intrinsic disorder throughout the sequence. To perform a detailed evaluation of its structural features, we designed protein constructs with the region genetically mapped as essential for the between GRF3 and GIF1, the SNH domain. Using NMR observables we gathered information on the degree of disorder and the residual structure in this domain. This information was integrated to obtain a conformational ensemble that describes the conformational free energy surface that explores the protein. Two short helical elements appear to be stabilized, that show independent behavior. These elements could provide an interaction interface for the recognition of specific GRF transcription factors.
Signal Analysis of Magnetic Resonance Data

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Key words: NMR well logging, Magnetic Resonance Sounding, Mathematical Transforms, PDE, Statistical signal analysis (PCA, ICA) Mathematical morphology, DTI Diffusion Tensor Imaging

ABSTRACT

Nuclear Magnetic Resonance (NMR) well-logging, Magnetic Resonance Sounding (MRS), Proton Magnetic Resonance, Surface Nuclear Magnetic Resonance (SNMR), and Underground Magnetic Resonance Sounding (UMRS) Signals are analysed for precise subsurface imaging to have information of formation evaluation. Acoustic NMR, Acoustic Nuclear Magnetic Resonance for rock acoustic emission detection, EMR, Electron Magnetic Resonance, Electron Paramagnetic/ Spin Resonance (EPR/ESR) are employed for petrophysical techniques, detecting petrophysical features. Geophysical well logging signals are nonstationary in character. nmrML is an open mark-up language for NMR data. For analysis of magnetic resonance signal mathematical transforms are employed. Statistical Signal analysis of magnetic resonance data mathematical transforms are employed. Statistical Signal analysis of magnetic resonance data is quite fashionable for spectral analysis and pattern recognition. MATLAB Wavelet toolbox, SPSS, and Python (GAMMA) are employed for NMR logging signal of ODP /IODP NMR data. Nuclear magnetic resonance imaging, Combinatorial Image Analysis, splicing, patch-cutting (quilting), texture quilting are done by using Onion Peeling Algorithm-Convex Onion Peeling Genetic Algorithm (COPGA). Principal component regression or Principal Component Analysis (PCA); factor analysis; Empirical Orthogonal Function (EOF) and Independent Component Analysis (ICA) are employed for statistical signal analysis. NMR signal analysis is applied for spin echo signals representative of a transverse relaxation time (T2) distribution, or signals representative of a longitudinal relaxation time (T1) distribution. In porous media there is a significant difference in the T1 and T2 relaxation time spectra of the fluids mixture filling the pore space. The main obstacle of Nuclear Magnetic Resonance (NMR) logging instrument is its very low signal to noise ratio as well as its overlapping resonances with different transverse relaxation time (T2) values. Noise in NMR logging instrument is mainly due to thermal noise induced by the movement of charged particles in the radio frequency coils and the small anomalies in the preamplifiers. The estimations of bulk volume irreducible, permeability, and fluid type depend on the accurate measurement of the spin-spin relaxation time (T2). Thus denoising is performed to improve the signal to noise ratio of the raw logging data. Wavelet transform is an effective time-frequency representation for noise reduction. Wavelet transform (WT) is employed for resolution of an overlapping NMR spectrum. Walsh functions consist of a complete set of orthogonal functions analogous to sines and cosines. They only assume the discrete values of 1 or -1. They are step functions in nature whose abrupt changes are reminiscent of the physical property changes in layered earth formations. Therefore, Walsh functions are better suited for describing the layered formation’s petrophysical properties than, say sines and cosines functions. Partial Differential Equation (PDE) Inverse Problems and Parameter Identification In Image Processing-Inverse Problems and Parameter Identification in Geophysical Signal Processing, Many problems in imaging are actually inverse problems. One reason for this is that conditions and parameters of the physical processes underlying the actual image acquisition are usually not known. Examples for this are the inhomogeneities of the magnet field in magnetic resonance images leading to nonlinear deformations of the anatomic structures in the recorded images. Superconducting Quantum Interference Device (SQUID)-Proton Precession Magnetometer (PPM), Quarks-Quantum Chromodynamics, Spintronics, Quantum Computing (QUBIT), etc. are emerging field of research interest. Geophysical signals are multiscale and nonstationary in character. Mathematical morphology-top hat transform, fractional fourier transform,
fractional wavelet transform, partial differential equations, etc. are employed for signal processing and image processing and analysis. Nuclear magnetic resonance Diffusion Tensor Imaging (DTI) is very efficient for NMR Image analysis.

References:


MORPHOLOGICAL CHARACTERIZATION OF PROTEINS AND MEMBRANE MIMETICS
BY A COMBINED NMR-SAXS APPROACH

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Molecular shape can be estimated based on the ratio between the radius of gyration (r_G) and the hydrodynamic radius (r_H). Diffusion NMR measurements enable calculation of r_H values from the experimentally determined diffusion coefficients, while the r_G values are obtained from the fitting of the Small-angle X-ray scattering (SAXS) curves. This approach was tested on selected proteins, and the effect of various parameters (concentration, temperature, buffer) was examined in order to find the optimal conditions for performing both the NMR and the SAXS experiments on the same sample, under the same conditions.¹² On the other hand the SAXS method can provide a low-resolution structure to describe the shape of proteins independently.

The method was applied further for DHPC micelles and neutral (DHPC/DMPC, DHPC/DPPC) and negatively charged bicelles (DHPC/DMPC/DMPG). We also tested the structural changes occurring upon addition of selected model peptides: a surface bound and a transmembrane segment.³⁴

The present study demonstrates the usefulness of the NMR-SAXS approach for the characterization of bicelle morphology and can be applied for analyzing the behavior of more complex lipid mixtures.

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References

Detecting a pH-gated conformational switch of bifunctional histidine kinases by $^{19}$F NMR

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Two-component systems are widely used as predominant signaling transduction systems in prokaryotes. TCSs regulate most of the important activities in the life of bacterial pathogens, which are the potential drug targets for treating bacterial infections. Conservatively, TCS is mainly composed by a histidine kinase and a response regulator mediated by phosphotransfer. Interestingly, the histidine kinases are normally bifunctional, which have both kinase and phosphatase activities towards the cognate response regulators. During the invasion to host cells, the bacterial pathogens would undergo environmental changes to stimulate the functional switches of histidine kinase. However, the mechanisms of the conformational and functional switches of histidine kinases are poorly understood (Fig.1.A).

Through a combined NMR and crystallographic study on the histidine kinase HK853 and its cognate response regulator RR468 from Thermotoga maritima, we elucidate the mechanism of a pH-gated conformational switch of HK853 and visualize the process that shuts off its phosphatase activity under acidic conditions. Using the phosphate analog beryllium fluoride (BeF$_3$) as a probe, we successfully detected the dynamics of conformational switch of HK853$^{19}$F-BeF$_3$-RR468 complex by $^{19}$F NMR (Fig.1.B). The phosphatase inactive state of HK853 was captured in the complex, and the structural details were elucidated at high resolution by crystallography (Fig.1.C). Our results show a novel conformational switch of histidine kinase that regulates the phosphatase activity without any aid of extracellular domains, metabolic secondary messengers, or other macromolecules. Based on the structural and functional investigations, we matched the biochemical mechanisms to the biology of histidine kinases of bacterial pathogens in response of pH changes.

Fig. 1. A: The bifunctional regulation of histidine kinase in cellular response. B: $^{19}$F NMR spectra of pH-gated conformational switch. C: Crystal structure of HK853$^{19}$F-BeF$_3$-RR468 complex at pH 5.0.

References


Secondary structure probing of the Hatchet ribozyme by site-specific $^{15}$N-labeling and solution NMR-spectroscopy

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We present a fast, reliable and economic synthetic access to $^{15}$N$_1$-guanosine and $^{15}$N$_1$-adenosine, and also $^{15}$N$_3$-uridine and $^{15}$N$_3$-cytidine RNA phosphoramidite building blocks. These compounds were used for atom- and site-specific labeling of RNA constructs with sizes up to 80 nucleotides. The main focus was on folding studies of ribozyme RNAs. Novel ribozyme classes such as the hatchet ribozyme were recently reported. For some of these novel ribozymes high resolution structures are available, but for the hatchet ribozyme structural information is rare limited to a secondary structure proposal. Thus, the $^{15}$N-labeled phosphoramidites were utilized to probe secondary structure elements of the hatchet ribozyme using state of the art NMR experiments. Some of the postulated secondary structure features could be confirmed, other base pairing interactions, however, could not be observed. Currently, a rigorous NMR study is carried out to establish a full secondary structure proposal for the hatchet ribozyme, a crucial pre-requisite for a full high resolution 3D structure elucidation.
Structure determination of the locally disordered state of ubiquitin by high-pressure NMR spectroscopy

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We have demonstrated that ubiquitin populates at least two high-energy conformations: an alternatively folded conformation N\textsubscript{2} and a locally disordered conformation I between the basic folded conformation N\textsubscript{1} and the totally unfolded conformation D [1][2]. The structure of N\textsubscript{2} stabilized by Q41N mutation and pressure was previously determined by using high-pressure NMR spectroscopy [3].

Here, we report the structure and dynamics of the I-conformer of the ubiquitin Q41N mutant. \textsuperscript{1}H-\textsuperscript{15}N and \textsuperscript{1}H-\textsuperscript{13}C HSQC measurements were performed at pH 4 and 278 K up to 2.5 kbar. The increase in pressure induced a loss in crosspeak intensities for amide groups preferentially at residues 28 and 33-41. In contrast to the amide groups, crosspeak intensities for C\textalpha\textsubscript{H} and C\textbeta\textsubscript{H} groups at these sites did not decrease with increasing pressure. \textsuperscript{15}N-spin relaxation experiments (R\textsubscript{1}, R\textsubscript{2} and hNOE) were also performed at 1 bar, 1 kbar and 2.5 kbar. Although the R\textsubscript{1} values for the amide groups were quite similar among different pressures, the R\textsubscript{2} values were substantially increased at residues 33-41 with pressure. These results indicate the presence of conformational heterogeneity at these amide groups due probably to heterogeneity of hydrogen bonds with water. To obtain distance constraints between the NMR-invisible amide and any other amide groups, we measured paramagnetic relaxation enhancements (PREs) for K33C/Q41N and Q40C/Q41N mutants, in which the paramagnetic probe MTSL was covalently attached to C33 or C40. We performed structure calculations using the program CYANA with NOE- and PRE-based distance constraints and the Bayesian structure refinement. The I-conformer showed a large displacement of the C-terminal $\beta\text{c}$-strand (Fig.1), providing an open conformation at the C-terminal region of the protein. The structural characteristics of I seem to be similar to those of N\textsubscript{2}, while the amide groups of residues 33-41 might be more heterogeneously hydrated in I.

Fig.1. The structures of N\textsubscript{1} (blue, 1d3z) and I (red). A displacement of the $\beta\text{c}$-strand is marked by an arrow.

References
Hetero peptides derived from mixed α/β-peptides mimic the α-helices and inhibit protein-protein interactions, providing the impetus to investigate such ‘foldamers’. α/β Hybrid peptides from alternating C-linked-carbo-β-amino acids (β-Caa), with sugar side chains and Ala residues display a variety of helical structures. The role of side chains in stabilizing the folds in such regular 1:1 peptides containing β-Caa containing deoxy arabinose (3-deoxy-L-ara) side-chain and the D-ala was recently investigated.\(^{[1]}\) It was observed that with no substituent at C3 position in the sugar ring, very stable helices were formed. Since electrostatic and steric interactions play an important role in the stabilization of the folds, it was felt worthwhile to investigate foldamers derived from β-Caa with 1,2-dideoxy furanoside side chain (β-Caa(dd)). α/β-Peptides (1-3) containing L-Ala and β-Caa(dd) were investigated to explore the role of side chains, without an acetonide group, in their folding propensities. The penta-peptide 1, with a C-terminus ‘α-β-α’ motif, folds as a regular 11/9-helix where as the hexa-peptide 2, with ‘β-α-β’ at the C-terminus, as expected,\(^{[2]}\) spawned a 11/9-helix at the N-terminus, which nucleated a ‘turn’ at the C-terminus, thus generating a ‘helix-turn’ (HT) motif. The turn in the HT motif was stabilized by a 13/7-membered H-bonded pseudo-ring. The octa-peptide 3, has two sequential β residues, with fragments ‘α-β-α-β’ and ‘β-α-β-α’. Such a design generated a ‘helix-turn-helix’ (HTH) super-secondary structure.\(^{[2]}\) The turn was stabilized by an 11/15-membered bifurcated H-bond and a 9-membered H-bond. The peptide provided the shortest sequence for an HTH motif.

1. Boc-L-Ala-β-Caa(dd)-L-Ala-β-Caa(dd)\(\rightarrow\)L-Ala-OMe
2. Boc-L-Ala-β-Caa(dd)-L-Ala-β-Caa(dd)\(\rightarrow\)L-Ala-β-Caa(dd)-OMe
3. Boc-L-Ala-β-Caa(dd)-L-Ala-β-Caa(dd)\(\rightarrow\)β-Caa(dd)\(\rightarrow\)L-Ala-β-Caa(dd)\(\rightarrow\)L-Ala-OMe

References


SOLUTION STRUCTURE AND DYNAMICS OF TWO NEW ANTIFUNGAL PROTEINS PAFB AND NFAP

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To understand the mode of action of small antifungal proteins we investigate their structure and dynamics using solution NMR. A new member of this family is PAFB (58 aa, produced by Penicillium chrysogenum), which we show to form three disulfide bridges resulting in a greek-key supersecondary and β-barrel structure like PAF [1-2], and it has a sequence similar to pgAFP [3]. According to ESI-MS and NMR spectroscopy, two more PAFB variants were detected that are shorter at the N-terminus by one or two residues. The second antifungal protein NFAP (57 aa, produced by P.chrysogenum) [4-6] also belongs to the group of defensin-like antifungal proteins. Using standard protein NMR techniques we determined the structures of sfPAFB (short form of PAFB, 56 aa) and NFAP. The Lipari-Szabó analysis of the 15N NMR relaxation data showed that sfPAFB and NFAP are as rigid as PAF on the ps-ns timescale.

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Conformational Exchange of Dengue Virus NS2B-NS3 Protease

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Dengue virus, belonging to the Flaviviridae virus family, is a positive sense RNA virus responsible of the dengue fever, a tropical infectious disease whose incidence has increased drastically over the last decades. In the infection, the virus RNA genome is translated into a poly-protein containing structural and non-structural components. NS3 protease (NS3p) is the N-terminal domain of the non-structural protein 3. It is essential for the virus maturation and the cleavage of the poly-protein into functional individual proteins. NS3p alone is unstable and needs its cofactor NS2B for protease activity. Previous crystal structures of NS2B-NS3p from the Flaviviridae virus family suggest that the complex exists in at least two states, one “open” state corresponding to the inactive conformation and one “closed” state corresponding to the active conformation. Conformational exchange between different states in solution is evident from previous NMR studies [1, 2], but no structural information is available for the “open” state and the kinetics for the conformational exchange process is unknown.

Here we present detailed studies on the conformational exchange using relaxation dispersion (RD) and chemical exchange saturation transfer (CEST). The data show that the region of I63-K87 (β2–β4) of NS2B undergoes conformational exchange between at least two different conformational states. The major state (~96%) corresponds to the folded state or bound state, while the minor state (~4%) to an unfolded state or unbound state. The lifetime of the minor state is about 0.3 ms. Different from β2–β4, the first strand of NS2B (β1) does not undergo conformational exchange on the NMR timescale (sub-second to sub-millisecond), suggesting that the first strand always binds to NS3p. Very likely, the unbound state corresponds to the “open” conformation in which the first strand of NS2B interacts with NS3p and the rest has no interactions. Deletion of β4 in the C-terminal region of NS2B renders NS2B-NS3p inactive, demonstrating that only the “closed” conformation is active.

References
NMR-based structural characterization of the intrinsically disordered non-histone chromosomal high mobility group protein A1 (HMGA1) – Insight into the regulatory mode of action of the structural ensemble through post translational modifications

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HMGA1a (high mobility group protein A1) is a highly abundant nuclear protein, which plays a crucial role during embryogenesis, cell differentiation, neoplasia as well as more specific during chromosomal organization, protein complex assembly, and DNA-repair¹. In this study we present the first NMR-based structural ensemble of the full length HMGA1a protein. The structural ensemble is based on 545 NMR-derived restraints including backbone chemical shifts, paramagnetic relaxation enhancement, \(^3\)J couplings, and \(R_2\) relaxation experiments. The ensemble was calculated with the software package ENSEMBLE and consists of 8 assigned clusters². Our data show that the negatively charged C-terminus of HMGA1a plays a crucial role for the regulation of the structural ensemble. Several contacts between the functional AT-hooks and the C-terminus could be observed. Additionally, the C-terminus folds back on itself and neighbouring regions in multiple clusters. To investigate the impact of posttranslational modifications of the overall structure of HMGA1a we analysed the CK2-, cdc2-, and the cdc2/CK2-form in detail³. Our data show that in the wildtype ensemble contacts between phosphorylation sites could already be identified. Upon phosphorylation, alterations of connected regions could be observed, which indicates that PTM sites are part of an electrostatic contact network that can alter the structural ensemble of HMGA1a by shifting the conformational pool towards different structural clusters. Furthermore, we present the very first structural data on the first AT-hook of HMGA1a. Our data show that AT1 can adopt a transient \(\alpha\)-helical structure, which serve as an additional regulatory mechanism in HMAG1a.

Quantitation of *trans*-fatty acids by NMR

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*Trans*-fatty acids occur naturally only in small quantities in food. Their concentration can be significantly increased in processed foods as a result of partial hydrogenation of oils, which is commonly performed to, e.g., extend shelf life or change texture. Adverse effects of *trans*-fats on health such as raised LDL and lowered HDL levels and, thus, an increased risk of coronary heart disease and stroke have been reported [1]. In 2006, the FDA instituted labelling requirements for all manufactured foods to assist consumers in making informed food choices [2] since intake of *trans*-fatty acids is cumulative [3]. The presence of *trans*-fats has to be indicated if one serving contains more than 0.5g. Similar requirements exist in other countries.

Total *trans*-fat content has been analyzed by GC, RP- and silver ion-HPLC, AgNO3-TLC combined with GC, IR, and Raman or NMR spectroscopy. Official methods validated by the Association of Analytical Communities (AOAC), the American Oil Chemists’ Society, and the IUPAC focus on GC and IR (including ATR) methods [4]. High variability of these methodologies frequently makes it difficult to obtain reliable data. However, GC analysis is often time-consuming and results depend heavily on sample pretreatment and derivatization. For the purpose of obtaining the total *trans*-fat content, detailed compositional analysis as provided by GC is not necessary. Newer FT-IR and ATR methods are quick, simple and easy to use but require calibration and usually rely on a primary method such as GC. Furthermore, FT-IR procedures show a drastic decrease in reproducibility below 5% *trans*-fatty acid content.

NMR is a quantitative, non-destructive technique that does not require additional sample preparation. *1H and *13C NMR methods for *trans*-fat quantitation have been developed and their suitability as primary methods confirmed [5]. *13C NMR exhibits a wider chemical shift range where *cis*- and *trans*-fat resonances can be distinguished. Long relaxation times and low natural abundance of *13C nuclei render this technique problematic for large numbers of samples in terms of instrument time and cost. *1H NMR spectroscopy, which offers the advantages of short relaxation times and high S/N, would therefore be preferable. Unfortunately, a much narrower chemical shift range results in significant peak overlap and chemometric methods (PSL regression) need to be employed for quantitation.

Here, we investigate the use of 2D NMR for the quantification of *trans*-fatty acids by NMR. This approach combines high sensitivity through *1H detection with better dispersion of resonances than in *13C alone. Results are compared with standard AOAC ATR-methods. We also investigated if quantitation by NMR allows for reproducibly lower detection limits (<5%).

References
New Long-acting Recombinant Human Insulin Analog
A22S-B3K-B31R

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Antidiabetic last generation drugs are becoming more efficient but the administration of human insulin or its various derivatives obtained by rationally designed sequence mutations are still the most effective way of pharmacotherapy in cases of extremely high hyperglycemia. There are known commercial preparations containing modified human insulins with long-acting or rapid-acting properties but there is still need to obtain new human insulin analogues for better activity profile and easier treatment in diabetics therapy.

As a result of our earlier studies in this area we have designed a promising human insulin analog A22S-B3K-B31R [1]. These modifications result in a shift of isoelectric point to pH 6.9 and thus decrease solubility at physiological pH. This insulin analog characterizes a long-acting, flat, almost no “peakless” course of regulating glucose in time and shows no fluctuations on concentration during long-term administration.

The determination of the oligomeric composition of A22S-B3K-B31R in the proposed pharmaceutical formulations was achieved by PFGSE NMR experiments and compared to nanospray ESI MS result. Systematic studies were performed by changing following parameters: ionic strength, concentration of glycerol, cresol and Zn. It can be concluded that routinely used components in pharmaceutical formulations have different influence on aggregation of new analogue and on human insulin. It was clearly evident that aggregation of human insulin comparing to its analog A22S-B3K-B31R is more pronounced regardless of the composition of the solution.

A monomer structure of A22S-B3K-B31R has been characterized by NMR in water/acetonitrile solution and compared with the structure of human insulin established in the same medium. T1, T2 relaxation times and hetero nuclear NOE at multiple magnetic fields were measured and analyzed by extended model-free approach. The deuterium exchange NMR measurements were performed using HSQC with time-resolved non-uniform sampling (TS-NUS) methodology [2].


References
WHAT A PLANT VIRUS PROTEIN CAN TEACH US ABOUT eIF4E REGULATION AND ITS IMPACT IN CANCER?

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The eukaryotic translation initiation factor 4E (eIF4E) is an oncoprotein that regulates many steps of RNA processing1. eIF4E interacts with the 7-methylguanosine (m7G) cap of a subset of RNAs in the nucleus and is exported to the cytoplasm using CRM12,3. eIF4E import in the nucleus is mediated via Importin 8 and occurs only in its RNA-free state4. The subcellular localization and activity of eIF4E must be highly regulated and in some cancers, nuclear accumulation of eIF4E leads to an elevated export of oncogenic mRNAs and increased production of oncoproteins5. To identify new regulatory eIF4E mechanisms, our project takes advantage of a recently reported interaction between the Viral Protein genome-linked (VPg) and human eIF4E (personal communication).

The regulatory site in eIF4E involved in VPg interaction is not yet understood. In the current study, we characterize the binding surface between VPg and eIF4E using NMR experiments. Interestingly, preliminary results suggest that VPg binds a similar surface in eIF4E as Importin 8 and similar to Importin 8, it interacts selectively with eIF4E only in its cap-free state. We hypothesize that VPg interferes with eIF4E subcellular localization by disrupting its nuclear import, inhibiting eIF4E-Importin 8 interactions. Future assays will help determining if VPg can prevent the association of capped RNA with eIF4E.

Our results provide additional clues in establishing a mechanism by which eIF4E is regulated and how such mechanism affect cancers with elevated eIF4E, thereby leading to potential new therapeutic strategies for cancer treatment.

References
Practical Strategies of Buffer/Salt Optimization for NMR Studies of Antibody-Antigen Interactions

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Protein NMR studies, especially epitope mapping of protein antigens, are often limited by the inability to prepare proteins with sufficient solubility and lasting stability. Protein samples must also be non-aggregating at sub-millimolar concentrations and need to be optimized for the narrowest linewidths without affecting the 3D structure of the protein. Here, we will present a practical strategy for exploring important influencing factors on protein solubility and stability, especially the buffer pH, salts, and other additives. The screening involves two major steps: (1) identification of an efficient lysis buffer solution by optimizing the level of extracted soluble proteins from bacterial cells; (2) purification and concentration of the target protein in the selected buffer/additives followed by verification of protein folding and sample stability using NMR spectroscopy.
Structural and Biophysical Characterization of Functional Implications of eIF4E-RNMT interactions

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Abstract

Eukaryotic translation initiation factor eIF4E is upregulated in 30% of human cancers including M4/M5 subtypes of AML and lymphoma. The oncogenic potential requires its ability to bind 7-methylguanosine (m'7G) cap of specific RNA transcripts, promoting RNA export and translation. To better understand the molecular basis for eIF4E dependent mRNA export, we identified a new nuclear eIF4E partner protein: the RNA 7-methyl guanosine methyltransferase (RNMT), a key enzyme involved in m'7G cap formation. We propose that eIF4E increases capping through direct interactions with RNMT, potentially modifying its enzymatic activity. We propose a study where the effective conformations/states of the eIF4E-RNMT interaction is probed via biochemical and biophysical techniques (GST pulldown assay, CD spectroscopy, NMR and AUC-SV) to enable us to understand the molecular basis of the effects of eIF4E on RNMT methylation activity, and of RNMT on m'7G cap binding by eIF4E. The aim is to examine the eIF4E and RNMT interaction and the structural features of the complex. The effects of enzymatic co-factors (SAM, SAH, sinefungin and m'7G) on the complex, and subsequent effects of RNMT on eIF4E m'7G capping affinity will also be investigated. To date there has been only one structure of RNMT with a binding protein, RAM. Our results depict that eIF4E binds RNMT, however there are no eIF4E consensus binding sites in RNMT (YXXXXLΦ, VpG domain or RING) making it a novel interaction. We will use the existing structural information of RNMT to decipher how RNMT structure is influenced by eIF4E.
structure and function of the pistol ribozyme by solution NMR Spectroscopy

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Over the past few years, the knowledge about the important roles, that RNA and DNA play in cellular processes, has significantly grown. In the past, DNA has been regarded as a simple storage molecule of genetic information and RNA as a linker/translator between the DNA’s information and proteins. Nowadays, this view is replaced by an unexpected wealth of additional and novel functions especially associated to RNA. There is a need for investigations addressing the structure and dynamics of nucleic acids and how function is encoded in these properties of a biomolecule. The presented research focus is on a catalytically active RNA – the pistol ribozyme – by solution NMR spectroscopy. Thereby, unprecedented insights on the interplay of structure and dynamics to fulfil the catalytic function are obtained.

Our group has strongly focused on the synthesis of atom-specific labelled RNA phosphoramidites and their incorporation into RNA. These building blocks are modified with 13C and/or 15N isotopes allowing state-of-the-art NMR experiments on RNA. The chemical solid phase synthesis makes site-specific labelling possible, which is not amenable with any other existing RNA production method.

Recently, we could also show that the novel RNA isotope labelling protocol is especially useful in studying dynamic properties of nucleic acids. A site-specific deuteration step allowed the application of so called proton relaxation dispersion experiments by reducing the size of homonuclear scalar couplings. Thereby, high energy conformations of the pistol ribozyme, which are populated to a low degree (< 10%) can be studied in unprecedented detail and hopefully high resolution 3D structures of this fleetingly populated states will become amenable soon.

Our main research goals for the pistol ribozyme research project are: (i) solution structure determination of the ribozyme/substrate complex and (ii) characterization of functional dynamics enabling the ribozyme to fulfill its catalysis. To this end, we first will produce stable isotope (SI) labeled pistol ribozyme constructs via the classical enzymatic method to introduce uniform 13C and 15N labeling for assignment purposes and to determine the high-resolution solution structure.

We will further capitalize on the site/atom-specific SI-labeling protocols we have earlier established to facilitate the resonance assignment process but also to introduce appropriate spin topologies to address functional dynamics via relaxation based NMR methods.

The preliminary data are very promising and will help to understand the mechanism of this class of ribozyme in more detail.
The apelin receptor (AR) is a class A G-protein-coupled receptor (GPCR) activated by two peptide hormones, apelin and apela. In addition to its high gyromagnetic ratio and broad chemical shift range, $^{19}$F is not naturally occurring within proteins. This allows for the incorporation of $^{19}$F-labels to characterize conformational changes in response to membrane mimetics, ligands, and other environmental factors. Using a recently introduced method showing efficient and cost-effective 5-fluorotryptophan protein labeling through addition of 5-fluoroindole to M9 minimal medium (Crowley et al. (2012) Chem Commun 48: 10681), we have incorporated tryptophan $^{19}$F-labelled at the 4-, 5-, 6-, and 7-positions into AR. Solution-state NMR spectroscopy was used to probe conformation and dynamics of two portions of AR: the N-terminus and first transmembrane (TM) segment (residues 1-55, AR55) and the first three TM segments of AR (residues 1-137, AR TM1-3). Our results demonstrate that $^{19}$F NMR chemical shift, peak pattern and dynamics are highly variable, depending upon both the NMR conditions and the $^{19}$F position on the indole ring. Perturbations in the $^1$H-$^{15}$N HSQC spectra of AR segments were also apparent in a $^{19}$F configuration dependent manner, primarily localized to cross-peaks corresponding to residues proximal to the Trp. Addition of AR ligand peptides resulted in perturbations to the $^1$H-$^{15}$N HSQC spectra, with corresponding ligand concentration-dependent changes in $^{19}$F peak intensity. From this study, strategies are suggested for effective fluorotryptophan incorporation and application for $^{19}$F NMR studies of membrane protein topology, structure, dynamics, and ligand binding.
Solution NMR techniques using amide moieties as probes are widely utilized for site-specific interaction, dynamics, and structure analyses of proteins. However, prerequisite assignment processes for such analyses are often difficult for large proteins and/or proteins in living cells due to their enhanced relaxation. Amino acid selective stable isotope labeling helps assignments even in such cases, as they enable amino-acid typing of each amide signal. Various combinatorial selective labeling methods have been proposed to reduce the required number of selectively labeled samples.

We developed an advanced combinatorial labeling strategy, named SiCode (Stable isotope encoding) [1]. SiCode is based on a concept that the information of amino-acid is encoded with a predefined labeling pattern and decoded from observed NMR spectra. To reduce the required number of labeled samples, i.e., to increase information content per labeled sample, we employed quantitative fractional labeling of $^{15}$N and $^{13}$C. Fig. 1 shows an example of labeling patterns, using three levels which act as ternary digits. By assigning each amino acid to a ternary-three-digit codeword, we can distinguish all 19 non-proline amino-acids at both i and i-1 positions with $^{15}$N-HSQC and HN(CO) spectra of 3 labeled samples (Fig. 2).

To apply SiCode to low signal-to-noise-ratio (SNR) spectra such as in-cell NMR, we established two methods. The first is to design the labeling pattern according to the coding theory that the minimum information distance should be maximized to achieve maximum noise-tolerance [1]. The second is a model-based decoding method to extract amino acid information from low-SNR spectra. Replica-exchange Monte Carlo computation allowed us to test virtually all possible amino-acid combinations to find the best to explain observed spectra [2]. Combining these two improvements, we succeeded amino-acid typing for amide signals of human Ubiquitin mutant protein solely with in-cell NMR spectra, which was difficult to accomplish with conventional selective labeling methods due to low SNR.

References
NMR Investigation of the Interaction between the plant protein CC1 and Microtubules

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d Abiotic stress, such as salinity, drought, and cold, causes detrimental yield losses for all major plant crop species. Understanding mechanisms that improve plants' ability to produce biomass, which largely is constituted by the plant cell wall, is therefore of great importance for agricultural activities.

The companion of cellulose synthase (CC) proteins sustain cellulose synthesis during salt stress by regulating the cortical microtubule network and cellulose synthase localization in Arabidopsis thaliana. Here, we provide detailed insight into the interaction between microtubules and the cytoplasmic tail of CC1 (CC1ΔC223) and the mechanism by which it regulates the microtubule array in vitro and in vivo.

We characterized the highly dynamic nature of the CC1ΔC223-microtubule interaction by NMR spectroscopy. The free CC1ΔC223 is essentially unstructured, but backbone carbon shifts hint for several regions with enhanced propensity for beta-strand secondary structure. The addition of microtubules to 15N-labeled CC1ΔC223 results in reversible and residue-specific line-broadening effects, stemming from the association of the free NMR-visible CC1ΔC223 to the microtubule surface, where it experiences near instantaneous transverse relaxation due to the long rotational correlation time of the complex. The results indicate, that CC1ΔC223 does not fold into a rigidly bound structure upon interaction with microtubules, but rather binds with conserved, short hydrophobic binding motifs that are connected by flexible linker regions. The dynamic nature of the CC1ΔC223 binding suggests that different regions in one CC1ΔC223 protein can bind to distinct tubulin dimers, thereby increasing the local tubulin concentration, connecting and stabilizing protofilaments or bundling microtubules. The mutation of key residues in the N-terminal binding region alters binding and bundling activity in vitro and results in disrupted protein function and a salt sensitive phenotype in vivo.

Our findings thus offer a mechanistic model for how CC proteins sustain microtubule organization and cellulose synthase localization, and thus aid plant biomass production, during salt stress.
NMR characterization of the backbone dynamics of the inactive and active small GTPase Rheb and their interaction with the regulatory protein FKBP38

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Ras homologue enriched in brain (Rheb) is a small GTPase regulating mammalian/mechanistic target of rapamycin complex 1 (mTORC1) and thus cell growth and metabolism. Rheb cycles between an active GTP and an inactive GDP state. The nucleotide binding state, GDP versus two different non-hydrolyzable GTP analogues referred to as GppNHP and GppCp, modulates the backbone dynamics of a truncated fragment of Rheb lacking the C-terminal CAAX box needed for farnesylation (= RhebΔCT). This is suggested to influence its interaction with regulatory proteins.

Rheb has been suggested to interact with the FK506-binding protein of 38 kDa (FKBP38), which inhibits its stimulating effect on mTORC1 [1]. However, later studies reported conflicting results [2]. Here, we show based on ¹H-¹⁵N-HSQC monitored interaction data that only RhebΔCT bound to the GTP analogue GppNHP shows very weak chemical shift changes in the presence of the FKBP38 binding region (FKBP38-BD), but not RhebΔCT-GDP. Real-time NMR rate analysis data suggests that this weak or transient interaction of RhebΔCT-GppNHP and FKBP38-BD stimulates the GDP-GTP exchange significantly if FKBP38-BD is present at higher molar ratios (~1:30).

Fig. 1. GTPase cycle of human Rheb (A) and its domain organization (B) and the superposition of the ¹H-¹⁵N-HSQC spectra of RhebΔCT bound to GDP or the GTP analogue GppNHP.

References
The automated identification of signals in multidimensional NMR spectra is a challenging task, complicated by signal overlap, noise, and spectral artifacts, for which no universally accepted method is available. Here, we present a new peak picking algorithm, CYPICK [1], that follows, as far as possible, the manual approach taken by a spectroscopist who analyzes peak patterns in contour plots of the spectrum, but is fully automated. Human visual inspection is replaced by the evaluation of geometric criteria applied to contour lines, such as local extremality, approximate circularity (after appropriate scaling of the spectrum axes), and convexity. The performance of CYPICK was evaluated for a variety of spectra from different proteins by systematic comparison with peak lists obtained by other, manual or automated, peak picking methods, as well as by analyzing the results of automated chemical shift assignment and structure calculation based on input peak lists from CYPICK. The results show that CYPICK yielded peak lists that compare in most cases favorably to those obtained by other automated peak pickers with respect to the criteria of finding a maximal number of real signals, a minimal number of artifact peaks, and maximal correctness of the chemical shift assignments and the three-dimensional structure obtained by fully automated assignment and structure calculation.

References
Structural characterization of the interaction between p53 and plakoglobin

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The tumor suppressor p53 maintains genome integrity and prevents oncogenesis. The core domain of p53 interacts with p53-specific promoters and promotes transcription of downstream genes. In addition to its DNA binding activity, the core domain also binds to numerous nuclear and cytoplasmic proteins[1,2,3,4]. Plakoglobin, a catenin family protein involved in cell-cell adhesion, is localized to the nucleus and activates p53 in vivo through direct interaction between C-terminus of plakoglobin and the core domain of p53[5]. Unlike many p53 core domain binding proteins in the nucleus that inhibit p53 activity, the interaction between plakoglobin and the p53 core domain activates p53[1,2,5].

In this study, NMR spectroscopy was employed to explore this interaction at the atomic-level using the p53 core domain (p53DBD, residues 94-312) and plakoglobin C-terminal domain (plakoTD, residues 686-745). Through standard triple-resonance assignment strategies, the plakoTD was successfully sequentially assigned. Circular dichroism spectorscry confirms that plakoTD is intrinsically disordered. Titrations of 15N-labeled plakoTD with unlabeled p53DBD monitored by 1H,15N HSQC experiments demonstrate that a methionine-rich motif is involved in the interaction. Interestingly, the titration of non-labeled, near full-length p53 (residues 1-312) into 15N-labeled plakoTD resulted in negligible chemical shift perturbations (CSPs) on plakoTD, suggesting that the N-terminus of p53 has a negative effect on plakoTD binding and may compete for the plakoTD binding site on the p53 core domain. Titration of non-labeled plakoTD into 15N-labeled p53DBD leads to CSP at many sites. Notably, these include K291, K292, and G293, found on the helix 2 which intercalates into the major groove of DNA duplex. plakoTD contains multiple serine and threonine residues which could serve as sites for phosphorylation. Mutagenesis (serine to glutamate and threonine to asparate) was performed to mimic phosphorylation. Titration of non-labeled plakoTD mutants into 15N-labeled p53DBD at a 1:1 molar ratio led to CSPs comparable to those observed at 2:1 molar ratio for wild-type plakoTD.p53DBD, suggesting that these phosphorylation mimics enhanced the interaction by approximately two-fold.

In summary, we demonstrate that plakoTD interacts with p53DBD through a methionine-rich motif, but that the N-terminal domain of p53 prevents this interaction. More interestingly, phosphorylation of plakoTD may regulate this interaction. Further experiments are underway to fully define the binding surface of plakoTD on p53DBD and to determine the structure of the protein complex.

References
Summary: By introducing a paramagnetic spin into biological macromolecules, dipolar coupling between the paramagnetic spin and nuclear spins will result in paramagnetic relaxation enhancement (PRE) and pseudocontact shift (PCS), which provides long-range distance restraints for structural determination. The paramagnetic spin may also align the molecule due to its anisotropic magnetic susceptibility, which results in residue dipolar coupling (RDC) and provides information on the motion of bond vectors. Here we report our effort on introducing lanthanide-binding tags to a 24-mer DNA. To achieve this, a nucleobase was chemically modified and incorporated into 24-mer DNA, such that different lanthanide-binding tags could be subsequently ligated to the modified nucleobase. Without isotope enrichment, a number of NMR experiments were performed on these samples to obtain PCS and RDC data, where PCS up to 1 ppm, H-H RDC up to 8 Hz at 950 MHz and C-H RDC down to -6 Hz at 800 MHz were observed. Different fitting models were tried, and we found that the rotation of lanthanide-binding tag relative to 24-mer DNA was necessary for an accurate modeling of PCS and RDC. Our model also predicts, that further rigidification of the tag would be beneficial for the application of lanthanide tagging in structural biology, as it could greatly enhance PCS and RDC.
The interaction of a quantum system with its environment causes decoherence, setting a fundamental limit on the suitability of a system for quantum information processing. However, we show that if the quantum system consists of coupled parts with different internal energy scales then the interaction of one part with a thermal bath need not lead to loss of coherence from the other [1]. Remarkably, we find that the protected part can become more coherent when the coupling to the bath becomes stronger or the temperature is raised.

The model system we consider is that of a pair of an electron and a nuclear spin. The electron spin is assumed to have the much stronger environmental coupling – i.e. a shorter $T_2$ – than the nuclear spin, such that when the two spins are coupled the nuclear spin coherence is primarily determined by the electron spin coherence [2]. We first develop an exact zero-temperature model of the coupled spin-environment problem, based on a modified Weisskopf-Wigner theory [3]. We then show that an approximate Born-Markov master equation model, without secularization, describes the zero temperature results accurately, and so we thus extend our model to high temperature.

Our results [1] show that by clever design of hybrid quantum systems, the coherence time of part of a system could be extended by heating it up. Our theory will enable the design of decoherence-resistant hybrid quantum computers, and is a further example that decoherence in bipartite quantum systems cannot be explained by considering each part separately [4].

**Fig. 1.** a) A coupled nuclear-electron spin system with electron Zeeman splitting of $\omega_0$ and hyperfine coupling $g$. We neglect the Zeeman splitting of the nuclear spin, and only the electron interacts with a thermal reservoir. b) The eigenenergies of the two-spin system and allowed transitions $\omega_{1,2}$. When the hyperfine coupling is small (c), the transitions overlap and nuclear spin coherences can survive. Increasing the hyperfine coupling (d) resolves the transition lines and coherences are lost.


Selective Addressing and Readout of Optically Detected Electron Spins

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Optically detected magnetic resonance (ODMR) is known to allow the ultrasensitive detection of a small number of electron spins for species such as nitrogen-vacancy (NV) centers in diamond, or silicon vacancies in SiC, down to the single spin level. This method was recently combined by our group and the group of Walsworth, with the pulsed magnetic resonance imaging (MRI) technique known as optically detected magnetic resonance imaging (ODMRI) to obtain images of NVs at resolutions ranging from the micron- to the nanoscale [1; 2]. The great advantage of ODMRI is that the quantum state of the spins can be acquired (i.e., read-out) in parallel from all parts of the sample without any loss of spectroscopic information. Here we further enhance the utility of our ODMRI technique by improving its spatial resolution and adding a spatially-selective spin addressing and manipulation capability. We demonstrate this capability by selectively manipulating a specific group of spins in our sample and subsequently imaging the entire sample to show this selectivity effect. Fig. 1 presents an example from the results of these type of experiments [3]. Such capabilities are of fundamental importance in the field of spin-based quantum devices and sensors.

Fig. 1. Example for selective addressing of a small number of spins. In this experiment we applied pulsed ESR with large pulsed magnetic field gradient to image a sample of electron spins (NV centers in diamond). The image on the left shows the entire sample, while the image on the right is the same experiment, but applied selectively only to a small subset of a few 100s of spins in the center of the sample.

References
RELATIONSHIP BETWEEN FAT HARDNESS, FATTY ACID COMPOSITION, AND IODINE VALUE OF PORK BACKFAT WITH SOLID FAT CONTENT DETERMINED BY TIME-DOMAIN NUCLEAR MAGNETIC RESONANCE

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Soft fat problems cause important economic losses in the pork industry for which there is no rapid detection method. Nuclear magnetic resonance (NMR) could be used efficiently for screening. Indeed, low-resolution NMR has been successfully used for rapid determination of composition and physical state of different components in biological material such as food. Time-domain NMR (TD-NMR) is an official method of the American Oil Chemists’ Society (AOCS) to measure solid fat content (SFC) in oils and fats. The goal of this work was to examine the relationship between iodine value, fatty acid composition, and fat hardness with SFC determined by NMR for rapid, non-destructive assessment of pork fat consistency.

Three series of experiments were performed. Fat hardness was measured on pig backfat with a durometer (series 1 and 2). Iodine value was calculated from the fatty acid profile obtained by gas chromatography. SFC measurements were taken on purified fat samples at several temperatures between 0 and 45 °C following the AOCS protocol for series 1 and with slight modification in the tempering scheme for series 2 and 3. Two methods of data analysis were used. In the first, melting curves were built from SFC data to give amplitude as well as melting temperature mean and standard deviation. Correlations were obtained with iodine value, individual fatty acid composition, and fat hardness. Alternatively, a multivariate regression was performed with SFC measurements at each temperature as independent variables and iodine value or individual fatty acid composition as dependent variable.

Good correlations (|R| ~ 0.8) were obtained between melting curve amplitude (or similarly SFC at 0 °C) and iodine value, proportion of some fatty acids (best for linoleic acid), and less importantly fat hardness (|R| ~ 0.6), but not for melting temperature. It was also shown that a longer tempering time at 0 °C induced a slightly more complete crystallization of animal fat.

Even though the SFC measurement itself is rapid (6 s per sample per temperature), pure fat is required for the AOCS method, and an alternate NMR approach bypassing the lengthy purification procedure is under development to obtain a usable method for soft fat detection.
NMR detection of oil trapped under Arctic sea-ice

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Oil exploration and production in the Arctic bring the chance for oil spillage and environmental damage. We have developed earth’s field NMR to detect the presence of spilled oil trapped underneath or in Arctic ice. The basic approach is to use large coils (6 m diameter) to penetrate beyond the bottom of the ice (1-2 m thick) and to enhance sensitivity with pre-polarization. The pre-polarization hardware is unique, supplying 12 kW from batteries and turning off the field in only 10 ms, to avoid loss from the rapid $T_1$ relaxation of the oil (100 ms or less). Nevertheless, the field is dropped adiabatically, to bring the spins into alignment with the earth’s field. The rf (audio) pulses that manipulate the spins must work over a wide range of $B_1$ field strengths. Suitable adiabatic AM/FM sweep pulses have been crafted that accurately excite or invert the spins over a 50:1 range of $B_1$, despite strong effects from the counter-rotating component of rf field ($B_1$ can be as large as $B_0$ here).

Two methods to suppress the overwhelmingly larger water signal rely on the shorter $T_1$ of oil (100 ms) compared to water (2000 ms); we note that chemical shift is unavailable at such low fields. The methods include a version of inversion-null recovery, modified for use with strong pre-polarization, and a more general method that allows for nulling after data are acquired. Both provide an overall rejection of water relative to oil of 200:1, allowing detection of a 1 cm thick layer of oil under the ice.
Nano-scale MRI: Spin-noise or Dynamic Nuclear Polarization?

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Technologies for detecting magnetic resonance with extreme sensitivity are bringing within reach the long-sought goal of visualizing single molecules via magnetic resonance imaging (MRI). These technologies include optical detection using nitrogen-vacancy defects in diamond and mechanical detection using attonewton-sensitivity cantilevers (magnetic resonance force microscopy, MRFM). These technologies have led to MRI experiments showing better than 10 nm resolution [1] and a sensitivity as good as 10’s to 100’s of proton magnetic moments [2, 3]. In these experiments the small sample size and the modest spin polarization ($p = 5 \times 10^{-3}$ for protons at 2.1 kelvin and 10 tesla) makes Curie-law magnetization too small to observe. Consequently, these nano-MRI experiments detected statistical fluctuations in the magnetization which exceed the Curie-law magnetization in small ensembles. Such “spin-noise” experiments avoid the need to polarize sample spins at the cost of measuring a signal proportional to $\sqrt{N}$, the square root of the number of sample spins, rather than $N$. In 2016, Isaac et al. demonstrated the transfer of electron spin polarization to nuclear spins in an MRFM experiment via cross-effect dynamic nuclear polarization (DNP) [4]. This created a small-ensemble signal proportional to $N$, but at the cost of adding an additional spin-polarization time to the experiment. This presentation will discuss the trade-offs in signal-to-noise ratio (SNR) and acquisition time for detecting and imaging hyperpolarized vs. statistically-polarized spins.

We begin by revisiting the SNR for the MRFM spin-noise experiment. In 2007, Degen and coworkers published a detailed analysis of this experiment and proposed a method to increase SNR through periodic randomization of the sample’s nuclear spins [5]. Their result, however, assumes a cantilever whose bandwidth is much larger than that of the spin-noise signal, which is unlikely to be the case for biologically interesting samples [1]. In order to obtain a more robust SNR estimate for the spin-noise experiment we develop an equation for the standard error of the sample variance of autocorrelated data. Using this equation, along with a model that accounts for force noise, detector noise, cantilever bandwidth, periodic randomization and software filter processing, we derive a revised SNR equation for the spin-noise experiment. This equation explains why magnetization randomization will likely not improve the SNR for biological imaging experiments; predicts parameters for optimal cantilever control; and predicts a SNR that can be compared with the SNR achieved by Isaac et al. in their hyperpolarization MRFM experiment [4]. We will generalize these findings and the results of Ref. 4 to obtain rules for comparing the SNR for detection and imaging of hyperpolarized vs. statistically-polarized spin signals from molecular-scale samples in a nano-MRI experiment.

References:
Superadiabatic pulses for NMR

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Amplitude- and frequency-modulated “adiabatic pulses” provide robustness against inhomogeneities in both the static and radio-frequency (rf) magnetic fields, finding applications in both high-resolution NMR spectroscopy and in vivo magnetic resonance imaging. Similar schemes have been used to enable robust control of quantum operations in many physical platforms.

We introduce an approach to systematically design pulses to maximize the adiabaticity of a unitary operation in a particular time interval, given the available controls. The method uses the superadiabatic quality factor ($Q_s$) as a performance metric to design robust, high-fidelity pulses for both single and multi-spin systems [1]. The idea of a superadiabatic Q factor was introduced by Deschamps et al. to explain the unexpectedly high fidelity of certain adiabatic pulses used in NMR [2]. We also introduce a scheme to find numerically-optimized pulses for multi-spin systems where analytical expressions for the adiabatic or superadiabatic quality factors are not available.

Figure 1 (left) shows the first 10 superadiabatic Q-factors for a $Q_1$ and a $Q_s$ optimized tanh/tan inversion pulse, as well as the performance of the pulse as the pulse length is reduced. Figure 2 (right) shows the robustness of different tanh/tan pulse to errors in the frequency ($\omega_0 \pm \delta$) and amplitude ($\sigma \omega_1$) of the pulse. Superadiabatic pulses are seen to have improved robustness to control errors. The results have been extended to two-spin systems.

References