# Program at a glance

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2 Abstracts for oral presentations
Practical, Long-lived, and Simple Hyperpolarization for NMR and MRI

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General applications of molecular hyperpolarization in magnetic resonance are limited by two fundamental challenges. The first challenge is that hyperpolarization decays back to thermal equilibrium at a rate given by the nuclear spin lifetime T1, which in solution is commonly seconds. The second challenge is that the best known hyperpolarization approach, dynamic nuclear polarization (DNP), only produces very large enhancements with drastically cooled samples in large fields (which in turn require mm-wave irradiation). As a consequence, the apparatus tends to be complex and expensive with slow buildup times, and is not readily scalable.

I will discuss recent work from our group which addresses both of these challenges. “Disconnected eigenstates,” such as the singlet between a single pair of inequivalent spins, or the state \((\alpha\beta – \beta\alpha)_{2N}(\alpha\beta – \beta\alpha)_{2H}\) between pairs of spins equivalent by symmetry, provide a powerful motif for reducing relaxation rates. Pulse sequences can load and unload such states in many different molecules. Recent work has focused on motifs with good biological compatibility, such as the three-membered C-N=N diazirine ring which can replace methylene groups in many different applications. At modest fields, such species can exhibit many-minute lifetimes.

We will also discuss a variety of promising new approaches to make hyperpolarization simpler, cheaper, and more efficient, building on the SABRE approach (catalytic transfer of parahydrogen order into other molecules in solution) pioneered by Duckett in 2009. This work was elegant and innovative, but as recently as two years ago the approach had several serious limitations. Signal enhancements were generally much lower than DNP, in part because the produced proton polarization was short-lived; only a small range of molecules (mostly heterocycles) were suitable; the polarization could not be done directly in the magnet; and good enhancements required non-biocompatible solvents. Since that time, work by multiple groups has surmounted all these limitations. Our two recent approaches, LIGHT-SABRE and SABRE SHEATH, permit this magnetization transfer either at arbitrary field strengths or directly from proton to nitrogen at extremely low fields. The SABRE SHEATH apparatus is stunningly simple (a shield and a single coil suffices to enable the catalytic transfer), with 10% nitrogen polarization demonstrated. Today it is clearly possible for essentially any NMR lab to use hyperpolarization without staggering effort or cost. In addition, the future of MRI may look very different from the past: low-cost (portable, or even wearable) magnets with convenient hyperpolarization have the potential to be transformative.

Figure 1. SABRE-SHEATH produces large nitrogen polarization in seconds. The chair and stool are a significant fraction of the apparatus cost.
Characterization of intrinsically disordered proteins and their functional, dynamic complexes

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Intrinsically disordered proteins (IDPs) are highly abundant in the human proteome and play key regulatory roles in biology. Here, quantitative and robust approaches will be presented for deriving ensembles of IDPs from experimental NMR data such as chemical shifts, residual dipolar couplings and paramagnetic relaxation enhancements allowing us to visualize in great detail both local and long-range conformational propensities in IDPs.1 Characterization of protein-protein complexes involving IDPs is an entirely different challenge as exchange between free and, potentially, multiple bound states often occurs on the micro- to millisecond time scale leading to extensive line broadening of the NMR signals. Here, NMR 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 measured in IDPs at sub-stoichiometric amounts of binding partner.2

The approaches are applied to disordered domains within the mitogen-activated protein kinase (MAPK) cell signaling pathways that assembles specific kinases into highly dynamic signaling complexes. Our results reveal how IDPs in the MAPK pathways rely on linear motifs for governing signaling specificity, and how different IDP binding modes may induce different functional states of the folded, binding partners (Figure 1).3


Figure 1. (A) Structural ensemble description of full-length mitogen-activated protein kinase kinase 7 (MKK7) derived from chemical shifts and residual dipolar couplings. (B) Characterization of the interaction between MKK7 and the c-Jun N-terminal kinase 1 (JNK1) using NMR exchange spectroscopy. (C) Crystal structure of JNK1 in complex with MKK7 peptide revealing two different binding modes.
Dynamic nuclear polarization (DNP) as a means to enhance NMR sensitivity has made significant strides in the past twenty five years, in particular due to the development of hardware, sample preparation strategies, and efficient polarization agents which have demonstrated its promise. The advent of commercially available high frequency microwave sources and auxiliary hardware compatible with high field NMR and MRI instruments has led to the transition of DNP-based research from proof of principle experiments to applications enabling spectroscopic investigation of samples previously beyond the reach of NMR. Key experiments measuring polarization enhancements in various field and temperature regimes coupled with EPR measurements have yielded a rough topological map for mechanisms of DNP and how they might be exploited to enable new applications in biomolecular NMR and real time in vivo MRSI of metabolism. Nonetheless, recent work highlights the need to continue to develop DNP approaches and a framework for polarization of “real world” systems. In particular, biologically relevant systems are both highly aqueous and naturally highly heterogeneous. Freezing samples for DNP adds another level of complexity. I will primarily focus on our recent efforts examining polarization at 5 T and 1.2 K for dissolution DNP applications as well as provide a few updates on DNP at 14.1 T and ~100 K for biomolecular MAS ssNMR applications. In both cases, pragmatic considerations of spin distributions and how they may be manipulated can improve observed polarization.

Figure 1. Polarization behavior of substrates for dissolution DNP prepared using rapid freezing without glassing agents enables a 1.5-3 fold time savings in polarization buildup while removing the need for toxic glassing agents. Achievable polarization using this approach and fully aqueous substrate solutions is equal to that observed using standard approaches and glassing agents. For further details, see Lama B, Collins JH, Downes D, Smith AN, Long JR, “Expeditious dissolution dynamic nuclear polarization without glassing agents”. NMR Biomed., 29(3):226-31 (2016).

Figure 2. Nitroxides (red) incorporated into membrane-intercalating lipids (green) provide a general, non-perturbing method of sample preparation for DNP enhanced ssNMR studies of biological membranes. We observe a more than two-fold signal enhancement for the membrane peptide KL4 (blue) polarized in this fashion when compared to standard DNP sample preparation. For further details, see Smith AN, Caporini MA, Fanucci GE, Long JR, “A Method for Dynamic Nuclear Polarization Enhancement of Membrane Proteins”. Angew. Chemie, 54(5):1542-1546 (2015).
High Time Resolution Nanoscale NMR with the Nitrogen-Vacancy Center Utilizing an FPGA

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Abstract—We present the FPGA-based solution for the high time resolution nanoscale nuclear magnetic resonance, with the Nitrogen-Vacancy center as a probe. The Xilinx FPGA is used to implement the high performance pulse generator, which contributes to a high precision measurement for the NMR signal. The pulse generator outputs continuous pulses with a time resolution of 50 ps and a dynamic range of 5 ns to 2.68 s. The central control and the readout logics for the NMR experiment are fully implemented in the FPGA. We observed the nanoscale NMR signals of the hydrogen atoms, through measuring the period of the Larmor precession with a 50 ps time resolution. The period of the Larmor precession, as well as the Larmor frequency and the calculated value of gyromagnetic ratio, are determined more precisely by increasing the time resolution. Furthermore, we demonstrate the ability to distinguish signals of adjacent frequencies, which is a great advantage in high resolution analysis for the nanoscale NMR.

![Figure 1. Diagram of the N-V center based nanoscale NMR system.](image-url)
Remote Hyperpolarization

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Nuclear spin hyperpolarization by dissolution dynamic nuclear polarization (d-DNP) [1] can be used to enhance the signals of 13C-labeled metabolites by several orders of magnitude. Given the short lifetimes of hyperpolarized magnetization (typically, T1(13C) < 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 T1(13C) becomes prohibitively short at low magnetic fields and high temperatures because of the proximity of the 13C spins to the statistically dispersed polarizing agents.

Here, we introduce a concept to dramatically extend the lifetimes T1(13C) 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 a suitable micro-particulate sample architecture where a “component containing polarizing agents” and a “component containing metabolites” are interleaved on a micron scale. Hyperpolarization is relayed to the protons of the metabolites by proton-proton spin diffusion and is transferred from 1H to 13C by cross polarization (CP).[2] Since the 13C 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 13C 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.[3]


Figure 1. Remote DNP concept.
Dynamic nuclear polarization (DNP) is a versatile technique for polarizing nuclear spins through cross polarization with unpaired electron spins. The unpaired electron spins are usually introduced in the form of persistent radicals inside a liquid sample containing the molecules with the nuclear spins to be polarized. It was shown that the high polarizations obtained after solid-state DNP can be maintained in the liquid-state solution recovered after a rapid dissolution process. Dissolution DNP is nowadays a well-established and widespread method that allows enhancing the liquid-state nuclear magnetic resonance (NMR) sensitivity by several orders of magnitude.

Dissolution DNP main limitation concerns the need to place the DNP polarizer as close as possible to the MRI scanner (or high resolution spectrometer) where the actual experiment is performed. Indeed, after dissolution the life-time of the hyperpolarized liquid is limited to spin-lattice relaxation that brings the nuclear spins population back to thermal equilibrium, generally in a couple of minutes. It was recently shown that photo-induced non-persistent radicals can be used to prepare radical-free hyperpolarized [1-13C]pyruvate for in vivo metabolic imaging. The photo-induced radicals are scavenged upon dissolution generating a radical-free hyperpolarized liquid. It was also demonstrated that the technique can be extended to polarize other 13C-labeled metabolic substrates and even other nuclei.

In the present work, we demonstrate how the photo-induced radicals can be eliminated in the solid state, prior to dissolution, thereby yielding radical-free highly polarized solid samples. This is done through a low-temperature thermalization process that briefly brings the temperature of the sample above the recombination temperature of the radicals, which is around 190 K. X-band ESR measurements performed at 77 K in UV-irradiated frozen pellets of 1:1 (v/v) water: pyruvic acid prior and after thermalization show that the radicals are efficiently scavenged. We also observed that the 13C polarization of [1-13C]pyruvic acid is conserved through the thermalization process since the DNP-enhanced NMR signal measured at 4.2 K is essentially unaffected (see Fig.2). The elimination of the photo-induced radicals following the thermalization process is further demonstrated by the more than 18-fold increase in the solid-state longitudinal relaxation time (prior and after thermalization) measured at 4.2 K (Fig.2). Because the DNP-enhanced solid samples do not contain radicals following thermalization, they can be extracted from the polarizer in their solid form as previously proposed in conjunction with a “brute force” polarization method, and it should be possible to consequently store and transport them.

References
DNP Enhanced Solid-State NMR Spectroscopy of Materials
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Although solid-state NMR (in conjunction with other methods) is the method of choice to describe at atomic level the structure of complex materials, it is plagued by its intrinsic low sensitivity. This limitation is further exacerbated for the characterization of surface species by the small fraction of active sites and by their often disordered and multi-site nature. One intriguing possibility for further increasing the sensitivity of solid-state NMR experiments is dynamic nuclear polarization (DNP). In a DNP experiment, the large polarization of unpaired electrons is transferred to surrounding nuclei providing a maximum theoretical DNP enhancement ($\varepsilon \sim 658$) for $^1$H nuclei. Although the principles of this effect have been known for many decades, its transposition to modern high-resolution NMR in liquids or solids has only started to become operational a few years ago. In this respect, in the past few years, high-field DNP-enhanced solid-state NMR spectroscopy under magic angle spinning (MAS) conditions has made great progress, and using state of the art biradical polarizing agents like AMUPOL or TEKPOL, enhancement factors of up to ~235 have been reported in aqueous or organic solutions, at magnetic fields of 5–9.4 T, sample temperatures of ca. 80–105 K and spinning frequencies up to 14 kHz.

Key to transposing the high enhancement factors observed for bulk frozen solutions to intrinsically radical free materials is the use of incipient wetness impregnation. In this approach, the powdered materials are wetted by a minimal amount of radical containing solution. Polarization is transferred from the radical to the $^1$H nuclei on the surface either directly or via proton spin diffusion through the surrounding solvent molecules. Cross-polarization is then used to selectively polarize surface nuclei ($^{13}$C, $^{29}$Si, $^{29}$Al, $^{119}$Sn, etc). Using this approach (called DNP SENS for DNP Surface Enhanced NMR Spectroscopy), we have shown that enhancement factors of between 10 and 200 could be routinely obtained at 9.5 T and 100 K on a wide range of materials such as zeolites, nanoparticles, oxides, metal organic frameworks or cementitious materials. Such amplification factors translate into spectacular reductions in experiment times (between 100 and 40000) and open the perspective to characterize the detailed structure of molecular species present at the surface of these materials.

In this presentation we will present new applications of DNP SENS in heterogeneous catalysis. In parallel we will present some recent experimental developments. In particular we will discuss results from high field (18.8 T) and fast MAS (~40 kHz) DNP NMR.

References:


NMR snapshots of a fluctuating ubiquitin structure

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We have demonstrated that high-pressure NMR is capable of analyzing structure and thermodynamic stability of high-Gibbs energy conformations of proteins. We have shown that ubiquitin populates at least two high-energy conformations, the alternatively folded conformation N₂ and locally disordered conformation I, between the basic folded conformation N₁ and the totally unfolded conformation D. Here, we present an NOE-based structural determination of the two high-energy conformers of ubiquitin using rational mutations and high-pressure NMR. We created the Q41N variant of ubiquitin, in which the hydrogen bond between I36 and Q41 is weakened. The Q41N mutation favors N₂: N₂ is ~70% populated in Q41N but only ~20% populated in the wild type at ambient pressure. Structure calculation for Q41N was performed at pH 7.0, 298 K, and 2500 bar, where N₂ is 97% populated, with 1245 distance and 72 torsion angle constraints. The N₂ has large displacement of the C-terminal β5-strand beyond residue 68 as well as some displacement of the α-helix and following loop regions. Because the N₂ conformation has close identity with the conformation of the protein bound to the ubiquitin-activating enzyme E1, the recognition of E1 by ubiquitin is therefore best explained by conformational selection rather than induced-fit motion. At pH 4.0, 278 K and 2500 bar, the conformer I is about 75% populated. Interestingly, under these conditions, the NMR signals of residues 33-41 in the folded polypeptide chain are preferentially weakened. The loss of NMR signals suggests that these residues have transformed into a heterogeneously disordered conformation exchanging just slowly enough to broaden out the NMR signals. To obtain distance constraints between the "NMR-invisible" region and the rest of the protein, we measured paramagnetic relaxation enhancements (PREs) for the Q40C/Q41N variant, in which the paramagnetic probe MTSL is covalently attached to C40 in the NMR-invisible region. More than 1300 NOE and PRE-based distance constraints and 42 torsion angle constraints were used to calculate structures. The I conformer shows a locally disordered conformation at residues 33-41 with large displacement of the C-terminal strand, providing an open conformation at the C-terminal region of the protein. We have provided two NMR snapshots, N₂ and I, of a fluctuating ubiquitin structure between N₁ and D. High-pressure NMR is a general method for elucidating the structure of protein conformations, which are thermodynamically accessible from the ground state.
Field-cycling photo-CIDNP MAS NMR
on a photosynthetic reaction center

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Nuclear spin hyperpolarization readily occurs during the light-induced photosynthetic charge separation processes in nature. It manifests itself, amongst others, in solid-state photo-chemical induced nuclear polarization (photo-CIDNP) in photosynthetic reaction centers [1]. So far, this phenomenon has been observed at a magnetic field range between 1.4 T and 17.6 T which allowed probing the field dependence of the solid-state photo-CIDNP effect via 13C MAS NMR [2, 3]. The study of the effect at different magnetic fields allows for determination of the mechanism producing the spin-hyperpolarization as well as of the electronic structure of the cofactors inside the proteins responsible for the charge separation process [2]. Since the effect has been predicted to have functional relevance in regulating electron transport, its study of even lower magnetic fields down to earth magnetic field is of further interest [4, 5, 6]. Since lower magnetic fields are accompanied by lower spectral resolution, field-cycling techniques need to be employed allowing to induce the effect at low fields, while recording the MAS NMR spectra at high magnetic fields.

Here we present a study of the solid-state 13C photo-CIDNP effect on selectively isotope labelled reaction centers of Rhodobacter (R.) sphaeroides WT between 0.25 T and 2.0 T by using an aerodynamic shuttle technique which allows inducing the effect in the stray field of the magnet and measuring it at 9.4 T under MAS conditions. The study revealed a surprising sign change of the effect not predicted by theory so far.

Conformational Dynamics of DNA

by orientation selective PELDOR/DEER and MD simulations

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Orientation selective PELDOR/DEER measurements on biopolymers with rigid spin-labels provide crucial information about their structure and dynamics. Experiments with the rigid spin label $\mathcal{C}$ have already yielded a mechanical model for the dynamics of dsDNA. Molecular dynamics (MD) simulations can act as a 'computational microscope' to resolve the dynamics of individual atoms, however, for a long time the application of MD to nucleic acids was hindered by the lack of accurate force fields. Recently a new force field\textsuperscript{2} for DNA (bsc1) has been published, which needs to be evaluated. This evaluation can be combined with answering the fundamental question of the conformational dynamics of DNA. Signals calculated from our MD simulations closely match the experimental PELDOR traces which confirm that the bsc1 force field is a significant improvement in the computational description of DNA.

We have analyzed the simulations with respect to our previously introduced mechanical models for DNA, which described the helix dynamics in terms of stretch-twist, radius change and bending motions. The DNA showed a negative stretch-twist-coupling, as several publications previously predicted \textsuperscript{3,4}. In addition, a previously unpublished 35$^\circ$ bending motion of the DNA was observed.\textsuperscript{1,3} Bayesian ensemble refinement\textsuperscript{5} enabled us, to increase the agreement between simulation and experiment further, which lead to somewhat shorter-spin-spin distance, highlighting the importance of explicitly considering the spin labels in future studies and will underpin future improvements of nucleic acid force fields.

Figure A) MD simulated 20mer dsDNA B) rigid spin label $\mathcal{C}$ C) Experimental (black) and calculated(red) PELDOR time traces for some double labeled dsDNA The calculations are done with the structures from the MD. The time traces for a sample differ in the offset $\Delta v$ between pump and probe pulse. $\Delta v$ is 40 MHz (bottom) to 90MHz (top).

Membrane proteins have garnered exponentially increasing research interests in both molecular and life sciences. Recently DNP-enhanced MAS SSNMR have shown its power and growing potential in contributing new knowledge in this field. In this abstract, we demonstrate that thanks to the superior sensitivity offered by recent developments in DNP SSNMR, the question/hypothesis-driven access to diverse membrane protein systems of high biological and pharmaceutical relevance is becoming feasible.

First, we demonstrate that “difficult” systems such as human GPCRs of nano mole amount recombinantly expressed in eukaryotic insect cells could be studied by DNP-enhanced SSNMR. We would focus on human peptide GPCRs, bradykinin receptors, and show that the peptide ligand conformation in its bound state could be determined along its binding chemistry. Up to now, such peptide receptors are still extremely difficult to be studied at high resolution even after the revolution in GPCR crystallography. Our DNP SSNMR approach represent an alternative strategy for investigating these receptors. We have obtained the full assignment of the peptide ligand bound to bradykinin receptor, determined its conformation and identified the hotspots upon binding by SSNMR with large DNP enhancement (> 100). Our results resolves a long-lasting puzzle about the ligand selectivity among peptide GPCR subtypes. The NMR data also explains a handful of SAR accumulated over decades and permits a joint modeling of the GPCR-peptide complex, which further matches biochemical studies on the receptor. Furthermore, we could also access the orthosteric and allosteric sites by SSNMR and visualize the role of these two sites in tuning agonism and antagonism of the receptor, which is of central importance in GPCR pharmacology.

Second, on “easy” systems such as a bacterial proton pump proteorhodopsin, DNP-enhanced SSNMR permits detailed investigations of protein architecture related to its functions. We will present an extensive collection of experiments designed to answer most relevant biochemical/biophysical questions. Most of our NMR approaches are also combined seamlessly with tailored labeling schemes to facilitate desired magnetization transfer. Such experimental designs balances spectroscopic selectivity, overall sensitivity, sample costs, and machine time economy. These NMR strategies together with mutagenesis and novel chemical biology methods allow us to map the cooperative network in a membrane protein and understand how nature engineers it for particular functional regulation. We will also demonstrate that DNP-enhanced SSNMR provides great opportunities in mapping protein-ligand interactions and even ligand electronic structure, e.g. by correlating CSA with dipolar interaction at site-specific level.

In summary, our efforts show that DNP-enhanced SSNMR opens up a vast and new space for extracting structural and functional information on challenging membrane proteins.
Pressure-based mapping of protein folding landscapes

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Detailed structural and energetic mapping of protein folding pathways is essential for revealing the sequence determinants of folding cooperativity and the population of excited states that may be important for function. Obtaining such information has proven experimentally challenging, as intermediates are generally sparsely populated and observables that report on detailed structural properties are few. Pressure leads to protein unfolding via the elimination of specific packing defects in the folded structure, unlike chemical denaturants which act due to interaction with exposed surface in the unfolded state. Hence, folding intermediates tend to be more highly populated in pressure denaturation experiments. NMR can provide very detailed local information across the entire protein, allowing structural characterization of these intermediates. We combined high pressure NMR unfolding experiments with structure based modeling to achieve a detailed and quantitative structural and energetic description of the folding landscape of the leucine rich repeat protein, pp32. Pressure-dependent decreases in the folded state NMR HSQC cross peak intensities at each amide were used to calculate for each pressure measured, a fractional contact map, where each fractional contact is the geometric mean of the experimental apparent fractional population of folded protein for the two residues involved in the contact. These fractional contacts were used to introduce bias in Structure Based Modelling. These course-grained simulations, constrained by the high pressure NMR data, allowed construction of a pseudo free energy profile characterizing the features of the folding free energy landscape of the wild type pp32 protein. Transformation of the generated conformational ensembles into a full atomistic models provided structural details of the folding intermediates and transition state ensemble throughout the p-T plane. We then explored the role of sequence on the characteristics of pp32 folding landscape by testing the effects of Leucine to Alanine cavity creating mutations and mutations that modify the capping motifs. We found that the apparent folding cooperativity of pp32 is strongly modulated by local structural stability of the different repeats.
I will describe the metainference method, a recently proposed Bayesian inference approach that integrates experimental information with prior knowledge and deals with all sources of errors in the data as well as with sample heterogeneity. I will then discuss how accurate and precise structural ensembles of proteins can be obtained using a combination of metainference with metadynamics, a powerful approach for the conformational sampling of proteins, and illustrate the application of this strategy to the calculation of the free energy landscape of intrinsically disordered proteins.

Pseudocontact shift from a non-local paramagnetic source

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Paramagnetic centres in biological molecules are a useful source of structural information because they generate distance- and direction-dependent pseudocontact contributions to the chemical shifts of the surrounding nuclei [1]. Paramagnetic shifts in general have multiple contributions, but at large distances from the paramagnetic centre only the pseudocontact shift (PCS) is significant.

PCS is commonly described using the point dipole approximation where the paramagnetic source probability density is assumed to be a delta function. For a given nucleus, the pseudocontact shift $\sigma$ depends on its position relative to the electron $\vec{r}$ and on the anisotropy of the magnetic susceptibility tensor $\chi$:

$$\sigma(\vec{r}) = \frac{1}{12\pi} \left[ \Delta \chi_{ax} \frac{2z^2 - x^2 - y^2}{r^5} + \frac{3}{2} \Delta \chi_{rh} \frac{x^2 - y^2}{r^5} \right]$$ (1)

The situation where the paramagnetic source cannot be assumed to be a point object is much less straightforward, but a partial differential equation has recently been proposed for an arbitrary distribution $\rho(\vec{r})$ of the source probability density [2]:

$$\nabla^2 \sigma(\vec{r}) = -\frac{1}{3} \psi^* \chi \vec{\nabla} \rho(\vec{r})$$ (2)

In this communication, this formalism is applied to commonly encountered PCS analysis problems. It is found to be particularly useful for the systems where the paramagnetic centre has some flexibility to move on the timescale of experiment and for those lanthanide-labelled proteins in which the position of the label is not well defined. In those situations the point model is not applicable and Equation (2) is the only way forward.

The inverse problem of recovering the probability density function and the susceptibility tensor from PCS data using Equation (2) may also be solved numerically with fast Fourier transforms and analytically using spherical harmonic expansion of the probability density function.

The techniques described in this communication are implemented in versions 1.8 and later of Spinach library (http://spindynamics.org) [3].

Residue-Specific Dynamics in HIV-1 Capsid Determined by Integrated MAS NMR, QM/MM, and MD Approach

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HIV-1 capsid (CA) protein is remarkably dynamic in the mature viral capsids. Internal motions of CA play critical roles in HIV-1 lifecycle, including viral maturation, capsid assembly and uncoating, and interactions with host factors. Anisotropic chemical shift tensors and dipolar order parameters, recorded residue-specifically in magic-angle-spinning NMR experiments on CA assemblies, provide a direct residue-specific probe of dynamics on nano- to microsecond timescales. We integrated NMR with molecular dynamics and Density Functional Theory calculations, to gain atomic-level insights into the internal backbone dynamics of CA. The results yield quantitative understanding into how the CSA and dipolar tensors are related to the local structure and the residue motions in CA. This integrated approach is applicable for dynamics studies of a wide range of biological systems beyond HIV-1 assemblies.
Sparse representations in NMR - what, when, how?

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NMR spectrum is a good example of a compressible data object. The reason is the sparsity i.e. the fact that major part of the spectrum does not contain peaks, but only noise. In other words, NMR spectrum can be equivalently represented by a relatively short list of peaks instead of hundreds of Mb large multidimensional dataset (e.g. fid or ser file). Yet, compressibility allows not only to save disk space, but also to effectively accelerate the measurement process. Nyquist sampling can be replaced with non-uniform sampling (NUS) and points of the signal omitted during the acquisition can be mathematically reconstructed under the assumption, that resulting spectrum is sparse. The sparsity-based reconstruction method, known as compressed sensing or compressive sampling (CS) has found numerous applications in various branches of science and technology. It is also implemented in most of NMR software. Moreover, sparsity constraint can be used in other fields of NMR, e.g. to regularize inverse Laplace transform used in processing of diffusion dimensions. This study will summarize the theory and selected applications of sparsity-based reconstructions in NMR.
Fokker-Planck equation as a unifying simulation formalism for all types of magnetic resonance that involve spatial degrees of freedom

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Fokker-Planck equations that describe the time evolution of probability distributions [1,2] were first used in a magnetic resonance context in the 1970s as part of the formalism that became known as the Stochastic Liouville Equation (SLE) [3-5]. To this day SLE remains the most general relaxation theory in magnetic resonance – it is non-perturbative and works at all magnetic fields and correlation times, from extreme narrowing to the solid limit.

Initial adoption of Fokker-Planck equations was complicated by large matrix dimensions and subsequent work had to rely heavily on sparse matrix libraries and Lanczos techniques [3], but the exponential rise in computing power in the following 40 years has removed the problem – the same calculations take just a few seconds on a contemporary workstation. The advances in computing power make the Fokker-Planck formalism worth re-visiting. One particular feature that to our knowledge remains unexplored in magnetic resonance is the possibility of treating any spatial dynamics that can be generated by a linear operator – the formalism itself is not restricted to diffusion. Although matrix dimensions do increase, the reduction in formulaic complexity is very remarkable: MAS, DOR, diffusion, PFGs, hydrodynamics, polychromatic irradiation and other methods that normally introduce inconvenient time dependence into the Hamiltonian become, within Fokker-Planck formalism, time-independent operators that are simply added to the background evolution Liouvillian. Even spherical grids are no longer needed – the Fokker-Planck formalism can solve directly for powder averages [6].

Allosteric Coupling in an Ion Channel: Solid State NMR Studies of KcsA

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Cell signaling in biological systems often involves key proteins in the lipid bilayer membrane, ion channels being an important example. Messages from the inside of the cell can be displayed on the outside, and vice versa, due to transmembrane allosteric changes in conformation and binding affinity. We have focused on allostery in an ion channel, full-length wild type KcsA channel in a native like membrane environment. New solid-state NMR methods are applied to obtain atom specific, structural and dynamic information on the channel opening and, inactivation. An important channel-inactivation process, known as C-type inactivation, is apparently universal in K⁺ channels, and partly controls the mean open time in a number of important channels in the human nervous system, thereby affecting the fidelity of signals. We recently showed that this activation mechanism has as its central step the evacuation of ions from the selectivity filter. Although there is still some controversy regarding this hypothesis, a number of insightful experiments form other laboratories lead to the same essential model. This slow spontaneous inactivation exhibits the classic signatures of transmembrane allostery, wherein the activated state is a metastable intermediate of the allosteric pathway. In ongoing work, we use NMR detected double titration experiments to show that K⁺ ion binding on the extracellular selectivity filter is strongly allosterically coupled to proton binding in the intracellular activation residues, over 30 Å away. These NMR titration studies also contain important information on the residues involved in the allosteric pathway. Functional studies of F103 in the hinge of the inner helix suggested an important role for its bulky sidechain in the inactivation; we recently showed that energetic strength of coupling of the gates is strongly altered when this residue is mutated to alanine, and characterized other mutants that are important for inactivation. These results provide quantitative site-specific measurements of allostery in a bilayer environment, and highlight the power of describing ion channel gating through the lens of allosteric coupling.
A combined computational and structural model of the full-length PRLR

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The prolactin receptor (PRLR) is an archetype member of the class I cytokine receptor family, comprising receptors with fundamental functions in biology as well as key drug targets. Structurally, each of these receptors represent an intriguing diversity, providing exceptionally challenging targets for structural biology. Thus, the molecular details of the cross-membrane signal transduction through these receptors remain obscure. The transmembrane domain (TMD) of the PRLR has been shown to be responsible for receptor homodimerization and is expected to be responsible for transmitting the signal that links extracellular hormone binding to the intracellular signaling platforms. In spite of these significant roles in the cytokine receptor systems, the membrane-embedded TMDs have remained largely understudied.

Using our new strategy for production of isotope-labeled single-pass TMDs [1] and with solution-state NMR spectroscopy as the main tool, we solved the NMR solution structure of the human PRLR-TMD. Furthermore, since structural studies of intact proteins with a globular domain in combination with a membrane embedded domain and an intrinsically disordered domain is exceptionally challenging, we accessed the molecular architecture of the full-length monomeric PRLR by combining experimental and computational efforts. [2] We collected structural data on overlapping fragments of the receptor with small-angle X-ray scattering, native mass spectrometry, and NMR spectroscopy. Along with previously published data these were combined by molecular modeling to generate a full receptor structure. The result provides the first full view of a class I cytokine receptor, exemplifying the architecture of more than 40 different receptor chains, and revealing that the extracellular domain is merely the tip of a molecular iceberg.

References


Interaction studies of the chemokine Interleukin-8 with the G protein-coupled receptor CXCR1

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G-protein coupled receptors (GPCRs) are the largest family of transmembrane receptors in eukaryotes. Although there are an increasing number of structures of GPCRs being determined, little is known about the exact conformational changes taking place in the receptor upon ligand binding. We are studying the interaction between the chemokine interleukin 8 (IL-8) and its receptor, the membrane protein CXCR1. Although a complete NMR structure of a GPCR complexed with a ligand is not available, it is known that residues of IL-8 have interactions with two sites on CXCR1: a high-affinity site on the flexible N-terminal domain and a secondary, lower affinity binding site on the extracellular loops.

We published the apo-CXCR1 structure and are currently studying the structure and dynamics of the CXCR1 - IL-8 complex, reconstituted in phospholipid bilayers. To understand the effect the different binding sites of CXCR1 have on IL-8, we have made 3 constructs of the receptor: wildtype CXCR1, 1TM-CXCR1 which contains only the primary binding site on the N-terminal domain as well as the first transmembrane helix, and NT-CXCR1 which is comprised of the full receptor without the N-terminal domain. Using solution NMR spectroscopy, MAS solid state NMR and proton detected fast MAS solid state NMR, we observed almost complete immobilization of IL-8 on 1TM-CXCR1 as well as on wildtype CXCR1 (figure 1). When looking at the receptor, we observe the same changes in dynamics; several residues in 1TM-CXCR1 and wildtype CXCR1 become immobilized upon addition of IL-8. We have also observed chemical shift perturbation, indicating a conformational change upon complex formation. Comparison of solid-state and solution-state NMR results will provide a valuable insight into understanding the interactions of the chemokine receptor and its ligand in the early stages of GPCR activation.

Figure 1. In A) solution NMR spectrum of IL-8. Comparison of proton-detected HETCOR spectra of IL-8 immobilized on CXCR1 (in B), 1TM-CXCR1 (in C) and NT-CXCR1 (in D).
An unrealized goal in structural biology is the determination of structure and conformational change at high resolution for membrane proteins within the cellular environment. Pulsed electron–electron double resonance (PELDOR) is a well-established technique to follow conformational changes in purified macromolecular complexes.1-3 Here we present the proofs of concepts for the use of PELDOR to observe conformational changes and protein-ligand interactions for a membrane protein in intact cells and native membranes.4-5 This method does not require detergent extraction, purification and reconstitution usually required for a membrane protein. With this approach, structure, function, conformational changes, and molecular interactions of outer membrane proteins can be studied at high resolution in native environments. Additionally, we show that the sensitivity of such measurements can be greatly enhanced by using orthogonal spin labels.

References
Loop dynamics of outer membrane protein OprG contribute to amino acid transport in *Pseudomonas aeruginosa*

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OprG is an outer membrane protein of *Pseudomonas aeruginosa* whose function as an antibiotic-sensitive porin has been controversial and not well defined. Circumstantial evidence led to the proposal that OprG might transport hydrophobic compounds by using a lateral gate in the barrel wall thought to be lined by three conserved prolines. In order to test this hypothesis and to find the physiological substrates of OprG, we reconstituted the purified protein into liposomes and found it to facilitate the transport of small amino acids like glycine, alanine, valine, and serine, which was confirmed by *Pseudomonas* growth assays. The structures of wild-type and a critical proline mutant were determined by NMR in dihexanoyl-phosphatidylcholine micellar solutions. Both proteins formed 8-stranded β-barrels with flexible extracellular loops. The interfacial prolines did not form a lateral gate in these structures, but loop 3 exhibited restricted motions in the inactive P92A mutant but not in wild-type OprG.
Spin labeling proteins by site directed spin labeling using Gd\textsuperscript{3+} chelates enables in-cell distance measurements by the DEER (double electron-electron resonance) technique because of the chemical stability of the labels in the reducing environment of the cell, and their high EPR sensitivity at frequencies higher than Q-band.\textsuperscript{1-3} Here explore the potential of in-cell DEER distance measurements to detect conformational states of proteins in the cell using Gd\textsuperscript{3+} labeled calmodulin (CaM). CaM is a Ca\textsuperscript{2+} binding protein expressed in all eukaryotic cells and it can bind and regulate a number of different protein targets, thereby affecting many different cellular functions. It has two domains, separated by a flexible hinge region and it contains four Ca\textsuperscript{2+} binding sites. In the Ca\textsuperscript{2+} free state the conformation is referred to as “closed”. After Ca\textsuperscript{2+} binding a conformational change to an “open” conformation takes place, enables binding the target protein and CaM undergoes a further conformational change to a “collapsed” conformation. This mechanism gives the target protein indirect Ca\textsuperscript{2+} sensitivity. We first explored the conformational states of CaM labeled with three different Gd\textsuperscript{3+} tags in solution on several double mutants of CaM. Two tags were conjugated via cysteine residues and one via conjugation to a genetically encoded unnatural amino acid.\textsuperscript{4} W-band DEER measurements on three of the mutants revealed all three different conformational states mentioned above for the following samples: CaM, Ca\textsuperscript{2+}-CaM and Ca\textsuperscript{2+}-CaM with a bound peptide. We then proceeded to carry the measurements in cell extracts and finally in HeLa cells, where the Gd\textsuperscript{3+} labeled CaM was delivered into the cells using electroporation.

Pulse EPR Dipolar Spectroscopy with High-Spin Mn$^{2+}$ Ions

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Pulse EPR spectroscopy is a valuable technique for the precise measurements of distances between paramagnetic species in the range of 1.8 up to 10 nm. Determination of such distances is useful for elucidating the structure and conformational flexibility of biomolecules. Since most biomolecules are diamagnetic, site-specific spin labelling with nitroxide spin species is commonly used for performing this kind of measurements. Recently, Gd$^{3+}$ and Mn$^{2+}$ high-spin systems have been introduced as spin markers for distance measurements in biological applications [1, 2]. From biological perspective Mn$^{2+}$ is interesting, because it is a natural catalytic active center in a number of enzymes and ribozymes. Furthermore, due to similar charge and ionic radius, Mn$^{2+}$ can replace Mg$^{2+}$, allowing to investigate nucleotide binding domains of membrane transporter complexes, G-proteins and metal binding sites in RNA riboswitches. We investigated the performance of dipolar spectroscopy experiments on synthesized model compounds, containing one or two Mn$^{2+}$ ions and nitroxide spin labels. PELDOR/DEER experiments and RIDME experiments have been performed at Q-band (34 GHz), W-band (94 GHz), G-band (180 GHz) and J-band (263 GHz) frequencies [3]. Distances and distance distributions determined by these experiments will be compared with the known structure of the model compounds. Modulation depths and signal quality achieved at high magnetic fields with the different methods will be also compared and discussed.


Figure 1. PELDOR experiments on a Mn$^{2+}$-nitroxide complex were performed. At 1.2 T the Mn$^{2+}$-nitroxide distance was determined by probing both spins. PELDOR obtained at 6.4 T provided as well the orientation of the nitroxide with respect to the dipolar vector connecting the spins.
Multi-Extreme THz ESR: Recent Developments in Kobe


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Recent developments of multi-extreme THz ESR (Electron Spin Resonance) in Kobe will be presented. Here multi-extreme includes the high magnetic field, the high pressure and the low temperature. At present we can cover the frequency region between 0.03 and 7 THz [1], the temperature region between 1.8 and 300 K [1], the magnetic field region up to 55 T [1], and the pressure region up to 1.5 GPa [2] simultaneously. Multi-frequency measurements are realized by the transmission measurement using various light sources such as Gunn Oscillators, multipliers, backward wave oscillators (BWO) and the optically pumped far-infrared (FIR) laser [1]. Pulsed magnetic field is used to apply up to 55 T.

In order to increase the pressure from 1.5 GPa, which was achieved by the transmission type piston cylinder pressure cell made by NiCrAl alloy and the ceramic piston parts, we have developed the hybrid-type pressure cell, which consists of the NiCrAl alloy inner cell, the Cu-Be alloy outer cell and the ceramic piston parts [3]. Although the magnetic field is reduced to 10 T due to the increase of outer diameter of hybrid-type pressure cell, we have achieved 2.7 GPa for our multi-extreme THz ESR [3]. High pressure THz ESR measurement up to 2 GPa at 2 K is applied to study the Shastry-Sutherland Model Substance SrCu$_2$(BO$_3$)$_2$ [3].

Moreover, highly sensitive mechanically detected micro-cantilever ESR, which enables the measurements of micrometer size sample, has been also developed [4]. Recently we have achieved the micro-cantilever ESR measurements of Co-Tutton salt up to 1.1 THz using the torque method and 15 T super conducting magnet. To our knowledge the micro-cantilever ESR measurement up to 1.1 THz is the world record for such mechanical detection of ESR [5]. In connection development on the magnetization detected ESR using SQUID (Superconducting Quantum Interference Device magnetometer (SQUID ESR) has been also achieved [6].

Pulse dipole-dipole EPR distance measurements in DNA duplexes at physiological temperatures

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Pulse dipole–dipole electron paramagnetic resonance (EPR) spectroscopy (double electron–electron resonance (DEER) or pulse electron–electron double resonance (PELDOR) and double quantum coherence (DQC)) allows for measurement of distances in biomolecules and can be used at low temperatures in a frozen solution [1]. In this work a physiological temperature distance measurements by pulse dipole-dipole EPR in DNA duplexes using different types of spin labels (triaryl methyl (TAM) and 2,5-spiro substituted nitroxides) [2-7] and different ways of immobilization of biomolecules (disaccharides, silica gels) [5,7] and as well as different structure of linker will be reported. We investigated a series of nitroxides with different substituents adjacent to NO-moiety including spirocyclohexane, cyclopentane, tetraethyl and tetramethyl groups. Electron spin relaxation times (T₁, T₂) of these radicals immobilized in trehalose were measured at room temperature at X- and Q-bands (9/34 GHz) [5]. In addition, a comparison was made with the corresponding relaxation times in nitroxide-labeled DNA immobilized in trehalose.

We compared the structure of native and TAM-labeled DNA duplexes by means of NMR analysis and MD simulations. MD and NMR showed that distance measurements at physiological temperatures by EPR allow researchers to obtain valid structural information on an unperturbed DNA duplex. Thus, we showed that distance measurements at physiological temperatures by the Q-band DQC method allow researchers to obtain valid structural information on an unperturbed DNA duplex using terminal TAM spin labels [6].


Figure 1. Comparison of structure of TAM-labeled (red), native (blue), and native NMR-restrained D1/D2 DNA duplexes (green) obtained by MD simulations.
MRI and EPR based Imaging Biomarkers to Guide Treatment in Tumor Bearing Mice

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Pancreatic cancer is a malignant neoplasm with an extremely poor prognosis. The 5-year overall survival rate is below 10%. A new drug TH-302 in combination with gemcitabine was approved for Phase 3 clinical trial in locally advanced or metastatic pancreatic adenocarcinoma in December 2012. The TH-302 is a kind of drugs known as hypoxia-activated prodrug (HAP), which activated under hypoxia (low concentration of oxygen) and exhibits anti-tumor effect. A question derived is how clinicians select patients who receive most benefit from treatment with this hypoxia targeting new drug, instead of classical radiation therapy or gemcitabine monotherapy. Electron paramagnetic resonance imaging (EPRI) can non-invasively provide 3D absolute oxygen images, which profile the tumors on a physiological basis. Similarly ¹³C MRI using Hyperpolarized tracers provide an assessment of the metabolic status of tumors. Here, we investigated if the EPR oxygen imaging and ¹³C MRI can predict treatment benefit of oxygen dependent or independent therapies in three different pancreatic cancer xenografts.

Three human pancreatic cancer cells Hs766t, MiaPaca2, and Su8686 were subcutaneously inoculated in hind leg of athymic nude mice. The tumor bearing mice were treated with TH-302 (80mg/kg, ip, 5 days), X-radiation (3Gy, 5 days), or gemcitabine (150mg/kg, ip, twice a week). Tumor oxygen imaging was conducted by a homemade 300 MHz pulsed EPRI scanner using an oxygen sensitive triarylmethyl probe OX063, followed by anatomic MRI scan. These animals were also studied using ¹³C MRI after injecting hyperpolarized ¹³C pyruvate.

Three pancreatic cancer cell lines showed large difference in tumor oxygenation. Tumor median pO₂ values are 9.1±0.7 mmHg for Hs766t, 11.1±1.0 mmHg for MiaPaca2, and 17.6±1.1 mmHg for Su8686. The lactate/pyruvate ratios from ¹³C MRI studies were also derived and found to follow Hs766t>MiaPaca2>Su8686. The imaging studies find that the most hypoxic tumor (Hs766t) was also the most glycolytic. TH-302 treatment provided survival benefic of 28.6 days in hypoxic Hs766t tumors but only 1.0 days in the most oxygenated Su8686 tumors. In contrast, tumor growth delay by radiotherapy was 10.3 days in Hs766t, 18.6 days in MiaPaca2, and 19.3 days in Su8686 tumors. Gemcitabine treatment was effective in both hypoxic and oxygenated tumors but there seemed to be most effective against the hypoxic Hs766t tumors.

Quantitative oxygen images by 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.
Protein Folding on the Ribosome

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The folding processes of nascent chains are intricately linked to their chain elongation, which occurs in a vectorial manner as the N-terminal part of the nascent chain emerges from the ribosome. While increasingly detailed pictures are emerging of the structures of ribosomes are emerging, little is known at the atomic level about the structural and co-translational folding properties of nascent polypeptide chains. The use of NMR spectroscopy on ribosomes and ribosome nascent-chain complexes (RNCs) is providing detailed structural insights of the conformations of protein chains while they are being created on the ribosome. By producing in-vivo derived RNCs in which the nascent polypeptide is selectively isotopically-labelled, recent work has allowed us to use NMR follow co-translational folding processes at a residue-specific level (1,2).

We have studied RNCs of a tandem of immunoglobulin-like domains (1) where NMR reveals a folded N-terminal domain and a disordered but compact C-terminal domain. Most significantly, in order to study how Dom5 acquires its native structure co-translationally, we shortened the RNC constructs by reducing the length of FLN6. We found that the ribosome modulates folding, as the complete Dom5 sequences emerges well beyond the tunnel before acquiring native structure, while in isolation it folds spontaneously, even when truncated. This suggests that regulating structure acquisition during biosynthesis may reduce the probability of misfolding, particularly as homologous domains emerge from the ribosome. We compare these findings to those of a model nascent chain system of misfolding prone, alpha-synuclein (2).

Our recent developments in the use of NMR (1-6) to produce the first experimentally derived structures (1,2) of ribosome nascent chain folding will be discussed. These combine data on the same samples from NMR and cryo electron microscopy. These studies are allowing us to describe structures sampled during the vectorial emergence, the interactions of the emerging chains with the ribosome (1, 2) and also how the molecular chaperone, the trigger factor (2) interacts with the nascent chain.

References:
Rapid membrane-transport kinetics of fluorinated sugars in human erythrocytes via $^{19}$F NMR

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Sugar-ring fluorination is an emerging strategy in the design of drugs that interact with carbohydrate-binding proteins. We explored this approach by investigating the interactions between several fluorinated analogues of glucose and the GLUT1 glucose transporter in whole human erythrocytes.

Due to the high sensitivity of $^{19}$F nuclei to the local molecular environment, they display separate NMR resonances from extra- and intra-cellular spin populations [1]. This fortuitous property allowed us to quantify transmembrane exchange rates of the studied fluorinated sugars using $^{19}$F NMR EXSY experiments (Fig. 1). Data analysis was performed using simulations of the 2D-EXSY spectra in the presence of scalar coupling networks using the software Spinach [2].

The differences in catalytic activity of GLUT1 on various fluorinated sugars were interpreted in terms of altered lipophilicity [3] and hydrogen bonding in the protein's binding site. The findings of this study will be discussed in terms of prospects to use fluorinated compounds to probe, enhance, or inhibit sugar-binding enzymes, transporters and receptors. The developed theory will be of general applicability to other proteins, substrates and cellular systems, including studies in vivo.

Macromolecular crowding inside cells reduce the $T_1$ of small carboxylic acids

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dDNP has since its invention in 2003 been applied in several fields ranging from molecular imaging to chemical engineering. The significant boost in sensitivity obtained by dDNP continuously leads to new application areas. The technique, however, introduces uncertainties in the NMR measurements that currently hamper the use of dDNP for quantitation.

One of the main challenges is knowledge of the longitudinal relaxation parameter, $T_1$. In the study of in vivo metabolism the incomplete knowledge of $T_1$ for different species in different environments leads to data that prevents quantitative kinetic models of cellular systems.

We have developed an assay for measurement of the intracellular $T_1$ for small carboxylic acids in living cells. Using this assay the intracellular signal of the $^{13}$C carbon in the carboxylic acid moiety of acetate, butyrate, keto-isocaproate and pyruvate could unambiguously be measured, Figure 1. The intracellular $T_1$ turn out to be up to 4 times lower than the extracellular $T_1$. Such large difference in $T_1$ inside and outside the cell is shown to have significant influence on the quantification of intracellular metabolism.

It is expected that the significant shorter $T_1$ of carboxylic moieties inside cells is a result of macromolecular crowding. An “artificial cytosol” has thus been made and used as a prediction tool for predicting $T_1$ of other carboxylic acids. We demonstrate the value of this prediction tool.

Figure 1. Time series of $^{1-13}$C-butyrate inside (pH 6) and outside (pH 4) yeast cells in suspension.
In-cell NMR observation of the biological events within living cells

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Recent advance in the NMR methodology, termed in-cell NMR, enables the observation of the NMR signals of the target protein inside the living cells. Using the in-cell NMR methods, one can investigate the post-translational modifications, protein folding stabilities, and protein maturations at an atomic resolution within the intracellular environments. However, due to the anaerobic environment within the NMR tube, the deterioration of the intracellular environments causes the increase of dead cell population during NMR measurements, thereby limiting the application of various NMR methods with a long experimental time. To overcome this problem, we developed a novel bioreactor system, in which fresh culture medium is continuously supplied to cells encapsulated in thermoreversible Mebiol gel inside the NMR tube (Angew Chem Int Ed (2013) 52, 1208-11). It was demonstrated that the intracellular ATP concentration, which was depleted within 30 min without the bioreactor, can be maintained more than 22 hours in the bioreactor. In addition, the population of the dead cell was significantly suppressed after 15 hours of incubation (Fig.1).

As will be described in this talk, we applied this bioreactor system to observe the biological events, including the intracellular protein-protein interactions, and the stress-induced response of the intracellular protein.

Figure 1. (A)Schematics of the Bioreactor system. (B) The intracellular ATP concentration and (C) the dead cell population before and after NMR measurements (left) without and (right) with use of the Bioreactor.
NMR in Cellular Structural Biology: from structures to functional processes

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NMR spectroscopy constitutes a unique tool for describing functional biological processes at atomic level and in a cellular context. NMR is indeed suitable not only for characterizing the structure and dynamics of biomolecules but, even more importantly, to describe transient interactions and functional events with atomic resolution, possibly in a cellular context. This approach requires the development of suitable methodologies capable of addressing multiple, specific, and sometimes non-conventional aspects and amenable to characterize functional processes in living cells, also integrating these data with those obtained in vitro.

Along a functional process, most interactions are transient in nature, suitably studied by NMR, which can also characterize processes in living cells with atomic resolution. Among processes involving transient interactions there are the metal transfer processes, in which metal ions are transferred from metal transporters to the final recipient proteins through a series of protein-protein interactions\(^1\). This transfer is determined by metal affinity gradients among the various proteins, with kinetic factors contributing to the selectivity of the processes\(^2\). The presence of paramagnetic centers, such as iron-sulfur clusters, dramatically affects the NMR spectra, requiring tailored experiments, which can also be effectively complemented with EPR spectra\(^3\). In-cell NMR can provide the description of these processes within living cells\(^4,5\). The cellular level characterization can be then integrated with optical and XRF imaging data which all together can provide the atomic resolution description at a cellular level\(^6\).

The power of this combined approach will be presented for a few pathways responsible for copper trafficking in the cell and for the biogenesis of iron-sulfur proteins. New major advancements in in-cell NMR\(^4,5\) and in the characterization of highly paramagnetic systems will be also discussed within an integrated approach where, from single structures to protein complexes, the processes are described in their cellular context within a molecular perspective.

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Silicon donors get down to bismuth –
Clock transitions and cavity-induced spin relaxation

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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]. Furthermore, recent studies have moved down Group V from more commonly studied donors, such as phosphorus, to bismuth which has been found to have several interesting properties, deriving from the large ($I = 9/2$) nuclear spin of $^{209}$Bi and large ($A = 1.47$ GHz) hyperfine coupling between the electron and nuclear spins of the donor.

First, Bi donors in silicon possess so called ‘clock’ or ‘ZEFOZ’ transitions whose frequency have zero first-order dependence on the magnetic field, making them robust to many sources of decoherence. At these points, coherence times up to 3 seconds have been measured for the electron spin in isotopically-enriched $^{28}$Si, while times approaching 1 second can be measured in natural silicon using dynamical decoupling methods [3]. Second, the large nuclear spin and hyperfine coupling yield allowed transition frequencies of approximately 7.3 GHz at zero applied magnetic field, making Bi donors in silicon of interest for coupling to superconducting aluminium circuits such as resonators and qubits. This has enabled studies of relatively small spin ensembles (~10,000 donors) coupled to micron-scale superconducting microwave resonators with Q-factors of approximately 100,000 [4]. In this regime, spin-relaxation by spontaneous emission can be enhanced by the cavity (by the Purcell effect [5]) to the point where it becomes the dominant relaxation mechanism [6]. We expect such cavity-induced spin relaxation will be important in future studies of ESR at mK temperatures, while the ability to accelerate relaxation of particular transitions may also have applications in DNP.

Magnet Technology Suitable for 30 T NMR & 20 T Human MRI

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Superconducting (SC) magnet technology suitable for both NMR and MRI is undergoing dramatic change at present for a variety of reasons. Dramatic increases in field are expected to be realized in the next few years. Preliminary coil designs and technological options for NMR magnets up to 30 T and human MRI magnets up to 20 T will be discussed.

**NMR:** The highest field available routinely for NMR experiments today is 23.5 T (1.0 GHz) which pushes the Low-Temperature Superconductors (LTS), NbTi and Nb\(_3\)Sn, to their limit. In 1986 the High-Temperature Superconducting (HTS) materials were discovered. There followed a 20-year period of development of a number of HTS materials in a number of formats, none of which were suitable for ultra-high-field (UHF) magnets.

In 2007 a new form of REBCO (Rare-Earth-Barium-Copper-Oxide) tape was produced by SuperPower. Unlike previous HTS materials, this product is fully processed at the factory, is very strong and stiff, and can be protected in case of a quench. This was the start of a 10-year period of development of HTS conductors suitable for UHF magnets and the development of the first such magnets. In 2013 a reinforced version of Bi2223 tape became available from Sumitomo and a reinforced version of Bi22212 was demonstrated this year. The first UHF SC magnet using an HTS section is now operational in Sendai, Japan, providing 24.5 T. The NHMFL expects to have a 32 T SC system as well as a 36 T, 1 ppm resistive-superconducting hybrid operational in mid-2016.

The next 5-10 years should be a period of propagation of HTS magnet technology into a number of magnet types, including UHF NMR.

**MRI:** Most clinical work is limited to 3 T. Recently 7 T MRI became a commercial product. The highest field available today for human head MRI research is 9.4T while systems in development are expected to reach 10.5 T and 11.7 T in the next few years. MRI magnets suitable for small animals exist at fields up to 21 T, and images from these systems demonstrate dramatic improvements in resolution and/or data-acquisition time via application of higher fields and justify a serious consideration of pursuing 20 T for human-head imaging.

Historically, the field available for human MRI has been primarily limited by safety regulations which are regularly being relaxed.

All of today’s head and whole-body MRI magnets rely on NbTi superconductors and most rely on copper for structural reinforcement. Going beyond 11.7 T will require use of Nb\(_3\)Sn. The NHMFL has built two magnets with field/bore combinations of 14.2T/60cm and 13T/50cm using Nb\(_3\)Sn cables reinforced with steel. Conceptual designs of 600 MHz (14.1 T) and 20 T NMR magnets using both Nb\(_3\)Sn and NbTi superconductors and steel reinforcement have been developed. Preliminary design calculations suggest that a 20 T head MRI system incorporating HTS materials would be of comparable size to the 11.74 T Iseult magnet presently under development. This surprising results is due to 1) using a 68 cm bore instead of 90 cm, 2) the higher current density of HTS materials at 20 T compared to NbTi as 12 T, and3) using steel to support the Lorenz forces instead of Cu.
Solid State NMR of Protein Assemblies and Related Topics: New Methods and Results


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Ongoing research in our lab includes structural studies of peptide and protein fibrils associated with neurodegenerative diseases, structural studies of HIV-1 capsid assemblies, and the development of new methods for low-temperature dynamic nuclear polarization (DNP) and magnetic resonance imaging (MRI). In the area of fibrils, we have characterized the diversity of 40- and 42-residue amyloid-β fibril structures in cortical tissue samples from a large set of Alzheimer’s disease patients, showing that most patients develop the same predominant 40-residue fibril structure, but that patients with rapidly progressing neurodegeneration develop a heterogeneous mixture of fibril structures. In the area of HIV-1 capsid assemblies, we have determined the molecular structure of the intermolecular dimerization interface within the mature capsid protein lattice and have obtained direct evidence for α-helix formation by a segment spanning the CA-SP1 junction within the immature lattice. In the area of low-temperature solid state NMR, we are developing and applying methods for characterizing transient intermediates in protein folding processes, using rapid freeze-quenching and DNP. We have also developed novel equipment for micron-scale MRI at low temperatures, compatible with DNP. In this talk, the most recent progress in these projects will be presented.
Diffusion MRI methods for complex anisotropic materials

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The structure of a porous material is imprinted on the pattern of translational diffusion of the liquids located in the pore space. Non-invasive MRI measurements of self-diffusion are thus an attractive means of studying the microscopic structure of non-transparent and sensitive materials such as the living human brain, e.g., to detect pathological conditions or to study normal brain development. Conventional methods give information that is averaged over the entire investigated sample or volume element, and fails to distinguish between properties such as pore size, shape, and orientation in case the material is heterogeneous. This presentation deals with recent progress in the field of advanced diffusion MRI methods to disentangle the confounding effects of the various pore space parameters, thereby enabling detailed characterization of anisotropic materials with complex architecture.

 Whereas conventional diffusion MRI relies on acquisition protocols where the size and orientation of the diffusion-encoding tensor \( \mathbf{b} \) is varied, our methods (see Figure 1) in addition utilize the \textit{shape} of \( \mathbf{b} \) [5,7]. As shown with the example results in Figure 2(a), analysis of data acquired as a function of the size, shape, and orientation of \( \mathbf{b} \) yields maps of microscopic diffusion tensors \( \mathbf{D} \) and orientational order tensors \( \mathbf{S} \), as well as conventional voxel-averaged diffusion tensors \( \langle \mathbf{D} \rangle \) wherein the information about local anisotropy and orientation is entangled [3]. In case of multi-compartment materials, the data can be converted to 2D size-shape distributions of microscopic diffusion tensors, see Figure 2(b) [6].


Figure 1. MRI pulse sequence for axisymmetric diffusion encoding. The images are read out with RARE or EPI while a spin echo block with smoothly modulated gradient waveforms encode the signal for translational motion. The gradient modulations are adjusted to provide diffusion encoding tensors \( \mathbf{b} \) spanning the range of shapes from linear to planar and spherical. The sequence has been implemented on microimaging equipment [1-3] and clinical scanners [1,2,4,5]. (Adapted with permission from ref. [3])

Figure 2. (a) Disentangling the effects of microscopic diffusion anisotropy and orientational order using axisymmetric diffusion encoding. The glyphs represent tensors mapped with 300 µm in-plane resolution for a lamellar liquid crystal in a 5 mm glass tube. (b) 2D size-shape diffusion tensor distribution for composite sample with a 5 mm glass tube with a reverse hexagonal liquid crystal inside a 10 mm tube with a yeast cell suspension. The glyphs symbolize the microscopic diffusion tensors of the three resolved components of the distribution (large sphere: extracellular water, stick: water in liquid crystal, small sphere: intracellular water) (Adapted with permission from refs. [3] and [6])
xSPEN: Single-shot MRI with exceptional resilience to field heterogeneities

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Central to MRI is Mansfield’s echo-planar imaging (EPI) proposition [1], a single-shot approach relying on oscillating gradients to rapidly scan the k-space. During recent years an alternative single-shot method has emerged, based on spatiotemporal encoding (SPEN) concepts [2, 3]. SPEN provides similar acquisition durations, sensitivity and resolution as EPI, but with an enhanced immunity to B₀ inhomogeneities and susceptibility distortions [4, 5]. Still, instances will arise where SPEN’s immunity is insufficient to deliver meaningful data; this study introduces an alternative modality that we denominate cross-term spatiotemporal encoding –xSPEN for short.

xSPEN possesses good sensitivity and remarkable resilience to field inhomogeneities despite involving only FT processing and demanding no a priori information.

xSPEN departs from conventional k-space scanning, and relies on a spatiotemporally encoding of the image being sought. Unlike hitherto proposed SPEN methods, this encoding does not utilize only a field gradient along the direction being probed, but adds an ancillary frequency broadening; for instance by adding an orthogonal field gradient –to which field inhomogeneities add– in order to both encode and decode the image (Fig. 1). Images along the “y” axis are thereby read out in xSPEN by applying a “z” gradient: the same inhomogeneities that encode the image thus decode it. 2D single-shot scanning incorporates an oscillating ±Gₓ readout gradient sampling the kₓy-space of one of the axes, and a constant gradient Gz charged with both the z-slice selection and the reading out of the y-axis dimension. Figure 1b illustrates how these oscillating and constant gradients transverse the resulting “hybrid” (kₓy) space. All that such sequence needs is to transform the resulting signals into xSPEN images, is splitting the resulting FID string into ±Gₓ-sampled segments, position these in their correct 2D space coordinates, subject these rearranged arrays to a 1D FT vs kₓ, and display in magnitude-mode the resulting matrix. Figure 2 illustrates xSPEN’s potential with experiments conducted under progressively degraded field homogeneity conditions.

Brain stem zoomed echo planar imaging with optimal control parallel transmit at 7T

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Background: Ultrahigh-field MR with field strengths at 7T and above are becoming increasingly available these days. Multi-channel radio frequency (RF) transmit setups, or parallel transmit (pTx), often accompany such ultrahigh-field systems due to the promising advantages provided by the extra degrees of freedom in comparison to conventional single channel transmit setups. Ultrahigh-field MR is challenged by considerable B1 inhomogeneity, strong magnetic susceptibility induced field variations, and shorter T2 relaxation times. By principles of Transmit SENSE1, advanced pTx RF pulses can exploit the spatial B1 variations for reduction of pulse durations making the pulses less sensitive to B0 inhomogeneity and T2 relaxation.

Aim: To obtain high-quality in vivo echo planar imaging (EPI) zoomed brain stem images on a 7T system by optimized 2D spatial-selective pTx RF pulses.

Methods: To design these advanced pulses we used a gradient-based, low-memory BFGS2 optimal control method.3 To assure clinically feasible pulse preparation times, we suffice with optimization gradients precise to first order.4 The system used an 8/32 transmit/receive head coil (Nova Medical, Wilmington, MA, USA) in a 7T scanner (Siemens Medical Systems, Erlangen, Germany) with gradient and slewrate limits of 70 mT/m and 200 T/m/s, respectively. The excitation k-space trajectory followed a gradient and slewrate limited (70%) spiral. We targeted pulses with one-, two-, and three-fold pulse duration reductions, i.e., pulse durations of 14.7, 7.6 and 5.2 ms. The spin echo EPI sequence with a GRAPPA factor 2, 16 averages, 

\[ T_F = 76 \text{ ms}, \ T_R = 3.7 \text{ s}, \]  

yielded 83x64 mm² reduced field-of-view (FoV) scans (voxel 0.5x0.5x1.0 mm³). Fat suppression was applied and bright cerebrospinal fluid was suppressed with an adiabatic inversion pulse (T1 3.5 s). B0 and B1+ maps were obtained with a dual echo GRE scan and the DREAM⁶ sequence, respectively.

Results and discussion: The reduced-FoV scans are shown in Fig. 1. We see an expected increase in the mean square error (MSE) of the scans with pulse reductions as compared with that of no reduction. However, structural similarity and details are well preserved throughout the scans, and the outer volume is greatly suppressed by our proposed sequence.

Conclusion: We have demonstrated a clinically feasible zoom-EPI method⁷ with pulse duration reductions for the challenging brain stem region.


Figure 1. Three 2D pTx RF EPI scans with no pulse duration reduction (a), 2-fold (b) and 3-fold (c).
Long-lived spin states as a source of contrast in magnetic resonance spectroscopy and imaging

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A method is proposed [1] to create Long-Lived spin States (LLSs) from longitudinal spin magnetization \( M_s \), which is based on adiabatic switching of an RF-field with properly set frequency. The technique is simple to implement with standard NMR equipment and provides an excellent efficiency of conversion of population from the triplet \( T_+ \) (or \( T_- \)) state to the singlet state of a pair of spins and back. The efficiency of the \( M_s \rightarrow \text{LLS} \rightarrow M_s \) conversion is equal to \( 2/3 \), being to the upper limit for the conversion efficiency of such type [2]. The method has been tested for the amino acid tyrosine and its partially deuterated isotopomer; for the deuterated compound, we have achieved an LLS lifetime, which exceeds the longitudinal relaxation time by a factor of 21. Furthermore, by slightly modifying the method, an improved contrast with respect to LLSs in NMR spectra is achieved; contrast enhancements of more than 1200 are feasible. This enables efficient suppression of longitudinal spin magnetization in NMR allowing one to look selectively at LLSs.

Additionally, we propose a modification of the method for a situation where the NMR spectrum is poorly resolved, namely, when a spin pair is strongly coupled forming an AB-system. The modification exploits the Spin-Locking Induced Crossing (SLIC) technique [3]; however, in contrast to the original SLIC technique, the \( M_s \leftrightarrow \text{LLS} \) conversion is due to adiabatic passage through a level crossing upon variation of the RF-field strength. The scheme works perfectly for pairs of strongly coupled spins. We envisage that this method is useful for generating LLSs in nearly equivalent spin pairs in specially designed molecules [4] and in low-field studies of singlet spin order.

The achieved spectral contrast with respect to LLSs opens new avenues in NMR spectroscopy. We have demonstrated that LLSs in CH\(_2\)-groups of the glycine residues in a pentapeptide Met-enkephalin and a protein ubiquitin can be created and selectively assessed, no matter whether the spin pairs are weakly or strongly coupled. Interestingly, not only spectral but also spatial contrast can be achieved: we have obtained spatial NMR images with strongly improved contrast originating from the difference of LLS lifetimes at different positions in the sample, see Figure 1.

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Imaging microstructure with double diffusion encoding

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Introduction: Diffusion magnetic resonance imaging is an attractive modality in neuroscience, due to its exquisite sensitivity to structure on scales far surpassing the nominal voxel resolution, and where biological changes associated with both normal and pathological plasticity occur. A recent extension to the diffusion toolbox is the double diffusion encoding (DDE) sequence¹⁻⁴ (see Fig. 1), where the classical Stejskal-Tanner⁵ diffusion encoding block is repeated after a variable mixing time. From the behavior of the signal as a function of the angle between the diffusion wave vectors, it is possible to distinguish macroscopically isotropic systems consisting of isotropic domains from those consisting of anisotropic domains in a model independent fashion. Furthermore, DDE can be used to distinguish crossing fibers from curving fibers, and has been used to measure dimensions of microscopic compartments⁶⁻¹².

Theory: As an example of the applications of the DDE, we consider the quantification of microscopic diffusion anisotropy $\varepsilon$, i.e. anisotropy within micro-domains. In the limit of long mixing time, the parallel perpendicular signal (S) difference is isotropic, giving the fractional anisotropy (FA) of diffusion within the micro-domains. When macroscopic anisotropy is present also, one has to powder average the diffusion signal over the rotation group $SO(3)$¹¹ for each pair of the diffusion wave vectors $(\mathbf{q}_i, \mathbf{q}_j)$.

$$\varepsilon = \left( \log S(\mathbf{q}_i \parallel \mathbf{q}_j) - \log S(\mathbf{q}_i \perp \mathbf{q}_j) \right) / q^4 = \frac{2 \Lambda^2}{5} ((\lambda_1 - \bar{x})^2 + (\lambda_2 - \bar{x})^2 + (\lambda_3 - \bar{x})^2),$$

where $\lambda_i$ are the eigenvalues of the single domain diffusion tensors. From Eq. (1) and the mean diffusivity, microscopic fractional anisotropy ($\mu$FA) can be computed, giving the fractional anisotropy (FA) of diffusion within the micro-domains.

Figure 1. DDE sequence. We consider the special case $\delta_i = \delta_i = \delta_1 = \Lambda_1$, and $\mathbf{G}_i = |\mathbf{G}_i|$. The diffusion wave vector is $\mathbf{q}_i = \gamma \mathbf{G}_i$. From¹¹.

Results: Fig. 2 shows $\mu$FA acquired in a phantom (liquid filled hollow fibers with mean diameter of 13.4 μm), demonstrating how $\mu$FA is unaffected by fibre distributions, in contrast to conventional FA. Fig. 3 presents maps of FA, $\varepsilon$ and $\mu$FA acquired in a fixed vervet monkey brain. Arrows point to locations where FA drops due to complex fiber arrangements, whereas $\mu$FA is unaffected.

Conclusions: DDE can provide access to microstructural features difficult to obtain with traditional diffusion encoding. An example is $\mu$FA, which could be used to disentangle distinct contributions to FA, such as fibre density and fiber dispersion.


Figure 2. Experimental DDE results from a physical phantom of a single fibre bundle (A), two crossing fibre bundles (AB) and 3 crossing fibre bundles (ABC). From¹¹.

Figure 3. DDE experiments in fixed vervet monkey brain yielding maps of conventional fractional anisotropy (FA), $\varepsilon$ and $\mu$FA.¹¹.
Structural investigations on channelrhodopsin and OmpG by MAS triple-resonance spectroscopy and DNP


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magic-angle-spinning, dynamic nuclear polarisation, membrane proteins, biradicals

Structural investigations of membrane proteins in native lipid environment by MAS NMR, including very fast spinning and the application of dynamic nuclear polarisation, are presented. Our studies involve channelrhodopsin and outer membrane protein G form e.coli, OmpG. A solid-state NMR structure of OmpG will is presented that deviates from the X-ray structure with respect to loop arrangements. It was obtained by making use of data from fast MAS NMR experiments at 60 kHz spinning, and from amino acid-selectively labelled samples. The value of the various approaches will be discussed.

In future, a major factor facilitating such investigations will be dynamic nuclear polarisation (DNP), which was introduced to increase signal-to-noise by one or two orders of magnitude. During the DNP process, electron polarization is transferred to the surrounding 'core' nuclei, subsequently to the bulk nuclei, and then further on to the molecule of interest. This process depends on several factors; among them are the relaxation behavior of the electrons and protons in the sample. In order to improve the quality of DNP spectra and to obtain maximum signal-to-noise, new radicals were synthesized and employed in measurements of protei samples around 190K. Enhancements in the range of 15-20 were observed in this temperature range while acceptable spectral resolution is observed. In this context, mechanisms of polarization propagation are analyzed and schemes for optimization of samples will be presented. Applications of DNP to a membrane protein system, channelrhodopsin will be presented. It was found that its ground state of the photo cycle is solely consisting of the all-trans form. Possibilities for tracking the proton pathway will be discussed.
Assignment and atomic-resolution structure of an Aβ(1–42) amyloid fibril

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Structure determination of amyloids by solid-state NMR is a powerful but also tricky method. In this contribution we focus on details of the NMR spectroscopy employed and critical points to be considered for data analysis.

Amyloid-β (Aβ) is present in humans as a 39–42 amino-acid residues metabolic product of the amyloid precursor protein (APP). Although the two predominant forms Aβ(1–40) and Aβ(1–42) differ in only two residues, they display different biophysical, biological, and clinical behavior. Aβ(1–42) is the more neurotoxic species, aggregates much faster, and dominates in senile plaque of Alzheimer’s disease (AD) patients. Aβ(1–42) amyloid fibrils are therefore a pathological hallmark of AD and play an important role in disease progression and cell-to-cell transmissibility. Here, we assigned all 38 visible residue and solved the 3D structure of a Aβ(1–42) fibril polymorph from solid-state NMR and mass-per-length measurements from electron microscopy. Residues 15-42 form a rigid cross-β-sheet entity with maximally buried hydrophobic side-chains. Residues 1-14 are partially ordered and in a β-strand conformation, but do not display unambiguous distance restraints to the remainder of the core structure.

The structure is compared to the one of other Aβ(1–42) preparation described in the literature and to the Osaka mutant Aβ(1–40) E22Δ.

PAR spectrum of Aβ(1–42) and backcalculation from 3D structure (crosses).
Structural insight to the interaction between a new Aβ fibril morphology and a Congo red derivative Alzheimer’s Disease marker

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Diagnosis of Alzheimer’s disease is still based on symptom development rather than direct visualization of Amyloid deposits in the brain.1 The major component of the amyloid deposits, Amyloid β (Aβ) fibrils, have therefore become a natural target for development of small organic markers like the Congo red derived FSB amyloid tracer.

We present a new fibril morphology of Aβ(1-40) and the characterization of its underlying atomic structure (figure 1A). Our model is based on chemical shift derived secondary structure and distance restraints from ssNMR and energy-filtered transmission electron microscopy. Based on both the assigned chemical shifts and the calculated structure, our fibril morphology displays clear differences to the existing deposited Aβ structures2,3,4.

To explore the binding of diagnostic amyloid-specific molecular probes, we investigated the binding of the FSB amyloid tracer by ssNMR using (13C, 2H)-labeled FSB5 (figure 1B top). Atomic resolution detail was enabled by 13C detection, polarization transfers and quadrupolar deuterium anisotropy that served as reporters of the fibril-ligand interaction. Our anisotropy data clearly showed that FSB strongly bound the Aβ fibrils and behaved as a solid (figure 1B bottom). Moreover, chemical shift perturbations of distinct residues revealed a structural defined binding site for the FSB ligand.

Figure 1. A) Structural model of Aβ fibril morphology. B) Chemical structure of the (2H, 13C)-labelled FSB ligand together with the ssNMR analysis of the fibril-bound FSB ligand (black). Simulated spectra (red) reveals very little dynamic of fibril-bound FSB. C) Spectral overlay showing the specific chemical shift perturbation of the two isoleucines among other residues.

Investigating Roles of Collagen Glycation by Solid-State NMR

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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 and consequential accumulation of advanced glycation end-products (AGEs), which are generally accepted as structural modifiers of proteins. Our main focus is effects of glycation of mammalian cell extracellular matrix (ECM), covering glycation of scaffold collagen and other essential ECM proteins. ECM has been proved to participate in regulating cell behaviour, such as proliferation and differentiation, and thus, glycation-modified ECM can be expected to lead to abnormal cell behaviours.1,2

This work studies the structural changes in glycated ECM, to identify common AGE species in tissues using two ECM models secreted by fetal sheep osteoblasts (FSOBs) and bovine vascular smooth muscle cells (BVSMCs) respectively. In addition, we have studied native tissues, such as bone, to uncover the presence of native glycation products.

A variety of solid-state NMR experiments are used on 13C, 15N-enriched, glycated and non-glycated ECMs and on native 13C-enriched tissues, including 1D 13C and 15N cross polarization (CP), 2D 13C-13C correlation experiments, e.g. PDSD and DARR, 15N-13C correlation experiments, e.g. NCA and NCC_DARR, to investigate structural changes and relaxation experiments to monitor mechanical changes. Figure 1 shows images from Inverse Laplace transform (ILT) of 13C T1 relaxation data from ECM before and after glycation. It is clear that T1 times of matrix components have changed due to glycation, suggesting altered molecular packing or ordering in the matrix as a result of glycation.

Figure 1. ILT contour plot of T1 relaxation data from VSMC sample before (top) and after (bottom) glycation with ribose for about two months

Solid State NMR studies of biomaterials developed for bone repair and drug delivery applications

Christian Bonhomme,1 Christel Gervais,1 Yang Li,2 Melinda Duer,2 Dinu Iuga,3 Mark Smith,3,4 John Hanna,3 Christèle Combes,5 Saad Sene,6 Dorothée Berthomieu,6 Danielle Laurencin6

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Over the past 15 years, solid state NMR has progressively emerged as a tool of choice for the characterization of complex biomaterials, such as those found naturally in tissues like bone, and those developed for drug delivery and tissue engineering applications [1].

The purpose of this presentation will be to illustrate some of the recent work we have performed in this context, along the following directions:

(i) the study of calcium phosphates like octacalcium phosphate and hydroxyapatite (which are closely related to bone mineral)[2], and of calcium pyrophosphates (which can be involved in pathological calcifications)[3]; here, emphasis will be made on 43Ca and 31P NMR analyses, and the first 43Ca…31P correlation experiments will be presented (Figure 1) [2].

(ii) the study of new formulations for an emerging family of organoboron drugs, benzoxaboroles [4]; here, the analysis of the interaction between the organoboron molecules and inorganic biomaterials like layered double hydroxides and hydroxyapatite will be described [5,6]; the importance of using combined experimental-computational approaches to determine the local structure around the organoboron molecule will be underscored.

Intermediates and Interactions in Photo- and Organocatalysis
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The detection and characterization of intermediates in catalytic reactions is crucial for the understanding of mechanisms and the rational optimization of reaction conditions. However, in many rapidly expanding fields of asymmetric catalysis, mechanistic studies as well as structural investigations on intermediates or intermolecular interactions are scarce compared to new synthetic applications. Therefore, in this talk mechanistic and structural studies about topics in photo- and organocatalysis will be discussed.

The talk will start with our recent results in the field of photocatalysis. First our LED based NMR illumination device for mechanistic studies on photocatalytic reactions will be introduced, which turned out to be versatile and simple, yet surprisingly powerful allowing even for time resolved CIDNP studies.1 Next as application of this LED device mechanistic studies about organophotocatalytic reactions with flavin will be discussed.2 Both NMR reaction profiles and CIDNP studies allow for new insights, which are not only complementary to UV/Vis studies but crucial for the understanding of the mechanism. Downstream intermediates, especially ion pairs, and processes accessible on the NMR time scale are decisive for the mechanistic pathway of this photoreaction. In addition, this study shows that the control of one- versus two-electron processes of flavin and potentially also other photocatalysts is possible without any protein just using solvent properties.

The second topic is ion pairing catalysis. There is a general lack of experimental structural data about small ion pairs in solution hampering the further development of this booming field in catalysis. On the example of Brønsted acid catalysis not only our recent results about the activation mode in achiral phosphoric acid/imine complexes3 will be presented but also new insights into the structures determining techniques, as well as the structures and hydrogen bond properties of famous chiral phosphoric acid/imine complexes.4

References:
CLIP-COSY: General InPhase Experiment for Rapid Acquisition of COSY-type Correlations

Martin R.M. Koos,1 Grit Kummerlöwe,2 Lukas Kaltschnee,3 Christina M. Thiele,3 Burkhard Luy1

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The COSY is an essential homonuclear 2D NMR experiment for resonance assignment. Its multiplet line shape, however, is often overly complicated, potentially leads to signal intensity losses, and is responsible for long minimum overall acquisition times. Here we present the CLIP-COSY, a COSY-type experiment yielding clean inphase peaks. It can be recorded within a few minutes with enhanced signal intensities for most cross peaks. The combination with non-uniform sampling allows further reduction of experiment times and the inphase multiplets enable the application of modern homonuclear decoupling techniques in both dimensions. As antiphase cancellations are avoided, the CLIP-COSY can also be applied to macromolecules and other samples with broadened lines.
Residual Chemical Shift Anisotropy: Robust and Powerful New Tool for Small Molecule configuration

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Abstract: Similar to residual dipolar couplings (RDCs), residual chemical shift anisotropy (RCSAs) provide information on relative orientations of different structural moieties within a molecule including non-protonated carbons.¹⁻³ While RCSA has been applied to the study of proteins and nucleic acids, its potential for the structural analysis of small molecules is yet to be explored. Herein, we present a method, in which compressions of chloroform compatible PMMA gel³ inside a 5 mm NMR tube are used for measuring ¹³C RCSA as ¹³C chemical shifts difference observed between two alignment conditions. The method is both robust and sensitive. In this work, main achievement achieved is the removal of obstacles for accurate measurement of RCSAs, making them available for routine applications.

Figure: Plot for the experimental RCSA vs back-calculated RCSA without and with isotropic shift correction. RCSA Q values are 0.11 and 0.44 for correct and incorrect configurations of estrone. The compression device for 1.7 mm tube, in which, only 25 µg of the sample will be sufficient under anisotropic conditions.

The PMMA gel does not touch the wall of the NMR tube in the compression device and therefore, a small fraction of liquid is most probably expelled from the gel under compression. Liquid leaving the gel increases the gel-to-analyte ratio causing an isotropic contribution that can be removed post acquisition in a new and robust way. After applying a correction for the isotropic shift, RCSA tensors are obtained that almost perfectly match the RDC alignment tensors. RCSAs were measured on five different molecules some rigid, some flexible and RCSA Q values are considerably smaller for the correct configurations. The most notable example is retrorsine in which flexibility aggravates the determination of the relative configuration. Furthermore, RDCs alone were not able to assign the correct configuration since one of the key chiral carbons is quaternary and therefore does not exhibit an RDC. Sensitivity with device is high and with a device for 1.7 mm cryo-probes, only 25 µg of the sample for the anisotropic condition would suffice.⁴ The simplicity of measuring just a simple ¹³C 1D NMR experiment should make this method available for every chemist.

Scalable chiral alignment in supramolecular lyotropic liquid crystalline phases

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Benzene-1,3,5-tricarboxyamides (BTAs) are known to self-assemble into rod-like and helical supramolecules.[1] These stiff aggregates act as mesogens to form thermotropic or lyotropic liquid crystals (LLC).[2, 3] Achiral substituted BTAs aggregate to racemic mixtures of (M)- and (P)-helices. One handed helices can be obtained by introducing enantiopure sidechains to BTAs or via a sergeant-and-soldier-principle (SaS) by mixing chiral and achiral substituted BTAs.[4] In this work several BTAs were synthesized and tested for their behavior as SaS-LLCs.

These are used as weak orienting media, which give access to residual dipolar couplings (RDCs). RDCs offer complementary structure information to the conventional NMR parameters for structure determination (as J-couplings and NOEs). They provide angular and distance information with respect to the external magnetic field. For structure determination of small organic molecules the LLCs should be compatible with organic solvents and introduce only a low degree of order.[5] Chiral alignment media can transfer the chiral information to the analyte molecule. As a result of diastereomorphous interactions with chiral alignment media enantiomers can be differentiated.[6]

We show here, that mixtures of chiral and achiral BTAs form LLCs in organic NMR-solvents. To investigate the capabilities of the SaS-LLCs as chiral alignment-media, THF-d8 was added to the phases. The characterization through H-NMR-spectroscopy indicated the SaS-LLCs to be a potential chiral alignment medium for small organic molecules with scalable chiral characteristics.

Scalar coupling is a key source of information on chemical structure, but is also an unwelcome source of complication. In $^1$H NMR, multiplets caused by homonuclear scalar coupling are often many times the width of a single line, making it very difficult to distinguish individual chemical shifts in crowded spectra. An efficient method to collapse the multiplet structure has long been sought, but only recently have experimental methods for such homonuclear broadband decoupling become practical. These “pure shift” or “chemical-shift resolved” methods give resolution improvements approaching an order of magnitude, far in excess of any gains realistically to be expected from increases in static magnetic field.

A variety of experiments are now available for measuring “pure shift” spectra, i.e. spectra in which all homonuclear couplings are inactive. The underlying principles involved will be described, practical results and applications compared, and some recent developments and extensions presented.

Solid-State NMR of Quadrupolar Nuclei

Philip J. Grandinetti

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For those more familiar with NMR of spin 1/2 nuclei, the spectra and solid-state NMR methods for quadrupolar \( (I > 1/2) \) nuclei can appear somewhat daunting. In this tutorial lecture I will discuss the origins of the nuclear quadrupolar interaction and its influence on NMR transition frequencies. The concepts of transition and symmetry pathways will be introduced and used to explain various methods such as MQ-MAS, DAS, and DOR for obtaining high resolution solid-state NMR spectra of quadrupolar nuclei in polycrystalline samples.

**Quadrupolar Frequency Contribution**

The battle between the EFG and \( B_0 \)

\[
B = 0 \quad |U_z| > |U_q| \quad |U_z| \gg |U_q|
\]

Increasing external magnetic field strength

Series expansion of the NMR transition frequency about the high magnetic field limit

\[
\Omega(m_i, m_f) = \Omega_z(m_i, m_f) + \Omega_q^{(1)}(m_i, m_f) + \Omega_q^{(2)}(m_i, m_f) + \cdots
\]

Zeroth-Order Contribution

First-Order Contribution

Second-Order Contribution

AMPERE tutorials (Store sal): Tuesday 15:30 - Philip J. Grandinetti
Product Operators as you possibly have not heard it before

Ole W. Sørensen

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You need to be familiar with the single-spin vector model and spin-1/2 operator definitions and relations, as included below, before the lecture. The lecture will focus on the product aspect.

Matrix representations of spin $\frac{1}{2}$ operators and products between them:

$$I_z = \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix}, \quad I_x = \frac{i}{2} \begin{bmatrix} 1 & 1 \\ -1 & 0 \end{bmatrix}, \quad I_y = \frac{i}{2} \begin{bmatrix} 1 & -1 \\ 1 & 0 \end{bmatrix}, \quad E = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

$$I^+ = \begin{bmatrix} 0 & 1 \\ 0 & 0 \end{bmatrix}, \quad I^- = \begin{bmatrix} 0 & 0 \\ 1 & 0 \end{bmatrix}, \quad I^a = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix}, \quad I^\beta = \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix}$$

Prove the following relations based on the matrix representations or other relations given:

$$I_z I_x = I_x I_z = I_y I_y, \quad 2I_z I_x = i I_y, \quad 2I_z I_y = -i I_x, \quad 2I_x I^a = I^a, \quad 2I_x I^\beta = -I^\beta$$

$$I^+ = I_x + i I_y, \quad I^- = I_x - i I_y, \quad I^a = \frac{1}{2} E + I_x, \quad I^\beta = \frac{1}{2} E - I_x$$

$$I_x = \frac{i}{2} (I^+ + I^-), \quad I_y = -\frac{i}{2} (I^+ - I^-), \quad E = I^a + I^\beta, \quad I_x = \frac{i}{2} (I^a - I^\beta)$$

$$I^a \xrightarrow{\phi I_z} I^a, \quad I^\beta \xrightarrow{\phi I_z} I^\beta, \quad I^+ = I_x + i I_y \xrightarrow{\phi I_z} I^+ \cos^2 \left(\frac{\phi}{2}\right) - I^- \sin^2 \left(\frac{\phi}{2}\right) + \frac{i}{2} (I^a - I^\beta) \sin \beta$$

$$I^- = I_x - i I_y \xrightarrow{\phi I_z} I^- \cos^2 \left(\frac{\phi}{2}\right) + I^+ \sin^2 \left(\frac{\phi}{2}\right) - \frac{i}{2} (I^a - I^\beta) \sin \beta$$

$$I^a = \frac{i}{2} E + I_x \xrightarrow{\phi I_z} I^a \cos^2 \left(\frac{\phi}{2}\right) + I^\beta \sin^2 \left(\frac{\phi}{2}\right) + \frac{i}{2} (I^+ - I^-) \sin \beta$$

$$I^\beta = \frac{i}{2} E - I_x \xrightarrow{\phi I_z} I^\beta \cos^2 \left(\frac{\phi}{2}\right) + I^a \sin^2 \left(\frac{\phi}{2}\right) - \frac{i}{2} (I^+ - I^-) \sin \beta$$
Biosynthesis of the Catalytic H-Cluster of [FeFe] hydrogenase as probed by EPR and other Spectroscopies

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Complex chemical reactions can be catalyzed by the 5′deoxyadenosyl radical produced in “radical SAM” enzymes (SAM=S-adenosylmethionine) [1]. For example in biotin synthase this radical chemistry pulls a sulphur atom from a [2Fe-2S] cluster and inserts it into the substrate dethiobiotin to form the biotin product [2]. HydG is another interesting radical SAM enzyme that is involved in synthesizing the catalytic H-cluster of [Fe-Fe] hydrogenases [3]. In a series of recent papers we and our collaborators have used advanced and other spectroscopies to show how the HydG radical chemistry drives the formation of a Fe(CO)₂(CN)L-cysteine organometallic intermediate in the assembly of the H-cluster [4-7].

In-cell NMR & EPR of human alpha-synuclein reveals a disordered monomer in mammalian cells.

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Intracellular aggregation of the human amyloid protein alpha-synuclein is causally linked to Parkinson’s disease. While the isolated protein is intrinsically disordered, its native structure in mammalian cells is unknown, with proposed conformations ranging from disordered monomers to folded helical tetramers. Here, we use in-cell NMR and EPR to show that the disordered nature of monomeric alpha-synuclein is stably preserved in non-neuronal and neuronal cells.

Figure 1. Cartoon models of alpha-synuclein conformations in mammalian cells.
Structural Polymorphism in Membrane Proteins, Fibrils, and Polysaccharides from Solid-State NMR

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Many biological molecules exhibit molecular-level structural polymorphism, as seen by multiple sets of chemical shifts in NMR spectra. However, the reason and the functional relevance of such polymorphism are often unclear. I will present our studies of three classes of systems where either polymorphic or single structures are observed and characterized using solid-state NMR, and will discuss the biological relevance of the structure distribution. 1) The amyloid-β (Aβ) peptide of the Alzheimer’s disease is well known to be polymorphic as induced by different fibrillization conditions. We have compared three Aβ40 sequences, the Arctic E22G mutant, the Osaka E22Δ deletion mutant, and the wild-type sequence. 2D correlation spectra show that the Arctic Aβ40 is extensively polymorphic under conditions where Osaka and wild-type Aβ40 show a single or a predominant conformation. Water polarization transfer, inter-residue contacts, and chemical shift comparisons suggest the overall fold of one of the major Arctic conformers. Fibril growth kinetics and fibril stability data suggest the reasons for the high structural polymorphism of Arctic Aβ40. 2) In comparison, a short de novo peptide that self-assembles into an amyloid fibril that catalyzes ester hydrolysis reactions exhibits a single conformation despite its short length. Using 2D correlation and distance measurements, we have elucidated the structures of two key histidine residues that coordinate with Zn²⁺ to carry out catalysis. 3) In the membrane-bound influenza M2 protein, the proton-selective residue, a histidine, exhibits multiple chemical and conformational structures at different pH and in different amino acid sequences. Chemical shift and dynamics measurements reveal how the cytoplasmic domain and the transmembrane domain amino acid sequences shift the complex structural equilibria of this histidine. The results point to the ubiquitous role of electrostatic interactions in regulating the histidine structure distribution and hence proton conduction. 4) Cellulose in plant cell walls forms microfibrils that are qualitatively different from the cross-β fibrils formed by amyloid proteins. We show that plant cellulose microfibrils exhibit significant structural polymorphism, and none of the polymorphs are the same as the principal conformations known in bacterial, algal, or marine celluloses. 2D correlation NMR together with DFT calculations gives insights into the spatial distributions of the various conformations in the microfibrils and the nature of these conformations.

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Conformational dynamics in biomolecular recognition and novel pulses for ultra-highfield NMR

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Solution NMR studies will be presented that highlight the role of conformational dynamics and population shifts in protein-protein and protein-RNA molecular recognition. We also report on novel radiofrequency pulses for ultrabroadband decoupling and excitation that will be important for heteronuclear NMR spectroscopy $\geq 21$ Tesla.

Binding of the human U2 auxiliary splicing factor heterodimer (U2AF65/U2AF35) to poly-pyrimidine-tracts at the 3' splice sites of pre-mRNA introns is an essential step for the assembly of the human spliceosome. We have reported that the tandem RNA binding domains of U2AF65 (RRM1,2) adopt closed/inactive and open/active arrangements free and when bound to Py-tract RNA ligands, respectively\textsuperscript{[1,2]}. We now show that a dynamic interaction of the linker with the RNA-binding interface of RRM2 plays an auto-inhibitory role to discriminate against weak (low affinity) Py tracts. Moreover, by combining FRET and NMR experiments, we demonstrate that U2AF35 induces a population shift of the conformational equilibrium of U2AF65 RRM1,2 towards the RNA-accessible open state, providing an unexpected molecular explanations for U2AF function.

We have previously shown that the Tudor domain of the Survival of Motor Neurons (SMN) protein recognizes symmetrically N-dimethylated arginine (sDMA) found in multiple copies in the tails of spliceosomal Sm proteins\textsuperscript{[3]}. Our structural studies showed recognition of the sDMA side chain by the aromatic cages of the SMN Tudor domain in an anti/anti conformation, in contrast to the binding in an anti/syn conformation reported for other Tudor domains. We have combined NMR and computational methods to rationalize and dissect the thermodynamic and kinetic features of the recognition of sDMA recognition by Tudor domains. Our data suggest a conformational selection mechanism for sDMA recognition and reveal the unique contributions of aromatic side chains in the SMN Tudor domain.

Together with the Glaser group (TUM) we are developing and implementing novel rf pulses with large bandwidths at low rf power for heteronuclear NMR spectroscopy at external magnetic fields beyond 21T. We have recently introduced BUSS decoupling pulses for very broadband decoupling required at ultrahigh magnetic fields at low rf power\textsuperscript{[4]}. Here, we introduce a pair of numerically optimized broadband excitation pulses, which have been designed based on the cooperativity principle. Simulation and experiment show that these COOP pulses cover an excitation bandwidth of 70 kHz with a maximum rf field of 10 kHz. This overcomes bandwidth problems at ultrahigh magnetic fields for applications in standard biomolecular NMR experiments, such as \textsuperscript{1}H,\textsuperscript{13}C HMQC, \textsuperscript{13}C-edited \textsuperscript{1}H,\textsuperscript{1}H NOESY and \textsuperscript{13}C-detected \textsuperscript{13}C, \textsuperscript{13}C NOESY experiments.

Deuterium Isotope effects on Chemical shifts as monitors of salt bridges

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Ammonium ions play a vital role in salt bridges. In addition, ammonium ions show fast rotation both in water and in the solid state. In the latter case this is demonstrated by self-decoupling of the N-H couplings.1 In the former case this is unexpected considering the four potentially strong hydrogen bonds to water molecules. Deuterium isotope effects on chemical shifts (DEIS) offer a strong tool in the study of such hydrogen bonds.

Deuterium isotope effects on chemical shifts (DEIS) are measured both in solution and in the solid state using in the former case the HISQC sequence or 1D spectra with 14N decoupling. The contribution will show that DEIS can be used to answer the central question salt bridge or no salt bridge in proteins in solution to established salt bridges in the solid state. The salt bridges are either between lysine or arginines and aspartic acid or glutamic acids: K..D, K..E, R..D or R..E types.

DEIS are one of the few tools that allows characterizing of the binding of the elusive ammonium ion as demonstrated using pseudorotaxanes or ammonium ions and crown ethers (Figure).

Deuterium isotope effects on 14N chemical shifts of ammonium halides are measured in the solid state allowing a correlation between 1∆N(D) and the heavy atom distances (N…Hal). These are used as reference points for compounds in solution.

DFT calculations of isotope effects allow to mimic a number of situation and show a decrease of 1∆N(D) in good agreement with experiment.

NMR for biomaterials (and vice versa?)

Enrico Ravera, 1 Tommaso Martelli, 2 Linda Cerofolini, 1, 3 Vladimir K. Michaelis, 4, 5 Ta-Chung Ong, 6, 6 Eric G. Keeler, 4 Stefano Giuntini, 1, 3 Alexandra Louka, 1, 7 Manuel Hafner, 7 Gil Goobes, 8 Marco Fragai, 1, 3 Christian F.W. Becker, 7 Robert G. Griffin, 4 Claudio Luchinat 1, 3

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Biomolecular solid state NMR has seen a profound improvement from the theoretical and hardware standpoints; up to the point that sample preparation has become the key determinant for the success of SSNMR characterization, and sample preparation is all but a trivial accomplishment. We will discuss the case of immobilized enzymes [1]. These systems may well represent the future for industrial, medical and analytical biotechnologies, yet their characterization of these systems has so far relied only upon activity tests or microscopic investigations. We have demonstrated that immobilized enzymes (may) yield high quality SSNMR spectra, that can contribute to the understanding of their structural features in this artificial but functional environment [2,3]. The NMR properties of these systems will be discussed, analyzing the implications for more advanced applications such as DNP [4].

This work has been supported by Ente Cassa di Risparmio di Firenze; by European Commission, Bio-NMR n. 261863, pNMR n. 317127 and COST action TD1103; the EU ESFRI Instruct through its Core Centre CERM/CIRMMP, Italy and one R&D award; FIRC though a triennial fellowship "Guglielmina Locatello e Gino Mazzega" (17941).

NMR Molecular Replacement, NMR²

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X-ray crystallography molecular replacement (MR) is a highly versatile tool for the detailed characterization of lead compounds and binding modes in the pharmaceutical industry.[1] The two major limitations of its application to drug research are (i) the availability of a similar protein structure, which, in the area of structure-based drug design, is most often a complex of the protein with a lead compound, and (ii) obtaining well-diffracting crystals of the ligand-protein complexes of interest. While nowadays the first point is often not a limitation anymore, obtaining well-diffracting crystals might be difficult. In such situations structure determination of protein-ligand complexes by liquid-state NMR is a good option. Unfortunately, the established standard structure determination protocol is in general time-consuming, and a shortcut using available structural data as in the case of MR in X-ray crystallography is not available.

Here, we present NMR², a MR-like approach in NMR[2] to determine the structures of the binding pockets of ligands at atomic resolution. The calculation of structures of protein-ligand complexes relies on the collection of unassigned semi-quantitative inter-molecular NOE distance restraints and on previously solved structures. The NMR² method uses a high throughput structure calculation protocol, rather than a docking-scoring simulation. It is fast since it requires only a few days of measuring time and bypasses the time-consuming sequential assignment steps for the protein. When applied to the cancer-relevant HDMX protein, the NMR² method yielded the structure of a ligand protein complex with an accuracy below 1 Ångstrom for the binding pocket irrespective of the starting protein structure templates used. We will present multiple NMR² applications covering a peptidomimetic inhibitor and small molecules that bind strongly or weakly to protein receptors. Our findings demonstrate that NMR² may open an avenue for the fast and robust determination of the binding pocket structure of ligand-protein complexes at atomic resolution without the need of diffracting crystals and high affinity ligands.

Improved NMR methods for IDPs and membrane proteins

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The goal of our research is to push NMR spectroscopy into new research areas and to provide insights at the molecular level into the factors that are responsible for the neurotoxicity of the proteins α-synuclein, Tau and Amyloid-β peptide during the course of Parkinson's and Alzheimer's diseases. In addition, we strive to obtain structure-function relationships for integral membrane proteins, such as the human voltage dependent anion channel (VDAC) and the 18 kDa translocator protein TSPO.

The aim of the presentation is to discuss recent improvements, which we made in the structural and dynamic analysis of intrinsically disordered proteins (IDPs) and membrane proteins. In order to achieve an efficient backbone resonance assignment of IDPs at physiological pH, we developed a six-dimensional alpha proton detection-based automated projection spectroscopy (APSY) experiment (1). In addition, we designed an improved protocol for the calculation of ensembles of IDP conformations, which is implemented in XPLOR-NIH and makes use of a wide range of NMR parameters including NOEs (2). We further show that an optimized Karplus equation for one-bond Ca-Ha scalar couplings improves the cross-validation of IDP ensembles (3). In case of membrane proteins, we demonstrated that it is possible to determine the sequence-specific resonance assignment of both the backbone and side-chains of the detergent-solubilized translocator protein TSPO and on this basis determine the structure of a helical, integral membrane protein at high resolution (4). More recently, we showed that when the membrane protein is even larger, \textsuperscript{15}N spin-relaxation rates provide critical information about the 3D structure and internal motions. Combined with an improved structure calculation protocol the relaxation rate-derived structure of the 283-residue VDAC protein revealed an anisotropically-shaped barrel with a rigidly attached N-terminal helix (5).

Why NMR Matters in Metabolomics

In principle, NMR is an ideal technique for metabolomics. It is non-destructive, non-biased, highly quantitative, requires no prior separation, permits the identification of novel compounds and needs no chemical derivatization. However, relative to other analytical techniques NMR is slow and relatively insensitive. Furthermore the identification and quantification of compounds in mixtures by NMR is manually intensive and often error-prone. Because of these limitations, NMR is being supplanted by mass spectrometry for many metabolomic applications. In this presentation I will show how NMR can be used to match or even exceed the speed, sensitivity and metabolite coverage claimed by various mass spectrometry methods. In particular I will describe a variety of instrumental and software techniques that my lab has developed to detect and quantify up to 210 different compounds in biological fluids. I will further show that when compared to existing mass spectrometry techniques, NMR is often superior in terms of speed, sensitivity, overall metabolite coverage and its ability to be fully automated. In particular, I will describe our recent efforts to completely automate NMR-based metabolomics using a software program called Bayesil. Bayesil is more than 95% accurate in terms of compound identification (for blood, cerebrospinal fluid, saliva, cell extracts) and its compound quantitation error (CV) is typically less than 10%. Bayesil is able to identify and quantify up to 60 compounds from an NMR spectrum in less than 3 CPU minutes. I will describe our efforts to extend Bayesil to the quantitative analysis of urine (with >120 metabolites), beverages (such as beer and wine) and lipoprotein fractions. These and other developments could make NMR-based metabolomics the method of choice for many metabolomics applications – both in the lab and in the clinic.
Overhauser DNP in supercritical CO$_2$

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Overhauser DNP in the liquid state is a promising technique to increase the NMR sensitivity. The large polarization of an unpaired electron is transferred to a nuclear spin via cross-relaxation. This cross-relaxation transfer is efficient when the translational correlation times are shorter than the inverse electron Larmor frequency. The correlation time of solutes in common solvents is usually in the order of 20-100 picoseconds, which makes Overhauser DNP using regular solvents at high magnetic field inefficient. Supercritical fluids have diffusion coefficients an order of magnitude larger than liquids. This property of supercritical fluids combined with a density comparable to a liquid makes them interesting as solvents for Overhauser DNP.

In our lab we have developed a hyphenation between a supercritical CO$_2$ chromatograph and a stripline NMR probe. This setup was used to mix solutions with CO$_2$ in an adjustable volume ratio and interrogate the resulting mixture using NMR. B$_1$-gradient diffusion experiments and relaxation experiments were used to investigate the translational dynamics of toluene in CO$_2$. The effect on cross-relaxation was investigated by adding various concentrations of TEMPO radical to the mixture. The diffusion and the relaxation data were then analysed using the Force-Free Hard-Sphere model. From this model we predict substantial Overhauser enhancements in supercritical CO$_2$ at high field. Preliminary DNP experiments were performed on a 95 GHz DNP setup on high pressure superheated water and toluene in high pressure CO$_2$. 

Altered region specific GABA neurotransmission in mouse model of Alzheimer's Disease: 1H HRMAS study

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In Alzheimer's disease (AD) GABAergic neurotransmission is severely disrupted which is believed to be involved in memory decline. Although region specific abnormal γ-Aminobutyric acid (GABA) release from reactive astrocytes has been established with advancing AD, a longitudinal region specific disclosure in GABA variation is mandatory for clinical investigation on AD patients. GABA has multi spin system and unambiguous assignment and separation of GABA signal in NMR spectrum from intact tissue is difficult. In this study we optimized and applied high resolution magic angle spinning (HR-MAS) NMR to probe the region specific GABA differences in the brain of transgenic Tg2576 mouse model of AD and their non-transgenic littermates (WT).

Figure 1. Schematic diagram for the 1H HRMAS NMR experiments. Mouse brains were isolated and brain parts were separated. Then they were transferred to 4mm zirconium rotor for measurements. In this case the anisotropic interactions are averaged out to zero at 54.7º which provides high resolution 1D (a) as well as 2D 1H-1H COSY spectra (b) allowing to resolve and quantify GABA signals. Upon assigning them, details of region specific GABA profile is achieved.

1D and 2D 1H HR-MAS NMR pulse sequences including 1H-1H COSY and J-resolved were optimized at 600 MHz NMR magnet. Using optimized sequences, we were able to separate and quantify GABA levels in a very small volume of brain tissue. The method has been implemented to probe changes in GABA levels in AD mouse brain in region and time specific manners. Interestingly, significant differences in GABA was observed already at the age of 6 month and continued to be further extended upto18 month of age in prefrontal cortex, parietal cortex, hippocampus and thalamus regions. Histological examination of GABA synthesizing enzyme (glutamic acid decarboxylase, GAD) were obtained along with glial fibrillary acidic protein (GFAP) and HuCD (for neurons) staining for further insight into the molecular mechanism behind region specific GABA change with AD. Our results show that, 1H HRMAS NMR technique can provide important localized information of GABA trafficking in the different brain regions unambiguously which could be directly linked to human patients for clinical diagnosis and treatment.

Acknowledgement: This work was partially supported by grants from Alzheimer Forschung Initiative e.V. (AFI, Grant Nr 13810).

Ultrafast 2D NMR on a benchtop spectrometer: a promising tool for on-line monitoring and rapid screening

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The amazing performance reached by high field (HF) NMR relies on strong magnetic fields, involving an expensive equipment with the need for dedicated laboratories. These economic and practical drawbacks have hampered the use of HF NMR in industrial sites and synthetic laboratories. A new generation of benchtop NMR spectrometers, more compact and cryogen-free, emerges as a relevant alternative to extend the scope of NMR in harsh environments.1 Obviously, the use of these permanent magnets involves a reduced frequency dispersion leading to crowded spectra with numerous overlaps. 2D NMR experiments offer an appealing solution, unfortunately their inherent long acquisition duration is incompatible with the time scale of applications such as reaction monitoring. In order to circumvent this time constraint, we developed Ultrafast (UF) NMR2 on a benchtop spectrometer, yielding homonuclear 2D spectra within a single scan.3 These developments were performed on a 43 MHz Spinsolve (Magritek) including a gradient coil originally designed for diffusion experiments.

We first highlight the relevance of the UF NMR at 43 MHz through the real-time monitoring of a Heck-Matsuda coupling reaction in an on-line fashion.4 In the course of the reaction performed in a conventional flask, the mixture is directly analyzed through a by-pass system connected to the benchtop spectrometer, which records UF COSY spectra with well-resolved cross-peaks (Figure 1). Another potential of this approach relates to a long-standing concern: the screening of edible oils. Relevant results were recently reported using benchtop NMR for the adulteration with cheap substitutes into olive oil. Albeit promising, the accuracy of the 1D approach remains limited by the overlapped resonances from glycerides protons and those arising from the unsaturated chains. The UF COSY experiments overcome this issue by delivering well-resolved cross peaks, whose integration provides an efficient authentication tool.4 These first results shed light on the potential of UF NMR at 43 MHz as a promising rapid screening tool.

Metabolomics in health research: friend or foe?

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A critical view of the uses and challenges of metabolomics in health research, and particularly in cancer research, will be presented, addressing the promise and pitfalls of this omic science in biological systems of varying complexity: cells, animal models and human studies.

In vitro studies benefit from well-controlled growth environments and metabolomics (both untargeted and targeted e.g. through pathway tracking) is invaluable in unveiling the metabolic adaptations of living cells in cancer onset/development or therapy. This is exemplified by a NMR study of anti-cancer drugs in osteosarcoma\(^1\), to explore potential new drugs and drug synergism. Translation of cell studies to in vivo and, eventually, to the human organism, will allow the important effects of tumour microenvironment and systemic responses to be considered. Several murine models are used presently for in vivo studies of cancer, either genetically modified (GEM) or xenograft-derived, some models approaching human behaviour very closely. Such studies are here exemplified by preliminary NMR metabolomics results on a GEM model of pancreatic cancer, demonstrating distinct metabolic signatures for early cancer stages and benign condition of pancreatitis, and for different cancer grades. Localised (tumour) metabolomics of animal models may unveil new markers of progression or prognosis, which may become detectable in vivo. On the other hand, animal biofluids carry the promise of non-invasive biomarker discovery, however, metabolic interpretation of biofluid profiles will require adequate consideration of systemic tumor-independent factors. This complementarity of information on localised and systemic metabolomes becomes particularly important in human studies. Cancer metabolomics in human patients carries an enticing promise of new possible tools (some non-invasive) for cancer diagnosis, prognosis, therapy tailoring and follow-up. However, important challenges still face both localised (tumor) studies and systemic (biofluids) studies of human subjects. These ideas and future prospects will be illustrated by NMR metabolomics studies of lung cancer and renal cancer\(^2,3\). The main take home message is that, ca. 20 years on, metabolomics still holds powerful promise in health sciences, now paved by the knowledge of some of the entailed pitfalls and challenges.


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Novel applications of permanent magnets and small coils in Nuclear Magnetic Resonance

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Permanent magnets (PM) can and have been used in Magnetic Resonance since its early days, to offer transportability and miniaturization. Modern materials offer new opportunities for generating highly compact homogeneous NMR and MRI systems. Over the last ten years we have developed a number of portable one-sided and enclosed high-resolution permanent magnet systems and recently a rotating MRI system for small animals. All these systems will be briefly discussed and novel PM systems, which can be combined with superconducting magnets, will be presented with particular emphasis on instrumentation advances towards sensor miniaturization.

Figure 1. Examples of permanent magnet assemblies for magnetic resonance. Ultra compact systems can be designed and built for generating strong (~2T) magnetic fields along a predefined direction, with a well defined homogeneity over the size of the sweet spot. This family of magnets includes enclosed, one-sided, and spinning magnets pointing along the magic angle.
Low-Field Thermal Mixing for Brute-Force Hyperpolarization

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Low-field thermal mixing (LFTM) can be used to hyperpolarize low-γ nuclear spins such as 13C and 15N, via the spin order originally established in a high-γ nuclei like protons.1,2 In recent experiments,2,3 brute-force pre-polarization of 1H nuclei was combined with LFTM to hyperpolarize the 13C nuclei of [1-13C] pyruvic acid, which is currently the prime molecule of interest for medical imaging investigations. LFTM took place on ejection of the sample from the polarizer through a very low field region (<100 G). Here, we report on measurements of LFTM in [1-13C] pyruvic acid under conditions comparable to those of the recent brute-force studies. Using fast-field-cycling NMR at cryogenic temperatures (4-15 K), 1H nuclei in neat [1,13C] pyruvic acid were polarized at 2 T and then equilibrated (~300 ms) with 13C nuclei by fast field-cycling to a low field (0 – 300 G) that activates thermal mixing. 13C NMR was recorded after fast cycling back to 2 T.

By varying mixing time (t_m) and field (B_{mix}), we determined field-dependent rates of 1H→13C transfer (1/τ) and decay (1/T_{1m}) during mixing. Near-optimum mixing occurred for t_m ~ 100 – 300 ms and B_{mix} ~ 30 – 60 G. Two glassy samples, (one well-deoxygenated, the other O2-exposed) were tested, as well as one sample pre-treated by annealing (also well-deoxygenated). Both annealing and the presence of O2 are known to dramatically alter high-field longitudinal relaxation of 1H and 13C (with up to 102 – 103-fold effects). Here, we found smaller, but still critical factors of ~2–5 fold on both τ and T_{1m}. Annealed, well-deoxygenated samples exhibit the longest time constants, e.g., τ ~ 30 – 70 ms and T_{1m} ~ 1 – 20 s, each growing with B_{mix}. A key requirement for effective LFTM is that T_{1m} must be much longer than τ. Our results validate this, consistent with our earlier observations that polarization can be transferred very effectively from 1H nuclei to 13C nuclei via the process of LFTM.

Finally, we also detail our brute-force systems and method, as well as newer hyperpolarization results in [1-13C] pyruvic acid, as assisted by nanoparticle relaxation agents.

Beyond Compact NMR

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Compact NMR refers to NMR with instruments that are small enough to fit on a tabletop or which are mobile to carry along to the site of interest [1]. Such instruments have been pioneered for time-domain NMR of food, polymers and porous media as well as for well-logging NMR. In the last few years they have become available also for low-field NMR spectroscopy. Current research sets the focus on making such instruments even smaller. While considerable progress has been achieved with designing single-chip electronics and micro-coils, challenges remain in boosting the signal and making permanent magnets smaller while maintaining the field homogeneity sufficient for resolving the $^1H$ chemical shift. Work on boosting the signal by selective transfer of spin-order from para-hydrogen is reported along with efforts to simplify the construction of small magnets with homogeneous fields. In particular, a SABRE catalyst is reported which is activated and functions in water [2], strategies to characterize and immobilize such catalysts are explained [3], and the way to build magnets from non-ideal blocks of permanent magnets is analyzed [4].

Decoupling, Selective Pulses and Higher Dimensions at Ultra-Low Field


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NMR detected at zero and ultra-low fields (ZULF) offers certain intrinsic advantages, primarily the ability to perform high resolution spectroscopy (line-widths are routinely 20-100 mHz), without worrying about susceptibility issues, using inexpensive, cryogen-free, benchstop-sized instrumentation. Over the last years we have worked towards adapting the catalog of advanced high-field experiments for use in the ZULF regime, increasing experimental flexibility and demonstrating that ZULF NMR has the potential to become a useful spectroscopic tool.

We have recently demonstrated the ability to selectively excite individual NMR transitions in ultralow field, and now we present two-dimensional spectra, detected in conjunction with different types of decoupling schemes. Decoupling in ZULF is challenging, because fast control pulses act on all spins in the system proportionally to their gyromagnetic ratios and because terms that are nonsecular in high field need to be taken into account here, increasing the complexity of the sequences.

Figure 1 shows the indirect detection of the zero-field spectrum of $[1]^{13}$C-acetic acid in the presence of a 740 µG residual field using a pulse sequence that simultaneously averages out carbon and proton Zeeman interactions while preserving the spin-spin $J$-couplings. The method allows detection of zero-field spectra under conditions of imperfect shielding of the background magnetic field. Figure 1b shows the $^{13}$C-decoupled, $^1$H-coupled $J$-spectrum of $[1]^{13}$C-propionic acid. Homonuclear $J$-only spectra have not previously been observed since zero-field signals require at least two different spin species, thus imposing a minimum level of complexity on the spectrum. However, using indirect detection and the appropriate preparation sequence we overcome this restriction.

Figure 1: 2D detected ZULF decoupling experiments, with the indirect dimension along the vertical axis, and the direct dimension along the horizontal. a: Effective zero-field spectrum of $[1]^{13}$C-AcOH detected in the presence of a 740 µG magnetic field using a field decoupling sequence. b: $^{13}$C-decoupled proton zero-field NMR spectrum of $[1]^{13}$C-propionic acid. In both cases the black lines correspond to simulated spectra and the red traces are integrated data from the shaded red slices.
Chemical analysis using low-field Magnetic Resonance

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The composition of complex mixtures is often analyzed using high-field magnetic resonance (MR) spectroscopy, which typically relies upon large and expensive superconducting magnets. Here we introduce three methods based on low-field MR measurements which can be performed using inexpensive permanent magnets and are therefore advantageous for low-cost and portable applications. Firstly, relaxation and diffusion are known to be intimately related to molecular sizes and these relations have been formulated as scaling laws for mixtures of hydrocarbons. These scaling laws can be used for non-invasive determination of molecular size distributions. Secondly, we show that low-field J-coupling measurements can be used to identify different molecular moieties. Finally, we discuss low-field MR hardware and the development of a non-resonant sensor for improved Nuclear Quadrupole Resonance (NQR) spectroscopy and 2D NQR of solid materials.
Investigating Composition, Structure and Disorder in Ceramics for Waste Encapsulation

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The crystal chemical flexibility of pyrochlore-based (A2B2O7) oxide materials has resulted in a wide range of applications, including energy materials, nuclear waste encapsulation and catalysis. There is, therefore, considerable interest in understanding the structure–property relationships in these materials, i.e., investigating how cation/anion disorder and local structure vary with composition. However, substitution can bring about a change in structure, with the pyrochlore phase predicted to be stable only when the relative ratio of the cation radii, rA/rB, is between 1.46 and 1.78. Below this, a defect fluorite structure is though to be formed, exhibiting disorder on the cation and anion lattices. Above 1.78, a layered perovskite-based structure is predicted to be observed.

Here, we combine 89Y and 119Sn NMR (MAS, CPMG and CSA-amplified PASS experiments) with density functional theory calculations to investigate the number, nature and composition of the phases formed in Y2(Sn,Zr,Hf)2O7 and La2(Sn,Ti)2O7 ceramics – materials with applications related to nuclear fission. NMR shows that in many cases two-phase mixtures are observed. A detailed analysis of peak intensities is able to determine the composition of each phase present, and provide information on cation and anion/vacancy disorder. Preferential substitution of cations onto specific sites within the layered-perovskite structure is also demonstrated (as shown in Figure 1). The use of 17O NMR spectroscopy to study pyrochlore ceramics (including the conditions required for quantitative enrichment and quantitative spectral acquisition) is also discussed.

![Figure 1. 119Sn NMR of La2Ti1.8Sn0.2O7, showing pyrochlore and layered-perovskite phases present. The coloured points represent the DFT-predicted shifts for Sn substituted into the four Ti sites in the latter structure.](image-url)
Solid-state NMR, DNP, and MD investigations of the organic/inorganic interface in silica biohybrids

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Organic/inorganic interfaces play a key role in catalytic processes, separation science, materials science, and bio-mineralization. Our recent studies on diatom biosilica using DNP[1] and 1H-13C-{29Si}-REDOR[2] provided information about the supramolecular organization at the interface and peptide secondary structure. Functional groups in close contact with silica were identified in diatom biosilica. For fully isotope-labeled biosilica, distance determination by REDOR is complicated by strongly overlapping signals.[2] Distances and spin system geometries can now be evaluated and verified using our well defined synthetic model systems employing selective isotope labeling. For example choline-silica nanocomposites exhibit experimental 1H-13C-{29Si}-REDOR fractions up to about 30% (see Figure 1) and changes in 13C chemical shift for strongly silica-adsorbed 13C-Choline. This indicates the formation of hydrogen bonds between the choline C1-OH and ionized silanols at the silica surface, which is verified by MD simulations and 1H-{29Si}-1H Double CP (DCP) experiments. The latter also show close contact of the choline methyl protons to the silica (see Figure 2). Furthermore, nanocomposites containing silica and selectively 13C and 15N labeled polyamines of similar structure as found in diatoms were prepared. Precise REDOR curves with maximum REDOR fractions exceeding 90% allow determination of the spin system geometry beyond the simple 2-spin-approximation. The corresponding triple resonance experiments on samples synthesized with sodium metasilicate of variable 29Si content (up to 100%) give reliable distance and spin system information.

Figure 1. REDOR fractions of strongly silica-adsorbed 13C-labeled choline. A 13C-29Si second moment of 9.1·104 Hz2 could be measured.

Figure 2. 1H MAS NMR and 1H-29Si-1H DCP of choline-silica nanocomposites. The OH and methyl groups are in close contact with silica in agreement with MD simulations.

Solid-State NMR reveals Li-ion transport mechanism in 15NaI·LiBH₄

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Solid electrolytes are one of the key materials to realize all-solid-state Li-ion batteries. So far, most of the solid electrolytes have been synthesized from compounds including Li-ions in themselves. Recently, we have synthesized Li-ion conductor by doping a small amount of Li salts (LiBH₄) into “Li-free” compounds (NaI).¹ Here, we present ⁷Li solid-state NMR analysis of NaI-LiBH₄ solid solution and propose the Li-ion transport mechanism.

NMR experiments were performed with a 4 mm T3 probe tuned triply at ¹H-⁷Li-²³Na resonances in a magnetic field of 14 T. NaI-LiBH₄ solid solution was prepared by ball milling NaI (>99.999% purity) together with LiBH₄ (>90% purity) with a molar ratio of 15:1. The samples were packed into sealed rotors in a glove box.

The ⁷Li MAS NMR spectrum of 15NaI-LiBH₄, which was found to be uniform from XRD,¹ showed two peaks (marked as P₁ and P₂ in Figure 1, top) unexpectedly. We assign P₁ to Li nuclei in segregated LiBH₄ from the chemical shift and P₂ to Li nuclei in solid solution. While the former is known to be Li-ion conductor,² it has not been clear whether Li-ions conduct in the latter.

To evaluate the transportation of the Li-ions in the solid solution, we applied ⁶Li ion current electrochemically through the sample and performed quantitative ⁷Li MAS NMR for the ⁶Li-substituted sample. We found that 40% and 75% ⁷Li ions were replaced by the ⁶Li ions in pure LiBH₄ and NaI-LiBH₄ solid solution, respectively. Therefore, the solid solution also conducts Li-ions.

To confirm the substitutions of the Li-/BH₄-ions at the Na/I sites in the solid solution, we performed ⁷Li-²³Na and ¹H-⁷Li CP/MAS NMR experiments, and found ⁷Li-²³Na and ¹H-⁷Li correlations for P₂. In addition, peak P₂ separated into two in the ¹H-⁷Li CP/MAS spectrum (Figure 1, bottom). The CP build-up curves indicate that the Li-ions occupy the Na sites not interstitial sites. Thus, the Li-ions ought to migrate in the NaI lattice via vacancies.

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References:
Three-Dimensional Structure Determination of Surface Species by DNP Enhanced Solid-state NMR

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Three-dimensional molecular structures determined from single crystals by diffraction methods transformed Chemistry in the twentieth century, leading to today’s structure based understanding of the field. However, if the system under investigation is located at a surface, as in many of the most interesting functional materials today, the problem of structure determination is largely unsolved. Such samples are encountered with increasing frequency, particularly in the area of energy and catalysis, so that there is a critical need to provide new methods for structural characterization of surfaces. Solid-state Nuclear Magnetic Resonance (NMR) spectroscopy would be the method of choice for surface characterization if it were not that the detection limit of NMR is too low to allow the study of many modern materials. The sensitivity of NMR is thus the major limitation to surface structure determination.

We have recently introduced an approach using Dynamic Nuclear Polarization (DNP) under Magic Angle Spinning (MAS) to greatly enhance NMR signals (1). With the recent introduction of polarizing agents of high molecular weight like TEKPOL (2), signal enhancement factors of two orders of magnitude (between 100 and 200 in mesoporous materials) are routinely obtained at magnetic fields of 5–9.4 T and sample temperatures of ca. 80–105 K.

Here we show that the gain provided by DNP surface enhanced NMR spectroscopy (SENS) is sufficient to enable the implementation with high sensitivity of a series of multi-dimensional correlation experiments (including HETCOR, $^{29}$Si–$^{29}$Si INADEQUATE, $^{13}$C–{$^{15}$N} and $^{29}$Si–{$^{15}$N} REDOR) that allow us to solve the three-dimensional structure of organic fragments incorporated on a silica surface. Using a total of 8 internuclear constraints, we could determine the structure of a Pt complex, used as prototypical metal site for molecularly-defined supported catalysts.


Solid-state NMR analyses of surface-mediated hydration and crystallization of inorganic materials

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Solid-state NMR, in combination with dynamic nuclear polarization (DNP) signal enhancement and X-ray diffraction analyses, yield new molecular-level insights on the adsorption of organic molecules at inorganic surfaces and their influences on hydration and crystallization of bulk inorganic solids. We have recently shown that DNP-enhanced NMR measurements provide crucial improvements in signal sensitivity that enable the detection of dilute (~0.1 wt%) organic molecules adsorbed on low-surface-area (1 m²/g) silicate surfaces,¹ which have previously been infeasible to characterize. New DNP-NMR analyses reveal the molecular origins of the surprisingly different adsorption behaviors of organic molecules at silicate and carbonate surfaces, which in competition with water, can favor metastable phases and mediate the rates of subsequent inorganic crystallization. Specifically, solid-state two-dimensional (2D) dipolar-mediated ¹³C{¹H}, ²⁹Si{¹H}, ³¹P{¹H}, ³¹P{¹³C}, and J-mediated ¹³C{¹³C} DNP-NMR measurements establish the interactions of adsorbed saccharides, phosphonates, and/or water molecules at inorganic surfaces in industrially important tricalcium silicate or calcium carbonate mixtures. The DNP-NMR spectra yield correlated signals that are well resolved, even at low temperatures (~100 K), and establish that the hydration of silicates and carbonates depends on the relative extents and types of organic-inorganic surface interactions, which are directly related to the architectures of the organic molecules. The results provide new understanding of surface-mediated hydration and crystallization processes that account for many of the macroscopic properties of cement-water mixtures and the carbonate products from carbon capture technologies.

Gaining insight into the ligand binding to chlorite dismutase from *Magnetospirillum* sp.

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The heme-containing chlorite dismutase (Cld) that catalyzes the reduction of chlorite to chloride and dioxygen, is an essential protein in (per)chlorate-reducing bacteria. Cld proteins are considered possible candidates in the development of chlorine oxoanion bioremediation processes. Besides photosystem II, Cld is the only example of an enzyme capable of catalyzing an O-O bond formation.

In order to gain insight into the inner working of the Cld protein, combined XRD, EPR and DFT studies are performed. Ferric wild-type Cld of *Magnetospirillum* sp (MaCld) forms a pentameric complex (Figure 1) for which EPR surprisingly reveals the presence of a multitude of ferric heme centers with different spectral signature. This heterogeneity seems to be related to the openness of the heme pocket. It largely disappears upon addition of salts, such as NaCl, or upon addition of axial ligands, such as azide, nitrite or imidazole, although pH-dependent changes in the EPR spectra are observed for the azide-ligated protein. The non-trivial analysis of the pulsed EPR data in terms of the heme-pocket structure will be discussed in detail.

**Figure 1. Pentamer structure of MaCld.**
Several classes of enzymes transduce the energy from ATP hydrolysis, taking place in the presence of the metal cofactor Mg$^{2+}$, into molecular motion. Mechanistic insights into this process, which is essential for their specific activities, can be obtained by elucidating the pathway through which ATP hydrolysis is coupled with conformational rearrangements. In the aforementioned systems the position along the reaction coordinate can be assessed by substituting the essential diamagnetic metal cofactor Mg$^{2+}$ with the paramagnetic Mn(II) ($S = 5/2, I = 5/2$) \[1\] and probing the hyperfine interaction between the unpaired electron spin of the Mn(II) and the nuclear spin of the $^{31}$P nuclei ($I = 1/2$) of the nucleoside phosphate coordinated to the metal ion. This hyperfine interaction, which was found to report on the hydrolysis state of the nucleoside phosphate \[2\], can be measured with electron paramagnetic resonance (EPR) techniques, specifically electron-nuclear double resonance spectroscopy (ENDOR) or electron-electron double resonance (ELDOR)-detected nuclear magnetic resonance spectroscopy (EDNMR).

Using a high field/high frequency spectrometer (94.9 GHz, ≈ 3.5 T), the proposed methodology is applied to follow the ATPase kinetics of (i) the E. Coli DEAD-box helicase DbpA \[2\] and (ii) the bacterial ATP-binding cassette (ABC) exporters MsbA and BmrCD \[3\]. We show that in $^{31}$P-ENDOR the ATP hydrolysis state is mainly contained in the spectral lineshape, whereas in $^{31}$P-EDNMR this information is mainly associated with the signal intensity. A direct proportionality is found between this latter parameter and the fraction of ATP in ATP:ADP mixtures; this allows extracting quantitative information about the reaction kinetics from the spectroscopic measurements.

\[1\] G. H. Reed et al., Met. Ions Biol. Syst. 2000, 37, 183.
\[3\] S. Mishra et al., eLife 2014, 3:e02740.
Time-optimal excitation of maximum quantum coherence:

Physical limits and pulse sequences

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The simplification and editing of complicated and overlapping spectra are highly desirable in many applications, such as the analysis of complex mixtures or of large biomolecules. To a certain extent, this can be achieved by using multi-quantum NMR spectroscopy. The largest simplification for homonuclear experiments is obtained by exciting and evolving the maximum quantum (MaxQ) order that can be created in a given spin system.

Here we present a study of the optimum efficiency of excitation of MaxQ coherence using analytical and numerical methods based on optimal control theory (OCT). The theoretical limit of the achievable MaxQ amplitude and the minimum time to achieve this limit are explored for a set of model systems of up to five coupled spins. In addition to arbitrary pulse shapes, two simple pulse sequence families of practical interest are considered in the optimizations and compared to the conventional approach using a 90°-Δ-90° pulse sequence element.

Based on the numerical results we show a novel analytical transfer scheme for two-spin systems using hard spin-selective pulses and isotropic mixing which allows double-quantum coherence generation twice as fast as conventional approaches. In addition, we are able to show that in a three-spin chain it is possible to excite MaxQ coherence three times more efficient using so-called geodesic pulse sequences than with the conventional sequence and approximately 10% faster than topology-selective excitation schemes introduced by Levitt and Ernst.

Figure 1: Comparison of the standard 90°-Δ-90° pulse sequence with a numerically found geodesic pulse sequence for a three-spin system. Shown is a comparison of the time-optimal (TOP) curve of MaxQ excitation (left), the best found pulse sequences (middle) and the DROPS representation of the MaxQ excitation efficiency of these pulse sequences (right).

References:


Optical Detection of Nuclear and Electron Spins in Diamond

Using Nitrogen-Vacancy Centers

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The negatively charged nitrogen-vacancy (NV-) centre in diamond is a colour centre that has a number of favourable properties including spin-dependent fluorescence, spin-coupling to its magnetic environment and long coherence lifetimes as compared with other similar electron spin systems, even at room temperature. Because of these properties, optically detected magnetic resonance (ODMR) of the NV- centre has proven to be a useful tool for exploring the rich dynamics arising from interactions between spin systems in bulk diamond as well as on the diamond surface at room temperature [1], [2]. Specifically, the NV- centre can be used to obtain quantitative information about nuclear quadrupolar coupling constants [3], about electron-nuclear hyperfine constants and about local paramagnetic spin concentrations in diamond [4]. In addition, we recently measured the fluorescence intensity of several diamonds with different concentrations of nitrogen-vacancy centres and paramagnetic nitrogen while continuously irradiating the diamond samples with 532 nm laser light and a radio-frequency magnetic field from 0.1 to 200 MHz. We observed changes in the intensity and structure of the optically detected cross-relaxation spectra as the concentration of the paramagnetic species in the sample was changed. We will review a few of these optical experiments and will discuss the feasibility of obtaining quantitative information about coupling constants and local spin concentrations of species adjacent to NV- centres via optically detected cross-relaxation at earth’s magnetic field.

References:

New EPR experiments with frequency-swept excitation

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Frequency-swept pulses can extend excitation bandwidth by more than an order of magnitude compared to monochromatic rectangular pulses at the same microwave power and resonator bandwidth. Our setup completely covers the spectrum of nitroxide spin labels at 34 GHz and can excite a sizeable fraction of the spectra of metal ions. Here we discuss pulse sequence design with these new capabilities. Distance distributions of pairs of Gd(III) centers can be measured with higher sensitivity and fidelity. Magnetization from a frequency band as wide as 2.5 GHz can be used with pre-polarization. At distances longer than 3 nm, two chirp pump pulses with individual bandwidths of 0.6 GHz provide modulation depths of \( \approx 20\% \) while detecting at the maximum of the spectrum and in the center of the resonator mode. Phase distortion from dynamical Bloch-Siegert shift, which is noticeable with a single chirp pump pulse, is largely canceled with such a pair of pulses. For shorter distances, the distance distribution is artificially broadened by admixture of levels with magnetic quantum numbers \( m_S \neq \pm 1/2 \) to the central transition. The effect is suppressed by pumping only spin packets with large first-order zero-field splitting. However, such pumping in the wings of the resonator dip and of the Gd(III) spectrum requires longer chirp pump pulses, which in the DEER experiment cause destructive interference of the dipolar modulation from spin packets with different resonance offsets at short distances. The chirp-induced dipolar modulation enhancement (CIDME) experiment solves this problem by temporary storage of observer spin magnetization as longitudinal magnetization.

![Figure 1: FT-EPR correlated SIFTER spectrum of a shape-persistent biradical with a length of 3.9 nm.](image)

Excitation of the complete nitroxide EPR spectrum allows for a two-dimensional experiment that correlates the dipolar spectrum to the Fourier-transform EPR spectrum. Such spectra contain information on the relative orientation of the two coupled spins and on the orientation of the spin-spin vector in their molecular frames, which otherwise can be obtained only by sets of orientation-selective DEER experiments with different pump/observer positions in the nitroxide spectrum.
Atomic insight into the function and activity of molecular chaperones

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

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

Three examples from diverse systems

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Biological NMR continues to be used in ever more challenging systems and the need to refine the structures of bio-molecules and complexes from ever sparser data continues to increase. Here we show novel approaches we have used for three diverse systems in which important structural insights have been gained without nOes or using highly ambiguous nOe data. These include the use of the XCamshift chemical shift force-field, our implementation of CamShift in XPLOR-NIH

For the transient association between the truncation mutant of human β2M-ΔN6 and its mouse homologue, which provides insights into the inhibition of amyloid formation1, we have for the first time used a chemical shift force-field (XCamshift), RDCs and paramagnetic relaxation enhancements (PREs) together to refine a complex without resort to the use of rigid body models (fig a). In the case of the FusB-EFG complex from S. aureus2, which mediates antibiotic resistance to fusidic acid (an important antibiotic), we used RDCs, PREs, and chemical shift ambiguous interaction restraints (AIRs) with the addition of solvent PREs to refine the complex, domain orientations and the internal structure of a system with limited solubility (200µM) and high mass (60kDa) without the use of nOes (fig b). Finally, for Hepatitis C we used membrane-CS-Rosetta and refinement with chemical shifts (XCamshift) along with highly degenerate nOe restraints to calculate the protomer structure of the heptameric membrane complex3, which was used to aid the design of new and potent inhibitors (fig c). Clearly the use of sparse data for structure calculation is set to flourish as the diversity of systems that can be targeted by them continues to expand.

a. β2Mm- β2Mh(ΔN6) b. FusB-EFG c. Hepatitis C P7 channel

De novo 100 kHz MAS structure determination of a viral nucleocapsid and insights from high field DNP

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The atomic-level characterization of large viral particles is one of the greatest challenges of modern structural biology, as well as a fundamental step for the design of effective antiviral treatments. Over the last decades, solid-state NMR (ssNMR) has developed into a powerful structural tool for studying structure and dynamics of solid biological samples at atomic resolution. However, the inherently low sensitivity and poor resolution of the technique has limited its applicability to small proteins that can be tightly packed at a high molar concentration, while large proteins or multi-domain assemblies were mostly inaccessible to site-specific ssNMR studies. This has been recently overcome by the introduction of faster spinning probes, which facilitate the use of proton-detected ssNMR experiments, as well as by dynamic nuclear polarization (DNP), which allows transfer of polarization from the unpaired electrons of a paramagnetic center to the surrounding nuclei, and can enhance the sensitivity of ssNMR experiments by several orders of magnitude.

Here we demonstrate the effectiveness of the recently developed ssNMR methods employing proton detection at high field and 100 kHz MAS by structure determination of the 2.5 MDa icosahedral capsid of the AP205 bacteriophage. We show that at this spinning regime spectral resolution is high enough to detect resolved correlations from amide and side-chain protons of all residue types, and to reliably measure a dense network of 1H-1H proximities that define the dimeric capsid subunit structure. The subunit structure is then used in conjunction with a low resolution EM map to construct an atomic-level description of the global capsid architecture. Additionally, we show that high quality DNP NMR spectra of the AP205 nucleocapsid can be obtained by combining high magnetic field (800 MHz) and fast magic-angle spinning (40 kHz). This enables assignment of aromatic resonances of the encapsidated RNA and the nucleoprotein, which are not observed at room temperature, opening up new possibilities for intermolecular interaction studies.

![Figure 1.](image)

Comparison of the aromatic region of the 800 MHz 13C-13C 2D NMR correlation spectra of AP205 nucleocapsids using DNP at 100 K and using conventional NMR at 280 K (right).
Molecular mechanism of spider silk formation and structural studies of artificially spun fibers

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Spider silk is one of the most outstanding biomaterials, combining high tensile strength with elasticity and toughness. It is made through association of spider silk proteins, called spidroins, which have a MW of ca. 300-600 kDa. More than 90% of the spidroin sequence is composed by repetitive domains (Rep), which confer mechanical properties to spun fibers and are flanked by N- and C-terminal domains (NT and CT). The spidroins are stored in the silk gland as dimeric proteins due to association of the CTs. During silk spinning, a spidroin solution is passed from a storage sac through an S-shaped duct, where changes in solution conditions induce polymerization of the spidroins through assembly of the NTs accompanied by structural changes in the Rep and CT domains.

To understand how rapid fiber production is ensured and at the same time premature association of spidroins in storage sac is prevented, we studied dimerization mechanism of NT produced by A. ventricosus spiders in minor ampullate silk glands. Determination of solution state NMR structures of monomeric and dimeric NTs, corresponding, respectively, to conformation of NT before and after spidroins have assembled into fibers together with site directed mutagenesis and tryptophan fluorescence studies allowed us to identify amino acids involved in control of dimerization process in response to changes in pH and salt concentration and to conclude, that dimerization of NT and hence its control of polymerization of spidroins, takes place via a three step mechanism that involves prealignment of NTs, uptake of two protons by each NT inducing formation of weakly associated dimer and formation of strongly associated dimer through an uptake of a third proton.

Additionally, we characterized the solution structure of the Rep domain in a spidroin construct that consists of the NT, two repetitive domains and the CT (NT-2Rep-CT). Our results show that each of the domains behaves in a highly independent manner and response of the NT and CT to changes in solution conditions is not influenced by presence of the other, nor the Rep domain.

Finally, we spun fibers from the NT-2Rep-CT proteins that were reverse labeled with amino acids present only in one of the domains. Using very fast MAS and proton detected experiments we characterized structural changes experienced by the Rep and CT domains when forming fibers and corroborate biological relevance of our previous structural studies on isolated terminal domains [1-2].

Solid-state NMR (SSNMR) Studies of Amyloid Assemblies for Aβ(1-42) and Ultra-Fast 2D Protein SSNMR

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This work entails two separate topics on solid-state NMR (SSNMR) methodologies and its applications to biomolecules. First, we discuss structural studies of amyloid fibrils and oligomers for 42-residue Alzheimer’s amyloid β (Aβ(1–42)). Increasing evidence suggests that formation and propagation of misfolded aggregates of Aβ(1–42), rather than the more abundant Aβ(1–40), provokes the Alzheimer’s cascade. Nevertheless, structural details of misfolded Aβ(1–42) have remained elusive. In this talk, we present unique structural features of the first atomic model of Aβ(1–42) amyloid fibril based on SSNMR data (Fig. 1) (1) with recent analysis results of other polymorphs of Aβ(1–42) fibrils that were isolated in our lab. Our ongoing efforts to capture structural features of metastable oligomers for Aβ(1–42) are also presented. The results provide insight into amyloid misfolding of Aβ(1–42) in Alzheimer’s disease.

Second, we discuss resolution and sensitivity enhancement in 1H and 13C biomolecular SSNMR under ultra fast magic angle spinning (UFMAS) conditions (≥ 80 kHz) in a high magnetic field (1H frequency: 750-800 MHz). Major challenges in biomolecular SSNMR are limited sensitivity and resolution. Here, we present an approach for extremely fast data collection of 2D SSNMR data (< 1 min) for a nano-mole scale protein sample (10–25 nmol) by using 1H detected SSNMR in an ultra-fast MAS condition at 100 kHz and paramagnetic condensed data collection (PACC) method (2, 3). The current sensitivity limit of multi-dimensional SSNMR is discussed with other subjects related to SSNMR experiments using UFMAS.

References:

Translating dDNP Hyperpolarized MR for Clinical Patient Studies in the Brain and Prostate

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Hyperpolarized Carbon-13 MR has the potential to become an important new radiological tool for cancer metabolic imaging by directly investigating key cellular enzymatic pathways in vivo. Hyperpolarized $^{13}$C imaging using the dissolution DNP (dynamic nuclear polarization) method provides a $>10,000$ fold signal enhancement for detecting $^{13}$C probes of endogenous, nontoxic, nonradioactive substances such as pyruvate to monitor metabolic fluxes through multiple key biochemical pathways. The hyperpolarization of $[1^{-13}C]$pyruvate has demonstrated the ability to not only detect pyruvate uptake but also the in vivo enzymatic conversion to $^{13}$C-lactate through the enzyme lactate dehydrogenase (LDH), $^{13}$C-alanine through the alanine transaminase (ALT) pathway; and $^{13}$CO$_2$ & $^{13}$C-bicarbonate through the pyruvate dehydrogenase (PDH) catalyzed metabolic pathway. The value of this powerful metabolic imaging technique for cancer imaging was shown first by Golman et al and we have applied it in a number of preclinical animal studies for detecting presence, progression and response to therapy in cancer models and in animal studies of liver and kidney disease. Our first-in-man Phase 1 clinical trial in prostate cancer patients, published in Science Translational Medicine (2013), demonstrated feasibility and safety.

We have conducted preclinical studies in multiple cancers including prostate, liver, kidney and brain. While $^{13}$C-pyruvate is the first to studied in humans, other hyperpolarized substrates including bicarbonate, glutamine, lactate, urea, alanine, bicarbonate, ketobutyrate, and dehydroascorbate have also been investigated preclinically to probe metabolism, perfusion and physiology. These studies have demonstrated significant metabolic changes with cancer presence, aggressiveness and response to therapy. The detection of a significant correlation with grade for up-regulated LDH-conversion to lactate by HP MRI is of great potential clinical value since there is no accurate current imaging technique to identify aggressive prostate cancers (which should be treated) from indolent cancers that may be managed through “active surveillance”. In addition to metabolic pathway information, hyperpolarized probes can provide valuable physiological information such as perfusion information (with HP $^{13}$C-urea) and pH (with HP $^{13}$C sodium bicarbonate).

In addition to basic science and animal studies utilizing HP $^{13}$C MRI, we have also focused on research to develop and translate hyperpolarized $^{13}$C methods for ongoing clinical trials. Specialized carbon-13 MRI techniques were developed to provide extremely rapid volumetric imaging and serial dynamic acquisitions to monitor temporal metabolic changes in cancer patients following the injection of HP $^{13}$C-pyruvate. Led by the UCSF Cancer Center Investigational Therapeutics group, our multidisciplinary research group designed and initiated the world’s first clinical trial of hyperpolarized carbon-13 MRI. This study received FDA-IND approval and a total of 31 patients were studied using this new metabolic imaging method. All exams demonstrating feasibility and safety with no dose-limiting toxicity up to 0.42ml/kg of 250mM HP pyruvate which is the selected dose for current and future trials that now have FDA and IRB approval to conduct clinical research HP C-13 MRI studies in prostate cancer, brain tumors, and metastases to liver.
Recent Developments in Paramagnetic Solids – Batteries and Fuel Cell Materials – In and Ex-situ

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This talk will describe recent applications of NMR spectroscopy to study battery and fuel-cell materials, starting with an overview of some of the materials challenges in this field. A major focus of this talk will be on new developments in the field of paramagnetic NMR. Specifically, the use of $^{17}$O NMR spectroscopy to investigate structural disorder, defects and dynamics in paramagnetic materials will be described, in compounds such as the mixed ionic and electronic conductor La$_2$NiO$_{4+x}$, where oxygen non-stoichiometry plays a critical role in the transport mechanism.

The assignments of the resonances are aided by first principles calculations of hyperfine shifts. Recent developments in approaches to calculate these shifts and how to relate 0K calculations to room-temperature data will be outlined. Finally, new hardware and applications of NMR in the battery area, to a diverse range of chemistries – from lithium, sodium to magnesium batteries, will be discussed.
Metabolic phenotyping: a revolution in diagnosis?

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Metabolomics profiling of body fluids by NMR can be obtained in minutes, has unsurpassed reproducibility, and low costs (a few tens of Euro when done in high-throughput mode). Even on a small scale, and including statistical data analysis and comparison with databases, metabolomic profiling can be performed at less than 200 Euro, i.e. significantly less than other profiling techniques.

We and others have shown that individual metabolic profiles exists, that they are stable over periods of many years, insensitive to alterations of lifestyles or mild disease conditions, but sensitive to the onset of major diseases from a very early stage.

Examples of successful metabolomics profiling from our laboratory are for the diagnosis of potential celiac disease, the prediction of relapse for breast cancer, the prediction of survival of metastatic colorectal cancer, and the early diagnosis of heart failure.

Typical diagnostic accuracies range between 80-90%, which is remarkable considering that they can be obtained in the absence of clinical symptoms, and that they can be obtained from a single NMR profile by comparing it with the databases of a number of different diseases. Diagnostic accuracies improve dramatically if the profile of an individual is compared with earlier profiles of the same individual. These evidences suggest that metabolomics by NMR can become a first-line, population-wide screening method.
Using protein side-chain dynamics to probe an enzymatic transition state and allosteric enzymatic regulation

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Whilst knowledge of the structure and dynamics of the protein backbone is essential to understand many aspects of protein function, understanding the dynamics and interactions of protein side chains is crucial. Side chains form important parts of many active sites of enzymes and side chains cover the protein surface and play instrumental roles in substrate recognition and non-covalent interactions. Two examples will be shown, where NMR studies of side chains have provided unique insight into the function of enzymes.

States along the phosphoryl transfer reaction catalysed by the nucleoside monophosphate kinase UmpK were captured and changes in the conformational heterogeneity of active site arginine side-chains were quantified by NMR spin-relaxation methods. In addition to apo and ligand-bound UmpK, a transition state analogue (TSA) complex was utilised to probe the transition state. The catalytically essential arginine side-chain guanidino groups were found to be remarkably rigid in the TSA complex, indicating that the enzyme has evolved to restrict the conformational freedom along its reaction path.

Histone deacetylases (HDACs) are key enzymes in epigenetics and important drug targets in cancer biology. While it is known that HDACs regulate a variety of cellular processes, less is known about the mechanisms by which these enzymes themselves are regulated. Using methyl-TROSY NMR spectroscopy and relaxation dispersion we show that histone deacetylase 8 exchanges between related conformations whose populations shift upon mutation or binding events. Structurally and dynamically, our results show that the active site is bi-directionally coupled to a distal allosteric region around 20 Å from the active site, where binding events at the active site trigger changes in the allosteric region and mutations in the allosteric region affect the active site and the enzymatic activity.

Figure 1. Conformational heterogeneity of UmpK arginine side-chains in the substrate-like AP5U complex (left) and in the transition state analogue complex (right). The side-chain order parameters $S^2$ were obtained from $^1$C and $^1$H detected spin-relaxation experiments and mapped on the respective crystal structures.

Figure 2. Structural representation of the chemical shift differences upon TSA ligand binding. The colour code ranges from blue (no chemical shift difference) to red (highest chemical shift difference).
Influenza A RNA polymerase is formed from three components, PA, PB1, and PB2. PB2 is independently imported into the nucleus prior to polymerase reconstitution. All crystallographic structures of the PB2 C-terminus reveal two globular domains, 627 and NLS, that form a tightly packed heterodimer that is apparently incompetent to bind Importin α and enter the nucleus.

Using a combination of solution-state NMR, small-angle scattering and single molecule FRET, we have discovered that in solution 627-NLS populates a temperature-dependent dynamic equilibrium between closed and open states. The closed state is stabilized by a tripartite salt bridge involving the 627-NLS interface and the linker, that becomes flexible in the open state, with 627 and NLS dislocating into a highly dynamic ensemble. Activation enthalpies and entropies associated with the rupture of this interface were derived from simultaneous analysis of temperature dependent chemical exchange saturation transfer measurements, revealing a strong temperature dependence of both open-state population and exchange rate.

SmFRET and SAS demonstrate that only this open-form is capable of binding to importin α and that, upon binding, the 627 domain samples a dynamic conformational equilibrium in the vicinity of the C-terminus of importin α. This intrinsic large-scale conformational flexibility, mediated by the flexible linker, therefore enables 627-NLS to bind importin through conformational selection from a temperature-dependent equilibrium comprising both functional forms of the protein, thereby allowing the influenza polymerase to enter the nucleus of the infected cell.


**A combination of exchange NMR (CEST), SAXS and FRET allowed the identification and structural characterisation of a temperature-dependent equilibrium between two conformations of 627-NLS and of its complex with importin α.**
Exact NOEs for ensemble-based structure calculations

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The structure-function paradigm is increasingly replaced by the structure-dynamics-function paradigm. All protein activity is steered by the interplay between enthalpy and entropy. Conformational dynamics serves as a proxy of conformational entropy. Therefore, it is essential to study not only the average conformation but also the spatial sampling of a protein on all timescales. To this purpose, we have established a protocol for determining multiple-state ensembles of proteins based on exact nuclear Overhauser effects (eNOEs), which gives ensemble averaged distance restraints with an accuracy of better than 0.1 Å.

When applied to the enzyme cyclophilin, which is a proline cis/trans isomerase, the ensemble description reveals the presence of an open and a closed state of cyclophilin, which is indicative of large-scale correlated motion. In the open state, the catalytic site is preorganized for catalysis, thus suggesting the mechanism of action to be conformational sampling, while the ligand-binding loop appears to act through an induced fit mechanism. This finding is supported by affinity measurements of a cyclophilin designed to be more open. Overall, more than 60-70% of the side-chain conformations of cyclophilin appear to be correlated.

When applied to the WW domain, which shows allostery between two protein interaction sides, the ensemble description reveals the presence of two states within the core of the protein connecting the two binding sides suggesting conformational sampling as the mechanism of allostery.

Figure 1. The two states of cyclophilin indicated by two colored bundle representations of the backbone.
Dynamics of membrane proteins in detergent micelles: the case of mitochondrial carriers

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Dynamic exchange between distinct conformations is a hallmark feature of many membrane proteins, and in particular of membrane transporters. Ironically, the overwhelming majority of high-resolution structures have been obtained by blocking the proteins in a single conformation. Despite the important insight that these structures provide, characterizing membrane protein dynamics, and identifying alternative structures is essential for understanding how transport processes occur. NMR spectroscopy is highly suited to determine such processes, and to detect “hidden” alternative conformations. Indeed, a growing number of studies have addressed conformational exchange in membrane proteins (1,2). Yet a fundamental point in such studies is how the chosen membrane-mimicking environment influences dynamics. Investigation of detergent effects on membrane protein dynamics requires systematic analyses, extending the very few reported cases (3, 4).

Here we study dynamics in a family of mitochondrial membrane carriers, which enable diffusion of many different substrates across the inner mitochondrial membrane. Recent NMR studies of structure and dynamics in DPC detergent have been reported (1, 5).

We investigate the structure, dynamics and interactions in three proteins of this family. Using CPMG RD experiments we find remarkably similar dynamics in these proteins, involving an exchange to an alternative conformation on a millisecond time scale. Remarkably, the time scale of exchange is similar to transport kinetics (6). By comparing dynamics in different mutants, and complementary functional tests, however, we establish that the detergent has an important impact onto structure and dynamics. Our studies show the importance of carefully investigating the effects that detergents exert on membrane proteins, and we discuss the possibly wider implications for biophysical studies.

Triggered by these findings, we show a novel procedure for studying membrane proteins in native nanodiscs (7), extracted directly from the bacterial membrane, using solid-state NMR. This first experimental report of ssNMR studies of polymer-based nanodiscs, shown for an alpha-helical and a beta-barrel protein, may become a general avenue for studying membrane proteins in close-to-native conditions.

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Dynamics from NMR: reaching a higher orbit with a sample shuttle

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Nuclear Magnetic Resonance is most often carried out in a fixed magnetic field. Historically, higher fields have always led to better sensitivity and resolution, which has opened the way to the characterization of molecular systems of increasing complexity. However, the quantification of nuclear spin properties at low magnetic fields can be extremely insightful. Relaxation rates, measured at magnetic fields that lie below the range of high-resolution NMR (7 T and below), provide information about motions occurring on nanosecond timescales, which are difficult to retrieve from high-field measurements. Here, we show that low-field properties can be measured, while retaining the sensitivity and resolution of high-field NMR. Our approach employs a high-field NMR spectrometer equipped with a shuttle system, which allows the sample to move swiftly in the stray field of a superconducting magnet.

Low-field measurements of relaxation rates in proteins offer unique information to identify and refine nanosecond time scale motions. We will show measurements of nitrogen-15 and carbon-13 relaxation rates at magnetic fields as low as 0.2 T. The analysis of relaxation rates over two orders of magnitude of magnetic fields allows the precise characterization of backbone motions in folded and disordered proteins alike. Motions of methyl-bearing side chains on time scales in the low picosecond to low nanosecond range can be quantified with a single set of carbon-13 relaxation rates recorded at 21 magnetic fields.

Relaxation rates are not the only properties that may benefit from measurements at low magnetic fields. Usually, measurements of chemical shifts benefit tremendously from higher magnetic fields. However, NMR spectra may be of poor quality at high field for some nuclei in the presence of line broadening due to chemical exchange or relaxation from chemical shift anisotropy. Over decades of methodological and instrumental developments, many NMR experiments have been proposed to probe physical and chemical properties at one particular magnetic field and observe the NMR signals at another field. Here, we introduce a unique two-field NMR spectrometer that permits the excitation of $^1$H, $^{13}$C and $^{15}$N spins and the observation of the resulting signals at two magnetic fields.

Chemical shifts can be measured at low magnetic field in a two-dimensional experiment, where polarization and detection are performed at high field. We show that good quality two-field NMR spectra can be measured with high resolution in all dimensions. In the presence of intermediate chemical exchange, signals of exchanging groups completely invisible at high field can be recovered in two-field correlation spectra, which are much less sensitive to the exchange process. Two-field NMR makes it possible to measure chemical shifts and other magnetic properties at the most suitable magnetic field. This approach allows the observation of molecular systems prone to enhanced chemical dynamics and paves the way for performing NMR on very high field spectrometers in the GHz era.
**Recent developments in $^{17}$O NMR spectroscopy of organic solids**

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In recent years, solid-state $^{17}$O (spin-5/2) NMR spectroscopy has been shown to be feasible for studying biological molecules [1,2]. In many cases, direct detection of the oxygen atom at the reaction center/active site is capable of providing important information about chemical bonding and molecular structure. The goal of our research program is to push the detection and resolution limits of solid-state $^{17}$O NMR and to demonstrate that the technique can be complementary to conventional NMR methodologies that rely on probing spin-1/2 nuclei such as $^{13}$C and $^{15}$N. For example, we have recently shown that high-quality solid-state $^{17}$O NMR spectra can be obtained not only for ligand-protein complexes [3] (Fig. 1a) but also for paramagnetic coordination compounds [4] (Fig. 1b).

In this talk, new experimental $^{17}$O NMR results will be presented in the following areas: (1) acyl-enzyme intermediates, (2) ligand-protein complexes, (3) paramagnetic proteins, and (4) detection of molecular dynamics.

**References**

A simple method to convert parahydrogen singlet order to magnetization on a nearby carbon nucleus

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Parahydrogen molecules are a repository of singlet order that can be observed if, after addition to a molecule, the protons become chemically inequivalent (PASADENA¹). Techniques to transfer this singlet order to magnetization on nearby heteronuclei have been developed to enhance the utility of PHIP. This feat was first achieved by Duckett, Newell and Eisenberg using an INEPT+ pulse sequence²,³. More recently, this transfer has been performed with different pulse sequences⁴, and by manipulating the external magnetic field⁵,⁶.

We present a novel technique to transfer parahydrogen singlet order in both AA’XX’ and AA’X magnetic systems to a carbon (X) nucleus through long-range J couplings. This new method can give 100% transfer in the absence of relaxation, and doesn’t require field cycling. A diagram of our spin systems is presented in Fig. 1. It is the difference in long-range heteronuclear J_{HC}-couplings that provides a symmetry-breaking route for population transfer.

![Diagram of spin systems](image)

**Figure 1.** Singly labelled maleic acid (left) and doubly labelled maleic acid (right), with the AA’X and AA’XX’ coupling diagrams below.

The primary molecules used in preliminary experiments were doubly and singly $^{13}$C-labelled maleic acid, followed by others. We have populated proton singlet order in these molecules using a standard pulse sequence, and demonstrated the transfer of singlet spin order to the carbon-13 nuclei in agreement with theory and simulations. Presently, we are preparing to complete the demonstration by performing the same experiment with parahydrogen as the source of singlet order.

**References:**

In-situ $^{51}$V NMR to follow formation of solid vanadium-based materials

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Vanadium-based materials are promising candidates for a wide range of applications such as batteries, catalysts, and microporous materials. Nevertheless, desirable properties depend strongly on the structure of the material. A common method to study structural features is powder diffraction, which however reflects only on long-range order whereas NMR gives valuable information about the local structure of the material.

Many vanadium-based materials are so-called mixed valence materials containing metals with various oxidation states, e.g., $V^{(IV)}$ and $V^{(V)}$. Despite the high natural abundance and good sensitivity, $^{51}$V NMR is complicated by the fact that $V^{(IV)}$ is paramagnetic. The result is that the $V^{(IV)}$ nuclei are not directly observable with NMR, whereas the diamagnetic $V^{(V)}$, which can be observed, have broadened signals due to the hyperfine interactions with $V^{(IV)}$. In addition, $V^{(IV)}$ may form during the course of the synthesis or already be present in the starting material.

Here, we applied in-situ $^{51}$V liquid and solid-state NMR to follow the formation of various hydrated vanadium pentaoxide nanosheets and vanadium borates. In addition, NMR experiments were complemented with EPR measurements to monitor paramagnetic species.

In-situ $^{51}$V solid-state NMR spectra revealed a loss in the $V^{(V)}$ signal as the formation of hydrated vanadium pentaoxide nanosheets progressed (see Figure 1) while additional features in $^{11}$B NMR spectra appeared during the evolution of microporous vanadium borates structures. Different $^{11}$B peaks were observed depending on the composition of the vanadium borates.

In-situ $^{51}$V in combination with EPR gives a detailed picture of the mixed-valence vanadium-based materials and thus allows us to determine local structural features, and in particular provides local information about the paramagnetic centers.

Figure 1. In situ $^{51}$V solid state spectra as a function of time monitoring the formation of hydrated vanadium pentaoxide nanosheets. The inset shows the normalized integral versus time.
Spotting single-molecule magnets with paramagnetic NMR

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Single-molecule magnets (SMMs), the term referring to chemical compounds exhibiting slow magnetic relaxation and magnetic hysteresis of purely molecular origin, have been discovered in the early 1990. Since then, they emerged as perspective components for information storage, quantum computing, spintronics and magnetic refrigeration. A necessary condition for a compound to be an SMM is a large axial magnetic anisotropy $D$ that splits the energy levels of a metal ion under zero magnetic field and gives rise to an energy barrier between the states with opposite directions of the magnetic moment.

The method of choice for detecting a large magnetic anisotropy is ac-magnetometry, which allows determining both the $D$ value and the effective barrier height $U$. This technique, however, is quite demanding and requires accumulation of large array of data at different magnetic fields and at very low temperatures. If the purity of the compound is not ideal, which is often the case of complexes with metal atoms in unusual oxidation states, the obtained results should be interpreted with extreme care.

In this report, we will speculate that NMR spectroscopy may be a fast and convenient tool for evaluating magnetic anisotropy of transition metal complexes in their solutions based on a linear relation with pseudocontact paramagnetic shifts. As modern DFT approaches can provide a reliable picture of spin density distribution, they are chosen to estimate Fermi contact shifts with the remaining part of the paramagnetic shifts considered dipolar in origin; the pseudocontact shifts are then used to extract the value of the magnetic anisotropy at different temperatures.

We have recently (Novikov et al., J. Am. Chem. Soc., 2015, 137, 9792) employed this protocol to identify a new class of single-ion magnets (cobalt(II) clathrochelates) with a very high Orbach relaxation barrier of 152 cm$^{-1}$. To do this, one only needs a high-resolution NMR spectrometer to be available and a reasonable number of $^1$H NMR spectra to be collected at different temperatures. The presence of impurities is also not a problem, as long as they allow detecting the signals of the compound under study. Therefore, this NMR-based approach paves the way to fast and inexpensive prescreening of possible SMM candidates.

The study was supported by RSF (project 14-13-00724) and CPRF (grant MK-6320.2016.3).
Application of Solid-State NMR to Pharmaceuticals, Polymers, Self-Assembly
and Plant Cell Walls

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Applications of advanced solid-state NMR methods for probing intermolecular interactions, notably hydrogen bonding are presented: Homonuclear $^1$H-$^1$H double-quantum (DQ) experiments reveal proximities (typically under 3.5 Angstroms) among pairs of hydrogen atoms, for example distinguishing between ribbon-like or quartet-like self assembly in guanosine supramolecular structures [1-3]. $^{14}$N-$^1$H spectra show one-bond NH connectivities or additionally longer-range NH proximities depending on the recoupling time employed. Applications to guanosine self assembly [1,2] and proving molecular level mixing in co-crystals [4,5] and an amorphous dispersion [5] are shown. Recent results applying $^{13}$C refocused INADEQUATE spectra to characterize biopolymer interactions in plant cell walls [6] and to identify molecular structure in novel polyaniline derivatives [7] will also be presented.

NMR Experiments for Two 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 1H and 19F nuclei not least because of the particularly important role that 19F 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.

![Figure 1](image-url)

**Figure 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.

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.

Quantum control of spins in solids and its applications

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The science of quantum control lies at the heart of modern physics. Various applications of quantum control have emerged and we witness great development in recent years, such as quantum computation, quantum simulation, and quantum metrology, etc. Spins of electrons and nuclei are among the most promising physical systems that can realize reliable and robust quantum control. They have a major advantage since the quantum coherence can be protected very efficiently against external noise, which represents the main challenge to the large-scale implementation of quantum control.

My presentation will mainly focus on our recent experimental study of quantum control over spins in solids. We concern on several respects such as decoherence suppressing with dynamical decoupling [1,2], precise spin control[3,4], efficient realization of quantum algorithms[5] and simulation[6], and ultrasensitive sensing with single spins in diamond[7,8].

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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](see Fig. 1A): 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. 1B. 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 highly sensitive low-noise detection techniques. We will also address applications of the experimental design to conventional (“non-exotic”) NMR.

![Figure 1: (A) Axion – nuclear spin couplings that give rise to a “pseudo-magnetic field” which can be utilized in place of $B_1$ RF irradiation for an NMR-based experiment. (B) A cartoon-level schematic of the CASPEr concept.](image)

High Resolution $^{14}$N Magic Angle Spinning Solid-State-NMR

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Here we report on our latest progress towards making the exploitation of $^{14}$N solid-state NMR routine for biomolecular systems, pharmaceuticals and natural materials.

The moderate quadrupolar constant of $^{14}$N provides a wealth of structural and dynamic information although ad-hoc methodology is required in order to obtain good sensitivity and resolution. Two approaches have recently become popular: the indirect detection of $^{14}$N fundamental transition via ‘spy’ nuclei [1-3] and the direct detection of $^{14}$N overtone (OT) transition [4-7].

The first method enable $^{14}$N spectral properties to be determined through detection along another channel, typically $^{13}$C or $^1$H. A variant of this method is here proposed and discussed. In our approach, coherences between the $^{14}$N site and the spy nucleus are mediated by the application of a moderate rf field on the $^{14}$N channel. Our approach allows looking at backbone quadrupolar couplings to extract structural information and to work on natural abundance materials to obtain fingerprint of their molecular structure.

The second approach relies on the detection of the overtone (OT) transitions: a double quantum transition with direct detection at twice the Larmor frequency. Because OT transitions are not affected by first-order quadrupolar coupling interactions, $^{14}$N-OT-NMR results in very narrow (few kHz) lines. This huge gain in resolution is balanced by a poor sensitivity in the direct observation of OT transition. Here we discuss the use of polarization transfer techniques [7] to improve sensitivity in $^{14}$N-OT-NMR. The analysis of results has been facilitated by the use of a new simulation strategy implemented within the Spinach library [8].

References:
Recent work has delineated the bandwidth of NUS,\textsuperscript{1,2} formalized the sensitivity of NUS,\textsuperscript{3ab} described how NUS can influence line shapes,\textsuperscript{3c} and developed the role of the point-spread-function in sampling noise.\textsuperscript{4a-d} Sophisticated strategies for choosing the subset of samples to acquire from the Nyquist grid have emerged\textsuperscript{4b,5-7}. Although the remarkable advances in understanding NUS NMR contribute to its wider adoption, spectroscopists face complex choices in NUS experiments.

We describe a new algorithm for choosing NUS samples which is easy to use, intuitive, and flexible for any probability density and any number of indirect dimensions (\textbf{Fig 1}). Based on quantiles, this approach incorporates a number of recommendations\textsuperscript{4b} that have been emerging for minimizing gaps\textsuperscript{5}, using BURST sequences\textsuperscript{2,4b}, ensuring coverage\textsuperscript{7}, and ensuring randomness\textsuperscript{4d}. Point spread functions and example data support the robustness of this technique, particularly for sparse sampling (\textbf{Fig 2}).

Next, the potential to devise rubrics to aid the design of effective NUS experiments is considered. While it may not be possible to answer the question of what ‘optimal’ sampling is for a given set of conditions, relatively simple decision trees may be able to guide the design of NUS experiments.

\textbf{Back to Basics: towards rubrics for nonuniform sampling and spectral quality}

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Molecular dynamics and NMR: A perfect match?

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Proteins are dynamical molecules and their ability to adopt alternative conformations is central to their biological function. Examples include motions that underlie allosteric regulation or ligand binding, or protein dynamics in enzymes that can modulate the overall catalytic efficiency. Protein motions can often be described as an exchange between a dominant, ground state structure and one or more minor states. The structural and biophysical properties of these transiently and sparsely populated states are, however, difficult to study, and an atomic-level description of those states is challenging. In an attempt to determine how well molecular dynamics simulations can capture slow, conformational changes in protein molecules we have studied several different protein systems, which are known to undergo conformational exchange on the millisecond timescale.

Using enhanced-sampling all-atom, explicit-solvent molecular simulations, sometimes guided by structural information from X-ray crystallography and NMR, we show that current force fields and sampling methods allow us to sample experimentally-determined alternative conformations with surprisingly high accuracy. In particular, we find that we can reversible sample both the ground state and minor state, at that the simulations capture the structure of the minor states also. Our simulations enable us to calculate the conformational free energy, and in favourable cases also the kinetics, between the two states, and comparison with experiments demonstrates a high accuracy.

Our simulations provide insight into the structural and biophysical properties of transiently populated minor states, and help reinterpret previous experimental measurements. Further, our results demonstrate that, at least in the three cases, which we have studied, modern simulation methods enable us to examine these otherwise “invisible” states of proteins and describe their structural, functional and thermodynamic properties.
The radical S-adenosyl-L-methionine tryptophan lyase NosL converts L-tryptophan into 3-methylindolic acid, a precursor in the synthesis of the thiopeptide antibiotic nosiheptide. Based on the identification of shunt products and by homology with the FeFe-hydrogenase matuarse tyrosine lyase HydG, a fragmentation – recombination mechanism that implied the Cα-Cβ bond cleavage of L-tryptophan has been proposed. Using CW and advanced pulsed EPR spectroscopic techniques and different L-tryptophan isotopologs, we trapped and characterized unanticipated radical intermediates that rule out this proposal. Instead, these radical species are evidence of an unprecedented carboxyl fragment migration. While NosL resembles its related tyrosine lyases, subtle substrate motions in its active site are responsible for a fine-tuned radical chemistry, which selects the C-C bond for disruption. An exhaustive EPR study combined with DFT calculations have shed light on different structural aspects strictly related to the bio-chemical synthetic aspects. Indeed, NosL is a remarkable example of evolutionary adaptation to structural constraints for an alternative chemistry. Taken together, our data allow for the full description of the catalytic mechanism for this enzyme.\(^{(a,b)}\) Furthermore, by providing insights at atomistic level, the EPR spectroscopic approach provides also interesting highlights on different mutations of this enzyme.

\[\text{Figure 1. An overview of EPR experiments recorded on isotopic labeled- and unlabeled-Tryptophan residues in NosL enzyme.}\]


Time-correlated motions in proteins: a basis for NMR-related models of internal dynamics

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NMR relaxation experiments in isotopically labeled proteins represent unique strategies to probe internal protein dynamics. Relaxation rates obtained from these experiments can be analysed to derive amplitudes and time scales of internal motions. However, it is well known that their interpretation in terms of dynamical parameters is not straightforward and requires, at least plausible, modeling of the motions and their statistics. These reasons are sufficient to justify the use of molecular dynamics (MD) simulations and theoretical models. We will present a novel approach based on the analysis of correlation functions obtained from MD simulations. In the proposed method, the clustering of effective correlation times allows to decompose protein structures in terms of time-scale dependent networks of dynamically correlated local domains. This segmentation of the protein on the basis of motion time scales should provide an adaptive strategy for coarse-graining internal motions, depending on the problem at hand, and could be used in the derivation of stochastic models for flexible macromolecules. This approach may serve as a basis for the development of a unified framework for the derivation of dynamic models that permit to extend the range of time scales accessed by MD simulations. This is of particular interest for the interpretation of magnetic resonance relaxation experiments, where dynamical processes at different time scales can be probed. Preliminary results obtained for several prototypical proteins will illustrate this approach.
Plasticity of a highly dynamic interaction between nucleoporins and nuclear transport receptors

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The MegaDalton sized nuclear pore complexes (NPCs) are among the largest molecular machines in eukaryotic cells and constitute a vital transport conduit between nucleoplasm and cytoplasm. Intrinsically disordered nucleoporins that are enriched in phenylalanine-glycine motifs (FG-Nups) form a selective permeability barrier in the center of the NPC and tightly control the passage of all macromolecules. At the same time, transport through the NPC is extremely rapid (few milliseconds) and mediated by the interaction between nuclear transport receptors (NTRs) and FG-motifs. Combining nuclear magnetic resonance, single molecule fluorescence, and molecular simulations, we show that a rapidly fluctuating FG-Nup populates an ensemble of conformations that are prone to bind NTRs with diffusion-limited on rates, as determined by fluorescence stopped-flow kinetics. 13C chemical shifts in combination with 15N spin relaxation and single molecule Förster Resonance Energy Transfer (FRET) suggest that the FG-Nup does not compromise its conformational freedom and remains flexible upon binding NTRs. Indeed, 15N transverse relaxation rates at increasing Nup·NTR ratios revealed that its multiple, minimalistic FG-motifs bind to NTRs with low, millimolar, affinities and are in fast exchange within the complex, allowing the FG-Nup to maintain this unexpectedly high plasticity in its bound state. These observations suggest that local binding and unbinding with NTRs may be achieved with relatively little energetic effort and can therefore be efficient. Local FG concentrations modulate the FG·NTR interactions, which we showed to be general for various human and yeast FG-Nups and NTRs. We thus propose that these exceptional physical characteristics that determine FG-Nup·NTR interactions eventually enable a rapid yet specific transport mechanism in the physiological context.

Probing functionally important motions in a half-megadalton enzyme by solid-state NMR

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Solid-state NMR allows overcoming size and solubility limitations which have severely hampered solution-state NMR spectroscopy. Of particular interest in this presentation, solid-state NMR allows studying very large (soluble) protein complexes at atomic resolution at all sites (i.e. backbone and side chains), and thus may provide functional insight into such large machineries.

Here we show recent results on a dodecameric enzymatic assembly of 12x39 kDa size. This protein encapsulates catalytic sites within a large enzymatic chamber, and many questions remain related to the entry and exit to/from this cavity, as well as the possible existence of gating mechanisms.

We show that it is possible to assign such large systems, exceeding the size of previously reported (assigned) proteins. This allowed us to protein interactions with inhibitors and reactants at the active site, and to identify a cluster of dynamic residues, which we show to be of functional importance. Our studies provides evidence that protein motions can be probed at individual residues even in very large systems, with a full coverage of backbone and side chains, and thereby reveal mechanistic details which are generally difficult to extract from crystal structures alone.
Studies of brain metabolism and function at high magnetic fields

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It is well established that with increasing magnetic field, the sensitivity of magnetic resonance increases, as do the challenges posed by substantially shortened radiofrequency wavelengths, requiring increasingly sophisticated approaches, as the behavior of radiofrequency in the human body is governed neither by the near- nor the far-field approximation of the Maxwell equations. Using suitable approaches with multiple coils and modulating the dielectric surrounding tissue, these effects can be modulated and exploited to increased performance, guided by 3D models of the organ under investigation. In addition, magnetic susceptibility changes not only at the tissue (diamagnetic) - air (paramagnetic) interface, but is also modulated by a number of factors linked to differential tissue composition, in particularly in the brain. Modulations in vascular susceptibility are exploited in brain functional imaging, due to the change from para- to diamagnetism in hemoglobin, as a consequence of the altered blood oxygenation during activation. At high magnetic fields, rapid data acquisition allows to measured whole brain activation in sub-second temporal resolution, with a spatial resolution that allows tiny activations to be detected, as well as the spontaneous fluctuations in brain activity. The mechanisms underlying the changes in blood oxygenation are intrinsically linked to changes in the brain's handling of its energy needs. The underlying metabolism generating the required energy in the form of ATP is currently difficult to measure, but recent studies in our lab have shown that activation results in consistent metabolic changes, implying that similar to the changes in blood oxygenation, ubiquitous changes in brain metabolism are likely. Current studies are ongoing, investigating the metabolism at the cellular level, by focusing on one hand on mitochondrial function and neurotransmission (likely to be the triggering event that modulates a brain cell's energy metabolism) in vivo, to on the other hand, investigating possible mechanisms of blood flow regulation in glucose transporter deficient mice, as well as measurements of glucose metabolism with subcellular resolution using x-ray fluorescent synchrotron imaging, suggesting that all major brain cells use glucose as a major source for energy production. We conclude that when combining an array of different disciplines, insights into the regulation of brain function are possibly, hitherto inaccessible to investigation.
The effect of magnetization transfer on $T_1$ weighted images of tissues

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In clinical scanners, $T_1$-weighed MRI of brain and spinal cord yield images with the white matter (WM) intensity larger than grey matter (GM). The common interpretation of this contrast is the shorter $T_1$ for the WM. In the current study we examined the possible contribution of magnetization transfer (MT) to this contrast\(^1\). In a saturation-recovery experiment using selective pulses (Fig. 1a), under the conditions: 90° selective pulses of duration of 2ms, TR=6s, short delay $t_{LM}$ fulfilling $t_{LM}<50\text{ms}<T_1$ and gradient echo (GE, TE=2ms) as imaging module, the ratio between the average intensities of the white and grey matters (W/G) was 1.4. Since the 90° (selective) pulses completely saturate the water magnetization and the $t_{delay}$ is much smaller than $T_1$, most of the observed signal originates from the immobile species (proteins, myelin). In that case the ratio of 1.4 is the result of the larger amount of immobile species in the WM (Fig. 2 left slide). The spinal cord image dependence on the suppressing pulse $s$ and TR are given in Fig.2.

The lack of contrast when non-selective pulses are applied is evident from the two right side slides. For this case as all species are suppressed the contrast is $T_1$ weighted and for short $t_{LM}$ no contrast is observed as expected. The lack of dependence on TR is consistent with the above since the water magnetization recovers only by the water’s $T_1$ and at $t_{LM}=0$ the water magnetization is zero regardless of TR. For direct evidence for the role of MT in the images obtained with selective pulses we repeated this experiment by preceding it with a long (1s) low power pulse (7 $\mu$T, Fig. 1c) applied at offsets in the range of 20-5 kHz from the water resonance. The obtained contrast was reduced from 1.4 to 1.04 for offsets declining from 20 to 5 kHz (see Fig. 3). As at an offset of 5 kHz the immobile fraction is completely saturated, and the magnetization transfer is suppressed, the 1.04 contrast may arise mostly from proton densities and $T_1$ effects.

Conclusion: In clinical MRI scanners, where the pulses are selective, on time scales shorter than $T_1$ the recovery of the longitudinal magnetization to equilibrium is dominated by magnetization transfer.
Multidimensional correlation of nuclear relaxation and diffusion tensor size, shape, and orientation

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Despite their usefulness in the study of porous materials, classical diffusion NMR techniques yield ambiguous results when the sample comprises multiple regions with different pore sizes, shapes, and orientations. The separation of contributions from the various types of water environments have thus been made by fitting mathematical models of increasing complexity to the acquired data. However, selection of a single model from all the ones that are able to reproduce the data is typically challenging.

Here, we present a novel experimental protocol where the heterogeneity of a given material is characterized by correlating the individual values of isotropic diffusivity \(D_{\text{iso}}\), diffusion anisotropy \(D_\Delta\), orientation of the diffusion tensor \((\theta, \phi)\), and relaxation rates \(R_1\) and \(R_2\), of distinct microscopic environments. Said correlations are constructed via a pulse sequence that encodes the NMR signal for both diffusion and nuclear relaxation (Fig. 1(a)) and a pseudo-random sampling scheme (Fig. 1(b)). The measured signal-decay is subjected to a model-free data inversion algorithm [1] that allows the extraction of \(P(R_1, R_2, D_{\text{iso}}, D_\Delta, \theta, \phi)\), the 6D probability distribution that characterizes the material’s heterogeneity. The different values of \((R_1, R_2, D_{\text{iso}}, D_\Delta, \theta, \phi)\) are determined and reported as correlation maps. All the presented methods are experimentally validated on materials with known diffusion properties.

Since the typical MRI voxel comprises multiple microscopic domains with varying chemical and diffusion properties, the presented method shows great potential for in vivo studies of the human brain. In particular, through the imposition of physiologically reasonable constraints, we expect that our protocol can serve as a basis for experiments capable of determining the composition of a voxel in terms of tissue and cell types.

References:

Figure 1. (a) NMR pulse sequence for encoding diffusion and nuclear relaxation effects. The bottom right panel illustrates the unit vectors \((n_1, n_2, n_3)\) of the three sets of gradient pulses which allow for isotropic and anisotropic diffusion encoding. (Adapted from ref. [2]). (b) Pseudo-random data sampling strategy for 6D correlation of \((R_1, R_2, D_{\text{iso}}, D_\Delta, \theta, \phi)\). The bottom right panel displays the orientation distribution of the diffusion-encoding vectors as an azimuthal projection of a sphere.
NMR spectroscopy of subnanoliter ova with ultra-compact inductive probes

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Due to sensitivity limitations, NMR spectroscopy of single cells was previously reported down to a minimum volume of 10 nl [1], a volume scale where only a few microscopic biological entities exist in nature. In this study we employed an ultra-compact 1 mm² single-chip high sensitivity probe (Fig. 1A) to sense endogenous compounds in intact single ova (Fig. 1B) having subnanoliter volumes down to 0.1 nl. At this volume scale, life development begins for a broad variety of animals, humans included (Fig. 1C). Despite a relatively low spectral resolution of about 0.3 ppm, in our experimental conditions a single scan limit of detection (LOD) of about 300 pmol of ¹H nuclei within a sensitive volume of about 250 pl is achieved [2,3]. 1D ¹H spectra of single ova of tardigrade Richtersius coronifer (Rc, 0.5 nl) and nematode Heligmosomoides polygyrus bakeri (Hp, 0.1 nl) are obtained, here shown after averaging times of respectively 12 and 36 hours (Figs. 1D and 1E). With a LOD of about 2 pmol of ¹H nuclei, lipids and other unassigned endogenous compounds are detected. Repeated experiments of eight Rc ova, and a study of spectra reproducibility, also suggest that Rc ova have visibly heterogeneous spectra. These results seem to indicate that miniaturized inductive high sensitivity probes are a promising candidate for the NMR-based analysis of single microscopic biological entities such as mammalian zygotes.

MRI of the pregnant mouse
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Mammalian reproduction, in which the embryo develops in the womb of the mother, offers a fascinating biological system for the study of vascular remodeling, which is robust, rapid, critical for life, and yet is not part of a pathological process such as wound healing or cancer. In contrast to the latter, remodeling of the maternal blood vessels to accommodate pregnancy is exquisitely well regulated so as to insure the wellbeing of both the mother and the offspring. It allows for proper transfer of oxygen and nutrients and removal of waste products, thus serving as the main gateway for development, and at the same time provides a tight barrier, protecting and isolating the embryo from the maternal immune system as well as from many circulating factors.

Over the last years we invested significant effort in development of MRI based tools for monitoring maternal vascular function in the mouse from embryo implantation to full term pregnancy (1-5). Within hours from implantation, maternal angiogenesis leads to the formation of the decidua, that can be detected by DCE-MRI, as enhanced permeability or per-embryo vessels to biotin-BSA-GdDTPA. Maturation of the vessels leads to enhanced blood volume surrounding the embryo, while the embryo-maternal barrier is maintained through remodeling of the extracellular matrix, and nutrients filter through by diffusion. At later stages, after development of the placenta, the embryo-maternal barrier is provided in the labyrinth where exchange of nutrient and oxygen occurs between the maternal and the embryo circulations. Biotin-BSA-GdDTPA based DCE-MRI detects uptake and aggregation of the contrast media by the trophoblast cells lining the maternal circulation, with no transfer of Gd to the embryo. This type of processing results in changes in T2* and can be used for analysis of compartmentation, particularly when combined with ADC measurements of flow and diffusion.

Perfusion of the placenta can also be followed by arterial spin labeling (ASL), showing the role of the two main arteries perfusing the uterus, the ascending uterine artery and the descending uterine branch of the ovarian artery. One of the key roles of the placenta is the transfer of oxygen from the maternal hemoglobin to the fetal fetohemoglobin. Using R2* BOLD contrast MRI for detection of hemoglobin saturation and oxygen enhanced R1 for detection of dissolved oxygen, we generated apparent P%) maps of hemoglobin saturation showing the function of the placenta in delivery of oxygen to the embryo. These studies shed light on a key process vital to the survival of mammals and suggest the potential for expanding the use of MRI for monitoring high risk pregnancies.

References:
Labile inclusion complexes with hyperpolarized $^{129}$Xe for molecular sensing

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Hyperpolarized NMR reporters are finding increasing applications in biomedical applications such as diagnostic MRI. Critically, the design of reporters that bind to specific molecular targets has been seriously limited by the finite lifetime of hyperpolarization. Xenon biosensors avoid this problem by combining a targeted empty reporter with reversibly bound hyperpolarized nuclei at the site of interest through spontaneous complex formation. Such host-guest inclusion complexes also allow for sensitive detection via chemical exchange saturation transfer with hyperpolarized nuclei (Hyper-CEST).

Initial biosensor designs for live-cell applications were based on cryptophane-A cages (CrA$^{2,3}$) as hosts which are characterized by high binding constants and moderate exchange rates. This leaves room for improvement with respect to the saturation transfer efficiency. More labile complexes provide the opportunity for more rapid build-up of the CEST effect. Herein, we compare the performance of CrA with more open macrocyclic hosts from the cucurbit[$n$]uril family (CB$n$). In particular CB6 and CB7 show distinct Hyper-CEST signatures in aqueous solution while their signals are difficult to identify in direct spectroscopy due to substantial line broadening. CB6 shows a ca.100-fold better gas turn-over constant that we derive as a novel parameter from quantitative Hyper-CEST measurements. However, translation of this potential into cellular environments is perturbed by the affinity of other competing guests. We therefore propose CB7 as an alternative host and demonstrate a displacement assay that senses enzymatic production of cadaverine via displacement of labile bound Xe.

‘Explosive’ production of hyperpolarized $^{83}$Kr and $^{129}$Xe MRI contrast agents

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Hyperpolarized (hp) $^{129}$Xe MRI / NMR of the lung and other biological systems, has gained increasing attention over the past two decades. Beyond $^{129}$Xe, hp $^{83}$Kr is an emergent MRI contrast agent [1] that has recently been demonstrated to serve as biomarker for an animal model of emphysema (Fig. 1) [2]. Generally hp $^{83}$Kr is promising for the diagnosis of pulmonary diseases affecting the surface of the respiratory system. However, the distinct physical properties of $^{83}$Kr that enable unique MRI contrast also complicate the production of hp $^{83}$Kr. Most significantly, cryogenic separation, used in the production of hp $^{129}$Xe, is not an option due to rapid quadrupolar relaxation causing the complete loss of the $^{83}$Kr hyperpolarized state.

Using molecular hydrogen as a buffer gas for spin exchange optical pumping (SEOP), a radically new approach for the production of hyperpolarized noble gas contrast agents becomes possible [3]. In the proof of concept study a spin-polarization of $P = 29\%$ and $P = 63\%$ were recorded for $^{83}$Kr and $^{129}$Xe respectively. Following SEOP, the H$_2$ buffer gas was reactively removed via catalytic combustion (Fig. 2.) without measurable losses in hyperpolarized spin state of either $^{83}$Kr or $^{129}$Xe. Highly spin-polarized $^{83}$Kr can now be purified for the first time to provide high signal intensity for the advancement of in vivo hp $^{83}$Kr MRI. The fundamental simplicity of the combustion process, should allow for on-demand continuous flow of purified and highly spin-polarized $^{129}$Xe.

Triplet-Singlet Imbalance in Pairs of Magnetically Equivalent Spins


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Long-Lived States (LLS) are spin states with lifetimes $T_{LLS}$ that can be much longer than the longitudinal relaxation time constant $T_1$. In a two-spin system, they correspond to a "Triplet/Singlet Imbalance" (TSI), induced by perturbing the high-temperature equilibrium between the average of the populations of the three triplet states and the population of the singlet state.

In recent years, it has been shown that it is possible to create a TSI by lowering the spin temperature well below the Zeeman splitting. This can be readily achieved by using Dissolution Dynamic Nuclear Polarization (d-DNP), either in pairs of inequivalent spins or in pairs of magnetically equivalent spins.

In our laboratories, we have generated TSI's in partly deuterated ethanol and in fumaric acid. In CD$_3$CH$_2$OD (with $^{13}$C in natural abundance), a TSI was observed through cross-relaxation into observable transitions of both $^1$H and $^{13}$C spins, in analogy to cross-relaxation in $^{13}$C-bearing methyl groups. In fumarate, a symmetry-breaking addition of D$_2$O catalyzed by fumarase made the two protons magnetically inequivalent, so that the TSI became observable.

In principle, our strategies should be applicable to H$_2$O, possibly for generating an excess of either ortho- or para-water with respect to the 3:1 ratio that prevails in the high temperature regime. However, H$_2$O appears more challenging because the TSI can relax through spin rotation and dissipate through fast proton exchange. We have found suitable conditions where proton exchange is sufficiently slowed down by dilution in aprotic solvents and we have adapted our d-DNP equipment to allow dissolution with such solvents. We have also studied longitudinal relaxation of H$_2$O in gas-phase, where spin-rotation is the dominant relaxation mechanism, finding relaxation times $T_1$ on the order of tens of milliseconds.

We will report here our latest results in this quest for the creation of ortho- or para-water, which we like to refer to as “forbidden fruits” of spectroscopy.


Cross effect dynamic nuclear polarization with bis-Gd(III) complexes

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Magic angle spinning (MAS) NMR is a powerful and indispensable technique in structural biology as well as materials science. Nevertheless, the inherently low sensitivity of nuclear spins is still one of the limiting factors in its application: the small thermal spin polarization often leads to prohibitively long acquisition times. Dynamic nuclear polarization (DNP) can overcome this problem by transferring the significantly larger spin polarization of unpaired electrons to the nuclei of interest during a typical MAS NMR experiment.

Generally, paramagnetic species such as nitroxides or other persistent organic radicals provide the large electron spin polarization to be transferred to the nuclei of interest. Recently, we have introduced paramagnetic metal complexes with high-spin states, such as Gd(III) and Mn(II),[1] as well as Cr(III) as polarizing agents.[2] The narrow central electron paramagnetic resonance (EPR) transition of these half-integer spin systems allows for well-resolved solid effect DNP at high magnetic field.

Nevertheless, cross effect (CE) DNP—where dipolar coupling within a pair of electron spins leads to highly efficient polarization transfer—requires energy splitting of the two electron spins matching the Larmor frequency of the nucleus to be polarized. In bis-nitroxide biradicals this energy splitting is provided by g-anisotropy; in contrast, all metal polarizing agents that have been shown to be DNP active thus far feature isotropic Zeeman interaction due to quenched spin-orbit coupling.

Here, we present that it is nevertheless possible to utilize paramagnetic bis-metal complexes as polarizing agents for CE DNP. We provide clear evidence of CE of 1H, 13C and 15N, with NMR signal enhancement factors exceeding 100 using compounds of the type Gd-spacer-Gd with well-defined Gd-Gd distances, originally introduced for dipolar EPR spectroscopy.[3] By variation of the spacer length we investigated the Gd-Gd distance dependence of DNP enhancements. Furthermore we will discuss influences of mutual complex orientation and individual zero-field splitting of the two Gd(III) ions on CE efficiency. These results will allow us to design improved bis-metal compounds for efficient CE DNP and might lead to DNP applications utilizing biomolecules doubly labeled with tags based on metal complexes.

References


Figure 1. Field dependent 13C DNP field profile (left) and chemical structure (right) of Gd-spacer-Gd Gd3y (blue) and the corresponding mono-Gd(III)-complex Gd0y (green).
Nano-NMR on a diamond chip

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Diamond has emerged as a unique material for a variety of applications, both because it is very robust and because it has defects with interesting properties. One of these defects, the nitrogen-vacancy center, has a single electronic spin associated with it that show quantum behavior up to room temperature. Our group is harnessing the properties of single NV centers for high resolution magnetic sensing applications.

In this talk, I will present recent efforts at implementing a nanoscale nuclear magnetic resonance (NMR) spectrometer with a diamond spin sensor. I will first introduce the basics of NV centers and show how they can be used for detecting ac magnetic fields, such as the spin noise produced by nuclear spins. I will then show examples of nuclear Fourier spectroscopy on single $^{13}$C nuclei within diamond, and small ensembles ($\sim 10^2$-$10^4$) of proton spins on the diamond chip. Finally, I will give an outlook on the steps needed to turn the technique into a tool for imaging nuclear coordinates in individual molecules.
The Magic Angle, Microwaves and Amyloid

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This presentation will selectively cover two closely related topics that employ magic angle spinning (MAS) NMR, dynamic nuclear polarization (DNP), or both.

First, we describe recent MAS experiments aimed at producing high resolution structures of amyloid fibrils. Many peptides and proteins form amyloids whose structures are of considerable pathological as well as functional importance. However, these assemblies do not diffract to high resolution and are insoluble, and therefore the two primary tools of structural biology, X-ray diffraction and solution NMR, are not applicable. Accordingly, we have developed a suite of MAS experiments that permit, together with cryoEM, determination of atomic resolution structures of fibrils. We demonstrate the methodology with a description of the high resolution structure of fibrils of monomorphic Aβ42, the protein associated with Alzheimer’s disease.

Second, we discuss dynamic nuclear polarization (DNP) experiments that utilize subterahertz microwaves (~150-600 GHz) generated by gyrotron microwave sources together with paramagnetic polarizing agents to enhance the sensitivity of MAS NMR experiments. Specifically, we irradiate electron-nuclear transitions that transfer the large electron polarization to nuclear spins via the Overhauser, cross and solid effects. In addition, we have recently initiated time domain DNP in order to circumvent the field dependence of CW DNP. We show that spin locking the electrons and matching the NOVEL condition serves as an effective approach to time domain DNP, and that the spin lock can be modulated to increase the efficiency of the polarization transfer.
Planning a Portable Head-only 1.5 T MRI System

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Functional magnetic resonance imaging (fMRI) continues to play a critical role in understanding the human brain. Yet current fMRI technology is far less than ideal for studying brain function due to the unnatural environment and restricting space of the magnet bore. Furthermore, due to its expense and infrastructure requirements, access to fMRI is restricted to mainly wealthier institutions, and this results in a highly biased subject sampling and a shortage of studies in non-western environments and cultures. In this project, we are planning a new way to image human brain function and structure using a small, lightweight, and portable magnet. The compactness and efficiency of this imaging system will make the study of human brain possible outside the unnatural laboratory environment and in subjects who previously were excluded from getting an MRI scan due to claustrophobia, obesity, and metallic implants. To demonstrate feasibility, this project focuses on three technical innovations essential to realizing this revolutionary new MRI scanner.

First, we sought to demonstrate feasibility of a portable magnet technology that will be small enough to allow the human body from the shoulders down to remain outside the magnet. The magnet is based on the high temperature superconductor, yttrium barium copper oxide (YBCO), which does not require liquid helium. YBCO tape was used to construct one of the 20 double pancakes of the planned 1.5 T head-only magnet. In a test at liquid nitrogen temperature (77 K), the double-pancake magnet was persistent and produced the predicted magnetic field. These tests demonstrate the feasibility of the planned complete 1.5 T YBCO magnet. In addition, these results support calculated weight estimates of the complete 1.5 T magnet of ~250 kg.

Standard approaches to generating MR images require a highly uniform magnetic field ($B_0$) and thus necessitate the use of magnets that are physically large. To overcome this limitation (so that brain MRI can be accomplished with our planned head-only magnet), a new way to perform MR imaging called STEREO was conceived and is being tested using computer simulations and experiments. Work performed this past year support our predictions of STEREO’s tolerance to $B_0$ inhomogeneity.

Finally, the capability to perform simultaneous radiofrequency transmit and receive (STAR) is a necessary technology for STEREO to work in extreme field inhomogeneity. STAR will also permit a drastic reduction in size, cost, and power consumption of our portable, head-only MRI scanner. Thus, we have focused our attention on developing the STAR electronics to allow RF transmission simultaneously with MRI signal detection. A prototype duplexer for this purpose was successfully designed, built, and tested. With it, we acquired the first ever images of human brain in vivo using simultaneous transmit and receive. As predicted, this approach required extremely low peak RF power (~ 30 mW).

In summary, this planning project is on track to demonstrate proof-of-principle of a portable, head-only 1.5 T MRI system. Making this system available to neuroscientists will open exciting new territories of investigation into the human brain and human behavior, in a wide range of conditions and populations of subjects worldwide.

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References:
3 Abstracts for posters in session "Biomacromolecular folding and dynamics"
Analysis of local dynamics in proteins using CP-VC under ultra-fast MAS

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Functional properties of most molecules are encoded in their motion. While several NMR methods are used to study dynamics in solution, there is a demand for high-throughput approaches to measure dynamics in solid-state. Since heteronuclear dipolar couplings are highly sensitive to local motions, we propose several ultrafast-MAS nD sequences to measure dynamics based on heteronuclear dipolar couplings. Previous studies have shown that a simple experiment, Cross-Polarization with a Variable Contact-time (CP-VC), is efficient at ultra-fast MAS to accurately measure the dipolar interactions corresponding to C-H and N-H short distances. CP-VC with indirect 1H detection allows a large gain in experimental time in case of small or perdeuterated molecules. CP-VC is robust with respect to (i) offsets, (ii) CSA, (iii) Hartmann-Hahn mismatch, and (iv) RF-inhomogeneity. These characteristics are related to the small rotor diameter allowing ultra-fast MAS (> 60 kHz) and large RF fields. CP-VC methods have been demonstrated in 2D with small unlabeled molecules and in 3D with labelled proteins, and the results have been compared with quantum mechanical calculations. Further studies are focused on the evaluation of local dynamics of each building unit of proteins including main skeleton (N-H and C=O), Cγ and side-groups (both aliphatic and aromatic). The challenging question is whether the dynamic processes in the crystal lattice of proteins are synchronized or not. It is also fruitful to carry out sequential analyses of proteins by dipolar line-shape analysis for each individual position in sequence H-N-Cα-Cγ-Side-Group. Our studies are also aimed at addressing for instance if jumps of aromatic residues promote the molecular motion of Cγ etc. in appropriate amino acid residues. For this purpose, we present several nD extensions of our previous 3D experiment on proteins. Experimental results obtained from several solids under fast spinning speeds (> 60 kHz) will be discussed. We will also show the way to extract the dynamics by fitting the dipolar line-shape with taking into account the dead-time, the apodization, the rf-inhomogeneity and the dynamic model.


Experimental and best-fit simulated CP-VC dipolar line-shapes for some aromatic C of LC8 protein extracted from the 3D CPVC-RFDR spectrum.
Dimerization interface and dynamic properties of the yeast ATPase inhibitor protein IF1 revealed by Site-Directed Spin Labeling EPR spectroscopy

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Mitochondrial ATP synthase is regulated by an inhibitory protein called IF1 whose role is to prevent the wasteful hydrolysis of ATP under anaerobic conditions. The oligomeric state of IF1 related to pH is crucial for its inhibitory activity. Although extensive structural studies have been performed to characterize the oligomeric states of bovine IF1 (bIF1), only little is known concerning the structural organization of yeast IF1 (yIF1). bIF1 can be found as an inhibitory dimer at low pH and a non-inhibitory tetramer at high pH (1). For yeast IF1, a monomer/dimer equilibrium has been described, high pH values favoring the monomeric state (2). The objective of our study was to determine the dimerization interface of yeast IF1 and to gain insights into the dynamics of its dimeric form using Site Directed Spin Labeling combined with EPR spectroscopy (SDSL-EPR).

Three yIF1 cysteine-substituted variants have been labeled to probe the dimerization interface and each of them has been studied at three different pH values. Continuous wave EPR was used to probe the dynamics of yIF1 and pulsed DEER experiments were performed to measure inter-spin distance distribution. Taken together the results show that the α-helical central part of yIF1 is involved in the dimerization interface and reveal the very flexible nature of the C-terminal region of the peptide.

Thanks to the combination of different SDSL-EPR strategies, this work brings the first structural characterization, at the residue level, of the dimeric form of yIF1 in solution. Our study demonstrated that the dimerization interface involves the central inhibitory region, as it is the case for bIF1 in its tetrameric interface. This result reveals that the dimeric form of yIF1 corresponds to the non-inhibitory state (3).

References

Figure 1. (A) Inter-spin distance distribution from Q-band DEER experiments for H39CMTSL and L54CMTSL at pH 5.0. (B) Model of yIF1 dimer deduced from the EPR measurements.
An Example of Asymmetry in a Protein Homodimer

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A key step in the maturation of microRNAs is the cleavage of the precursor miRNA by the RNase III enzyme Dicer. In humans, Dicer associates with two double-stranded (ds) RNA binding proteins, TRBP and PACT, via an interaction mediated by repurposed RNA binding domains. The Dicer interaction domain of PACT (PACT-D3) is a non-canonical type-B dsRNA-binding domain (dsRBD) that has lost the capacity to interact with dsRNA. As well as interacting with Dicer, PACT has been shown to form homodimers and to interact with TRBP and the viral dsRNA-sensing kinase PKR, all via the type-B dsRBD. There is relatively little information available about the mechanism of dsRBD-mediated protein/protein interactions. Using SEC-MALLS and analytical ultracentrifugation, we have demonstrated that PACT-D3 is a homodimer and that the mechanism of homodimerisation is different from a previously reported dsRBD homodimer from Staufen1. NMR exchange spectroscopy revealed that the PACT-D3 homodimer exists in two equally populated states in slow exchange. The largest chemical shift differences between the two states map to a surface that is predicted to interact with Dicer. High-pressure NMR studies revealed differences in the pressure dependence of amide proton resonance frequencies with the largest differences also mapping to the same surface. Mutation of a conserved leucine residue on this surface generated a monomeric variant of PACT-D3 that exists in a single state. Our data suggest that PACT-D3 forms an asymmetric homodimer and that homodimerisation involves the same interface as Dicer binding.
Investigating Photocontrol of Trp-Cage Folding

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Consisting of only 20 amino acids, the Trp-cage is one of the smallest peptides with a stable secondary and tertiary structure at physiological conditions. Its fold is stabilized by hydrophobic interactions leading to the eponymous encapsulation of the Trp-residue.

Our goal is to achieve photocontrol of the Trp-cage fold by crosslinking it with a photoswitchable moiety. For this reason, several new Trp-cage variants are designed and synthesized. Different azobenzene derivates serve as photoswitchable crosslinkers, which are covalently connected to the side chains of residues at varying positions in the sequence.

Due to their reversible cis-trans-isomerization and photostability, azobenzene derivates are predestined as ideal crosslinkers. Their photoinduced structural change is intended to produce enough strain to induce complete unfolding of the Trp-cage. Two different approaches are pursued to achieve the switching of Trp-cage conformation. In the “pull-approach”, the longer (trans-) form of the linker supports a stable protein fold. Upon isomerization to the shorter (cis-) form, the linker contracts and pulls the junction points closer together to effectively disrupt the fold. In contrast to this, in the “push-approach” the peptide is folded when the distance of the junction points matches the end-to-end distance of the short (cis-) form of the linker. Isomerization to the longer (trans-) isomer then destabilizes the peptide by pushing the two junction points apart.

The Trp-cage fold was analyzed using two-dimensional NMR techniques. Furthermore, NMR spectroscopy was used to determine half-lifes and photostationary states of the crosslinkers and crosslinked peptides.
DECONVENS: Deconvoluting protein structural ensembles without deconvolution

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Proteins are dynamic molecules sampling multiple conformations over various spatial amplitudes and timescales and are best described by structural ensembles obtained by NMR and SAXS techniques for example. However due to the large number of free parameters in multi-structure models, mapping protein conformational free energy landscape heavily involves sophisticated computer modeling approaches optimally combined by extensively cross-validation. Here we introduce DECONVENS, a novel method allowing the qualitative and quantitative description of the free energy landscape of proteins exploring multiple states with minimal theoretical inputs. This general approach is based on the sensitivity of protein conformational (sub)state stability to changes in buffer environments (ionic strength, pH or other physico-chemical parameters). A limited number of experimental data are collected under various conditions and collectively analyzed to provide a comprehensive and unambiguous structural and thermodynamic description.

We demonstrate the utility of the methodology on a diflavin protein¹. The interdomain interface in this bidomain protein is highly stabilized by many electrostatic interactions. We collected NMR and SAXS data for NaCl concentrations ranging from 0 to 1M and showed that the protein explores two substates: the locked state, stable at low ionic strength and characterized by a well-defined interdomain interface, and an highly flexible unlocked state, stable at high ionic strength and in which the two domains almost freely tumble. Substate populations retrieved from chemical denaturation theory provided thermodynamics of the interface contacts over the whole range of conditions, including at physiological ionic strengths. We also show that rapid Thermal Shift screens allow optimizing conditions before collecting the time- and sample-demanding NMR/SAXS data. DECONVENS is general and illustrates how the exploration of protein conformational states beyond physiological conditions can help to deconvolute complex structural ensembles under physiological and non-physiological conditions.
Physiological and pathological regulations of the molecular chaperone BIP
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The endoplasmic reticulum (ER) is an essential organelle in eukaryotic cells responsible for folding and maturation of the majority of secreted and membrane proteins. Binding Immunoglobulin Protein (BIP), the only Hsp70 chaperone in the ER lumen, is a key player of the ER protein quality control system. BIP main functions include: (i) assisting folding and maturation for newly synthesized proteins, (ii) preventing accumulation of aggregated and misfolded proteins in the ER, and (iii) fast (reversible) regulation of the unfolded stress response (UPR), the ubiquitous eukaryotic signalling pathway, enabling real-time adaptive adjustments of the ER folding capacity. All these processes aims at better cell survival under physiological and pathological stresses and failure of BIP function can lead to many pathological processes, including several cancers, diabetes, and neurodegenerative diseases.

The majority of BIP functions rely on its ability to cycle between several functionally and structurally distinct conformations under control of ATP binding and hydrolysis; accumulation of unfolded and misfolded proteins inside the ER; communication with other components of the ER quality control network; and ER environmental factors, such Ca\textsuperscript{2+} flux and oxidative potential. Our research is focused on the understanding of how this chaperone system is regulated at the molecular level by these physiological and pathological factors. We are using solution NMR (including chemical shift perturbation analysis and relaxation dispersion measurements) and a wide range of biophysical and biochemical techniques as well as molecular dynamic simulations to explore how functional conformations of BIP are regulated by posttranslational modifications, Ca\textsuperscript{2+} binding, and interactions with other components of the ER protein quality control network. Our preliminary results provide the mechanistic understanding of how redox potential and Ca\textsuperscript{2+} alter the conformational landscape of the nucleotide-binding domain of BIP, including its thermodynamical, structural and functional features (Figure 1). We believe that these results provide new insights into the mechanism of physiological and pathological regulations of the BIP chaperone and will potentially open a new avenue in the design of new allosteric drugs that selectively target BIP functions.

Figure 1: Characterization of the BIP NBD landscape:
(A) Conformational flexibility of the BIP NBD monitored by methyl NMR in the presence of different ER environmental factors (shown in different colors). (B) Thermodynamics of nucleotide binding (ITC) under different conditions, mimicking different ER physiological states. (C) ATPase activity of BIP in the present of ER environmental factors.
Insights into the Chaperone-Dependent Delivery Mechanism of Unfolded Outer Membrane Proteins to the Bacterial Outer Membrane

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The biogenesis of bacterial Gram-negative outer membrane proteins (Omps) involves multiple chaperones to transfer the unfolded Omp polypeptide chains from the inner membrane (IM) to the outer membrane (OM). These chaperones prevent aggregation of the Omps during their passage across the aqueous periplasm. It has been proposed that the periplasmic chaperones SurA and Skp form two parallel pathways, where SurA may act as the primary path and Skp as an alternative rescue path. The SurA pathway is dependent on the interaction of SurA with the soluble β-barrel assembly machinery (BamA) POTRA domains to deliver the unfolded Omps to the Bam complex for insertion in the OM. Despite the importance of the SurA and Skp pathways, the specific mechanism of interaction and transfer of Omps to the outer membrane is still poorly understood.

Here, we use solution NMR spectroscopy combined with complementary biophysical techniques to investigate the interactions between chaperones and the soluble BamA POTRA domains at atomic resolution. Our data reveal the presence of a distinct binding sites for each of the chaperones on the POTRA domains. Chemical shift mapping distinguishes the binding sites of the chaperones. Chaperone binding triggers a conformational change in the POTRA domains that might be connected to the transfer of the Omps into the OM. Upon the interaction with the POTRA domains, we observe substantial structural rearrangements of the chaperones that are consistent with a possible mechanism for substrate release. Overall, our data reveal a possible mechanism for chaperone-dependent delivery and transport of Omps along POTRA domains that lies at the center of the essential Omp biogenesis pathway.

Structural and mechanistic studies of misfolding and amyloid nucleation by polyglutamine proteins.

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Neurodegenerative diseases are often associated with the aggregation of proteins or peptides that undergo misfolding and aggregation reactions following a delayed onset that reflects a pivotal nucleation event from the initial soluble ensemble or folded state. This rate-limiting step is seen as a crucial target for inhibiting or modulating the disease-causing misfolding process [1,2]. Via a combination of structural and mechanistic studies we have been investigating the molecular mechanism behind the aggregation of expanded polyglutamine (polyQ) proteins. Diseases such as Huntington’s Disease (HD), spinal and bulbar muscular atrophy (SBMA), and various kinds of spinocerebellar ataxia (SCA) are associated with the expansion of a polyQ domain in a disease-specific protein. Despite extensive studies, the mechanism of aggregation and the nature of the toxic species remain under intense debate. On the one hand, the disordered ensemble of unaggregated polyQ (or polyQ-containing proteins) remains under investigation, with some suggesting that monomeric β-hairpins act as toxic species. On the other hand, the literature features conflicting accounts of the structure and polymorphism of aggregated polyQ. Studies of both soluble and aggregated states are complicated by the degenerate sequence, the cost and difficulty of preparing the aggregation-prone polypeptides, and (expected) structural heterogeneity. We have probed the mechanism of polyQ aggregation by integrating mechanistic and structural studies, with the latter enabled in large part by advanced magic-angle-spinning NMR. The MAS NMR reveals that intramolecular β-hairpins form the core structure of aggregated polyQ in disease relevant huntingtin exon1 fibrils (Fig. 1) [1]. To probe the mechanistic role of β-hairpins, we also studied the structure and aggregation behavior of polyQ model peptides with and without β-hairpin stabilizing mutations. Our results point to a key role for β-hairpin formation in the nucleation process. We discuss implications for the characteristic polyQ-length threshold seen in polyQ expansion disorders, and for attempts to modulate polyQ misfolding by targeting the nucleation process.


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Interdomain dynamics in the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger investigated by NMR spectroscopy

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The Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX) is one of the main Ca\textsuperscript{2+} extrusion mechanisms in excitable cells. Besides transporting Na\textsuperscript{+} and Ca\textsuperscript{2+}, the NCX is regulated by these ions. The NCX is a large protein (\textsim 100 kDa), consisting of transmembrane and cytosolic domains connected by flexible linkers. It has two cytosolic Ca\textsuperscript{2+}-sensor domains called CBD1 and CBD2. Binding of Ca\textsuperscript{2+} to CBD1 activates the exchanger. However, the Ca\textsuperscript{2+} allosteric activation mechanism remains unclear. The Drosophila Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, CALX, has anomalous behavior. In contrast to all other NCX proteins characterized to date, CALX is already active in the absence of intracellular Ca\textsuperscript{2+}. Binding of Ca\textsuperscript{2+} to CBD1 inhibits this exchanger. This behavior is odd because the NCX and the CALX are similar proteins. In order to get insights on the mechanism underlying the Ca\textsuperscript{2+} allosteric regulation of the exchanger, we used NMR spectroscopy to investigate the effect of Ca\textsuperscript{2+}-binding to a construct corresponding to the CALX-CBD1 covalently linked to the CALX-CBD2 (CALX-CBD12).

It was found that CBD1 and CBD2 are flexibly linked to each other in the absence of Ca\textsuperscript{2+}. Binding of Ca\textsuperscript{2+} to CBD1 restricts the CBD1-CBD2 interdomain motions, and stabilizes a slightly tilted interdomain arrangement (Figure 1) (1). This behavior is analogous to what we observed previously for the NCX-CBD12 construct (2). A model is proposed to explain how the binding of Ca\textsuperscript{2+} to the CBD1 domain can inhibit the CALX while at the same time it activates the NCX exchanger (1).

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(2) Salinas et al. (2011) J. Biol. Chem. 286: 32123-32131

Figure 1. CALX-CBD12 interdomain arrangement in the apo state (top) and in the Ca\textsuperscript{2+}-bound state (bottom) based on \textsuperscript{1}H-\textsuperscript{15}N RDCs measured in two different alignment media (2).
Sensitivity-optimized measurements of cross-correlated relaxation rates in amide and methyl spin systems, with application to dynamics within ribosome–nascent chain complexes

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NMR measurements of nuclear spin relaxation provide extraordinarily powerful probes of polypeptide dynamics across a range of timescales. In particular, measurements of cross-correlated relaxation processes provide a valuable description of ‘pure’ dynamics within a spin system, free from contributions due to chemical exchange or interactions with external spins. However, such measurements have typically been associated with low experimental sensitivity. Here, we report on the development of sensitivity-optimized pulse sequences for the measurement of N/NH (CSA/DD) cross-correlations in amide spin systems, and CH/CH (DD/DD) and C/CH (CSA/DD) cross-correlations in methyl spin systems. We also describe the application of optimal design theory to implement ‘on-the-fly’ adaptive sampling schemes, calculated in real time during acquisition, that maximize the accuracy of the measured rate constants. Following validation on ubiquitin and other small proteins, we are beginning to apply these methods to study the rotational diffusion of folded and unfolded states in translationally-arrested ribosome–nascent chain complexes of the FLN5 filamin domain (Cabrita et al. (2016) Nat. Struct. Mol. Biol.).
EXPLORING THE CONFORMATIONAL NETWORK OF THE WW DOMAIN USING ENOE DATA

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Exact distance restraints (eNOEs) \cite{1-3} can be obtained from quantitative analysis of NOE buildup rates. A multi-state structure calculation protocol that is reflective of the occupied conformational space \cite{4-6} also reveals concerted conformational exchange on an atom per atom basis.

The WW domain folds into a small three stranded, anti-parallel β-sheet structure. Together with a N-terminal PPlase, the WW domain comprises the Pin1 cis-trans isomerase. Here, we compare the apo and holo forms with phospho-Cdc25c or phospho-cyclin-E1 binding fragments with our eNOE-based ensembles. This enables us to dissect the dynamics and long-range correlations of the WW domain. Upon ligand binding to the first loop, we find an allosteric redistribution of states in the second loop, which is implicated in communication with the PPlase domain.

Further, we use our technique to study WW domain folding \cite{7}. We compare 5°C and 30°C temperature multistate structural ensembles. The temperature increase causes a loss of structural correlation, whereby an increase of the averaged distances by 8% suggests an overall inflation of the domain.

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Stabilizing Short α-Helical Protein Sequences

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In this work we present a novel strategy for stabilizing short α-helical sequences by using the Trp-Cage miniprotein as a stabilization unit. The Trp-Cage is the smallest stably folded protein (20 amino acids) and comprises a short N-terminal helical fold of eight amino acids. Its fold is induced by hydrophobic interactions including a tryptophan side chain. By creating an N-terminal protein chimera or fusion protein of a Trp-Cage and biologically active peptides with α-helical propensity, we aim at propagating the existing helical fold to the biological peptide, thus stabilizing the peptides fold and increasing their activities.

For the chimera approach we chose a protein with one active side, the α-helical antifreeze protein type 1 from winter flounder. This protein consist of 37 amino acids with a repetitive pattern of eleven amino acids. Without any external stabilization, shortening its sequence by a few residues results in a dramatic decrease of the helicity and abolishes activity. We combined a shortened inactive version of this AFP with the Trp-Cage in three different designs with varying orientation of the ice binding side in respect to the cage fold. By standard protein NMR techniques the propagation of the helical fold without any signs of N-terminal fraying is observed for all three designs. Furthermore, the three de-novo antifreeze proteins display significant ice binding activity.

Encouraged by the results, the strategy is extended to fusions of the TRP-Cage with single-helix antimicrobial peptides in order to test the effect of helical stability on antimicrobial activity.
Amyloid peptide aggregation and co-aggregation mechanisms

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The assembly of proteins into amyloid fibrils is a central phenomenon in several human diseases. We use a combination of NMR, optical spectroscopy and mass spectrometry to study the mechanism of peptide self-assembly into amyloid fibrils. Our studies are focused on amyloid β peptide (Aβ) from Alzheimer's disease and α-synuclein (α-syn) from Parkinson's disease. The goal is to find the underlying microscopic steps in the aggregation process and their molecular driving forces. In other studies we seek to find the composition and structure of the formed aggregates. We study the process starting from pure monomers, or monomer supplemented with a defined amount of pre-formed fibrils. We also study monomers mixtures including length variants, or monomers supplemented with inhibitors or bilayer membranes in the form of with phospholipid vesicles. The results provide insights into the specificity of various microscopic steps in the aggregation process and the formation of co-assemblies at different stages along the aggregation process. In particular, we have found that secondary nucleation of monomers on the surface of existing aggregates is the dominant nucleation mechanism for Aβ at several solution conditions, including neutral pH and physiological salt, and for α-syn at mildly acidic pH relevant for endosomes and other intracellular compartments. We have found that this process has a very high level of specificity. We have also found that lipids get incorporated in the aggregates formed by α-syn in the presence of membranes.

Figure 1. Summary of the aggregation process in mixtures of Aβ40 and Aβ42, with interaction at the level of primary nucleation only, leading to formation of separate homomolecular fibrils.
Pico- to Nanosecond Motion in Protein Side-chains Explored by High-Resolution Relaxometry

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Understanding protein function requires the characterization of both structure and dynamics at atomic resolution. NMR relaxation is an efficient technique to study protein dynamics both in liquid and solid state. Here, we use a combination of high-field relaxation measurements and high-resolution relaxometry to probe pico- and nanosecond motions of methyl-group bearing side-chains in the protein ubiquitin.

Methyl groups have been shown to be a valuable probe for protein sidechains dynamics. Deuterium, proton and carbon-13 relaxation rates can be used to probe the spectral density function describing the motions of methyl groups. High-field measurements of relaxation rates do not provide information on the spectral density function at low frequency. However, relaxation measurements at low magnetic fields would be impractical because of poor resolution and sensitivity. Combining the advantages of relaxometry and high-field NMR on proteins is possible with the use of a sample shuttling apparatus that allows for relaxation to take place at low field while polarization and detection are carried out at high field1,2.

The selective labelling of isoleucine δ₁ methyl groups \(\{^{13}\text{C}^2\text{H}_2^1\text{H}\}\) in an otherwise fully deuterated protein provides simplified relaxation pathways in an almost isolated \(^{13}\text{C}^1\text{H}\) pair. We have used high-resolution relaxometry to measure both high-field and low-field relaxation in such selectively labelled ubiquitin. The parameters for overall rotational diffusion were evaluated using the measurements of \(^{15}\text{N}\) relaxation rates at 600 and 800 MHz. The spectral density functions at low frequency have been probed with measurement of \(^{13}\text{C}\) longitudinal relaxation rates over two orders of magnitude of magnetic fields: from 0.24 T to 18.8 T using high-resolution relaxometry. All relaxation pathways in a \(^{13}\text{C}^2\text{H}_2^1\text{H}\) spin system have been taken into account to carry an adapted Iterative Correction for the Analysis of Relaxation Under Shutting (ICARUS2). This extensive dataset allows for the quantification of motions from low picoseconds (methyl group rotation) to low nanosecond (transitions between rotamers).

Solution structure of the apo- and metal-bound form of the heavy metal binding domain of a PIB-type copper-ATPase

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Heavy-metal homeostasis and detoxification is crucial for cell viability. P-type ATPases of the class IB (PIB) are essential in these processes, actively extruding heavy metals from the cytoplasm of cells. Previously, the structure of a PIB-ATPase, a *Legionella pneumophila* CopA Cu⁺-ATPase, in a copper-free form, was determined by X-ray crystallography, but noticeably the structure of the N-terminal heavy metal binding domain (HMBD) could not be resolved due to missing density. Knowledge of the structure of this HMBD is crucial for understanding the mode of action for the ATPase. Here we present the solution structure of the HMBD in the metal-free form as well as the metal-bound form using NMR spectroscopy. The chemical shifts were analyzed to discern structural features, provide dynamical characterization and follow the binding of Ag⁺. We find that the HMBD structure is composed of two flexible ends and a compact structured core built of an anti-parallel beta-sheet with three strands connected by two loops. The metal ion is bound sandwiched in between the two loops coordinated to three sulfur atoms in two methionines (M44 and M61) and a cysteine residue (C59). The HMBD structure rearranges slightly in the loops to accommodate the metal ion.

*Figure 1. Structure of the Ag⁺ bound form of HMBD with Cartoon representation showing Ag⁺ and coordinating S-atoms as grey*
High pressure NMR and stability of protein deposits in neurodegenerative diseases

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Protein aggregation is a pathological hallmark of neurodegenerative diseases such as Alzheimer (AD) and Parkinson (PD) diseases. A remarkable feature of neurodegenerative pathology is its rapid spreading in human brains often through particular routes. A key mechanism in the spreading of neurodegenerative pathology is prion-like propagation of protein aggregation, which its efficiency is largely dependent on the stability of protein aggregates. Recently, we have used high pressure NMR to investigate the stability of amyloid-beta (Ab) aggregates against pressure-induced monomer dissociation (1). Using Ser-8 phosphorylation as an example of a posttranslational modification, it was shown that the Ab modification could modulate the stability of Ab deposits, even when the site of modification was outside the structured core of Ab fibrils. Quantitative analysis of Ab monomer release data from phosphorylated and non-phosphorylated Ab aggregates showed that the Ser-8 phosphorylation increased both the kinetic and thermodynamic stability of Ab aggregates against pressure-induced monomer dissociation. The volume change upon monomer release was around three times smaller in phosphorylated than the non-phosphorylated Ab aggregates. Molecular dynamics simulation data showed that, in the absence of Ab monomer release, the phosphorylated Ab fibrils had higher compressibility than the non-phosphorylated fibrils, suggesting a potential mechanism for their lower susceptibility against pressure-induced monomer dissociation. Our data demonstrate the power of high-pressure NMR for quantitative determination of the stability of neurodegeneration-related protein deposits. In addition, they support an important role of posttranslational modifications in modulating the stability of protein deposits and thereby influencing the initiation and spreading of neurodegenerative pathology.

Reference:
Polypyrimidine-tract binding protein, a dynamic folding platform for adaptive RNA recognition

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Polypyrimidine Tract Binding (PTB) must fulfill many tasks in RNA metabolism such as enhancing or repressing splicing, or activating IRES mediated translation. To achieve these functions it must recognize a given RNA target in the appropriate structural context and potentially remodel it. Defining the changes in dynamics of both RNA and protein binding partners plays an important role in understanding this process.

Structural studies have demonstrated that the RNA recognition motif domains (RRMs) of PTB interacts with ssRNA through the β-sheet surface and the loops connecting the β-strands.1 Interestingly, upon binding to a stemloop RNA containing a UCUUU pentaloop conserved in picornavirus type II Internal Ribosomal Entry Sites (IRES), the N-terminal RRM of PTB forms an additional helix α3 extending the βαββαβ RRM topology. This helix docks to the β2-strand at one edge of the beta sheet. We performed 15N and 13C NMR relaxation studies to characterize the dynamics changes of RRM1 and the SL-RNA upon complex formation.

We found that the free protein is highly dynamic exhibiting motions in the C-terminal half of the domain which are characteristic of partly folded proteins. Millisecond timescale motions detected in α1, β2 and the C-terminus suggest the C-terminal helix transiently folds and docks to α1 and β2 both of which contact α3 in the SL-RRM complex. NOESY experiments support the presence of a dynamic equilibrium involving this α3-docked state. Relaxation dispersion measurements performed with the PTB RRM1-stemloop RNA complex revealed μsec dynamics at the binding interface of the protein and RNA apical loop that could be fitted to a global exchange model. Our study suggests that PTB RRM1 explores partially unfolded conformations and that binding to a structured RNA is a cooperative folding process. This allows PTB to adapt to a wide range of structured RNA targets and may be important for the assembly and rearrangement of multicomponent RNA-protein complexes. Potential implications for modulation of PTB function will be discussed.

4 Abstracts for posters in session "Biosolids"
Pyroglutamyl-Modified Aβ Fibrils Exhibit a Similar Secondary and Tertiary Structure and Dynamics as Wildtype Aβ Fibrils

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Fibrillation of the different species of amyloid β peptides and deposition as senile plaques in the brain are the hallmarks of Alzheimer’s disease. Among these peptides of different length, also N-terminal truncated variants seem to play a major role in the development of the disease. These variants include forms where the glutamate residues at position 3 or 11 are converted into cyclic pyroglutamate. Here, we compare the molecular structure of mature Aβ(1-40) fibrils with fibrils grown from pE3-Aβ(3-40) on the single amino acid level.

ThT-Fluorescence, electron microscopy, and X-ray diffraction reveal the general morphology of amyloid fibrils. With special attention to the unstructured N-terminus, which may play a role in the fibrillation process, we measured ¹³C and ¹⁵N chemical shifts of different amino acids in Dual-Acquisition experiments (¹³C-¹³C DARR in combination with ¹⁵N-¹³Cα, see figure 1). So far, we found overall a good agreement between the chemical shifts and therefore the secondary structure of pE3-Aβ(3-40) and wildtype Aβ(1-40) fibrils. Some alterations occur in the direct vicinity to the pyroglutamate. Also, the existence of the well-known interresidual contact between the two β-strands of the Aβ fibrils was confirmed by detection of cross peaks between the side chains of Phe 19 and Leu 34.

Furthermore, the molecular dynamics of the peptide backbone was investigated by means of order parameters using the separated local field experiment DIPSHIFT. Mature Aβ fibrils exhibit an interesting increase in the order parameters for the first amino acids of the N-terminus. Comparing the order parameters of pE3-Aβ(3-40) and Aβ(1-40) fibrils no significant differences were observed.

Overall, a very similar structure and backbone dynamic of amyloid fibrils grown from pE3-Aβ(3-40) and Aβ(1-40) has to be assumed.

![Figure 1](https://example.com/figure1.png)

*Figure 1. Scheme of a Dual-Acquisition experiment to detect a ¹³C-¹³C DARR- and ¹⁵N-¹³Cα-Spectrum at the same time.*
Antimicrobial Peptide-Lipid Interactions and Lateral Diffusion in Lipid Bilayers via $^{31}$P CODEX NMR

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Lateral diffusion of phospholipids is a process essential to membrane function, and its accurate determination can provide insights into kinetics of membrane-associated biochemical reactions. Here we describe the application of CODEX (Centerband-Only Detection of Exchange)$^1$ and our powder-average model to measure lateral diffusion of phospholipids in lipid bilayers$^2$ assembled into large unilamellar vesicles (LUV). CODEX is an ideal experiment for these systems because $^{31}$P NMR can be measured in natural abundance, eliminating the need for synthetic labels.

The plasma membrane in many types Gram-negative and Gram-positive bacteria contains anionic phospholipids, as well as neutral phospholipids. The difference in charge arises from differing phosphate head groups on the lipids, which will appear as separate narrow resonances in a $^{31}$P CODEX spectrum. Unhindered, the two lipid species will experience free diffusion at comparable rates. Many antimicrobial peptides selectively bind to anionic lipids, causing them diffuse at a slower rate than the unbound lipids. By comparing differences in diffusion rates between the lipids bound to the peptide of interest versus the bulk lipids, we will learn valuable information about these peptide-lipid interactions and gain insights into the various mechanisms governing the efficacy of antimicrobial peptides in disrupting the bilayer, which ultimately results in the death of bacteria. We investigated the binding between the cationic peptide polylysine and the model antimicrobial peptide KL14 with liposomes consisting of the neutral phospholipid POPC and anionic phospholipids PPG, DOPS, and cardiolipin. This proof of concept illustration proves to be a springboard for a number of other possible experiments. Our method can also be used in investigating how peptides and proteins interact with the cell membrane, and answer important questions regarding various disease pathways.


Solid-state NMR chemical-shift perturbations indicate domain reorientation of the DnaG primase in the primosome of Helicobacter pylori

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We investigated the interactions between the DnaB helicase and the C-terminal domain of the corresponding DnaG primase of Helicobacter pylori using solid-state NMR. As for HpDnaB alone¹, the difficult crystallization of this 387 kDa complex, where the two proteins interact in a six to three ratio, can be circumvented by simple co-sedimentation of the two proteins directly into the MAS-NMR rotor. While the amount of information that can be extracted from such a large protein is still limited, we can assign, using the available chemical shift information from the isolated N-terminal domain², a number of amino-acid residues experiencing significant chemical-shift perturbations upon helicase-primase complex formation. The location of these residues is used as a guide to model the interaction interface between the two proteins in the complex. Chemical-shift perturbations also reveal changes at the interaction interfaces of the hexameric HpDnaB assembly on HpDnaG binding. A structural model of the complex that explains the experimental findings is obtained ³.


Quantification of inorganic polyphosphates in activated sludge

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Phosphorus (P) is a limited resource that is essential for all life, as it is found in DNA, phospholipids and ATP. Recycling of P will be necessary, as the supply of P from phosphate mining is expected to decrease in coming years due to depletion of the known phosphate rock reserves1. Recovery of P from waste water treatment plants is an attractive way to recycle P, as it simultaneously minimizes P pollution in the environment, and the development of new strategies for P recovery requires deeper insight into the different P forms present in waste water and sewage sludge.

Polyphosphate-accumulating bacteria incorporate phosphate into polymeric, inorganic polyphosphates (poly-P) with a general chemical formula \(\text{H}_2\text{PO}_4-[\text{PO}_4]_n\text{H}_2\text{PO}_4\) in biological P removal in waste water treatment plants. The large amounts of poly-P in waste water treatment plants means that development of methods for quantification of poly-P is an important step to understand the speciation and flows of P in a waste water treatment plant. Solution state \(^{31}\text{P}\) NMR is often used for identification of organic P species in the environmental sciences and has earlier been used for identification of poly-P2-4. Our objective is to develop an extraction method for solution state \(^{31}\text{P}\) NMR, which can precisely identify and quantify the poly-P present in waste water treatment systems.

Solution state \(^{31}\text{P}\) NMR is advantageous with regards chemical information and experiment time, but the P compounds in the environmental sample must be extracted before measurement. This implies risks of incomplete extraction or poly-P hydrolysis3,5. To investigate the efficiency of the extraction method with regards to poly-P, \(^{31}\text{P}\) solid state NMR (SSNMR) provides directly insight into the speciation of phosphorus in the sludge prior to extraction. The combined results from \(^{31}\text{P}\) solution state NMR and \(^{31}\text{P}\) SSNMR indicate that the optimized extraction method extracts all poly-P present in the sample without hydrolysis of the poly-P.


Orthophosphate
\text{P mono- and diesters}
Calcium-P
Polyphosphate

\[\delta(\text{P})\text{ (ppm)}\]

\[\nu_L = 242.9 \text{ MHz and spinning speed } 15 \text{ kHz}\]

Figure 1 \(^{31}\text{P}\) solid state NMR spectrum of freeze-dried activated sludge from a Danish waste water treatment plant. Spinning side bands are marked with asterisks.
Radiofrequency fields in solid state MAS probes

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Modern multi-dimensional solid state NMR experiments designed for applications in structural biology rely on multiple magnetization transfer steps. These are typically implemented using homonuclear and heteronuclear dipolar recoupling experiments, including, for example, cross polarization, DCP, DREAM, MIRROR, or PAR. To meet different recoupling criteria of these sequences, it is crucial to match radiofrequency (rf) fields with respect to magic angle spinning (MAS) frequency. Most of MAS probes use solenoidal coils which generate inhomogeneous rf fields. It implies that recoupling cannot occur homogenously in all parts of a sample. To remedy such problems, ramp or adiabatic sweeps are used to broaden the resonance conditions.

In my contribution, I review possible methods to map rf fields which are based on using a $B_0$ gradient across the sample for imaging purposes. Such gradient can be produced either by additional gradient coils installed in a MAS probe or by shim system of a magnet itself. A simple nutation experiment [1] should provide direct calibration of rf amplitude distribution. Cross polarization experiment [2] correlates rf fields on two channels according to Hartmann-Hahn match. Using spin-lock experiment to search for rotary resonance recoupling conditions [3] offers another method to calibrate rf amplitudes towards MAS frequency. The comparison is completed with theoretical calculations based on Maxwell equations accounting for finite wavelengths [4]. The results confirm the common experience that rf calibrations from determination of 180° pulse are not precise enough for recoupling periods spanning several milliseconds, and that DCP experiments suffer from about 50% loss of sample volume located at rotor ends.

Figure 1. Calibration of DCP experiment in a presence of $B_0$ field gradient. The $^{15}$N rf field kept constant while $^{13}$C rf field amplitude ramped (70-100%) and changed in linear steps. The pseudo-2D spectrum provides information on which sample regions contribute to the total signal. The lower spectrum represents a trace taken at maximum and is compared to single-pulse $^{13}$C spectrum. It confirms the known fact that only 50% of the sample from the middle of the rotor is excited. Other conditions: $^{13}$C$_2$, $^{15}$N-glycine, 1.9mm N/D/C/H probe with a gradient coil, 30kHz MAS, 700MHz NMR system.

Acknowledgement

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References
Solid-state NMR structural studies of Antimicrobial Peptides LPcin Analogs with Enhanced Activities

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The availability of antibiotics has allowed for the successful treatment of many bacterial infections as well as the ability to perform invasive medical procedures including surgery and chemotherapy. However, their wide use has led to pathogens’ increased drug resistance and the need to find novel classes of antimicrobial peptides as alternatives to antibiotics. Lactophoricin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, corresponds to the carboxy terminal 113–135 region of component-3 of proteose-peptone. LPcin is a good candidate as a peptide antibiotic because it has an antibacterial activity but no hemolytic activity. Three different analogs of LPcin, LPcin-yk2 which has mutant amino acids, LPcin-yk1 and LPcin-yk3 that has shorter mutant amino acids are recently developed by using peptide engineering techniques in our laboratory. These three LPcin analogs show better antibiotic activities than wild-type LPcin and no toxicity at all.

In order to understand the structural correlation between LPcin analogs structure and antimicrobial activity under the membrane environments, we tried to express and purify as large as amounts of LPcin and three different LPcin analogs. We finally optimized and succeed to overexpress in the form of fusion protein in Escherichia coli and purified with biophysical techniques like Ni-affinity chromatography, dialysis, centrifugue, chemical cleavage, and reversed-phase semiprep HPLC. In here, we will present the optimizing processes with high-yield expression and purification of three LPcin analogs and solid-state NMR structural studies to figure out antibacterial killing mechanisms.
Interaction between N-terminal hPrP and Aβ oligomers studied by solid state MAS-NMR

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Both prion protein (PrP) and Amyloid beta (Aβ) play an important role in the pathogenesis of neurodegenerative disorders as they misfold or form amyloid fibrils. Misfolded PrP is responsible for a number of diseases, e. g. bovine spongiform encephalopathy and Creutzfeldt-Jakob disease [1]. Aβ oligomers have been identified as neurotoxic factor of the pathogenesis of Alzheimer’s disease (AD), whereas fibrils are known to be the main component of insoluble plaques [2].

In contrast to the secondary structure of fibrils, secondary structure elements of the oligomers are relatively unknown, due to their transient nature [3], [4]. Recently, it has been shown that oligomeric Aβ binds human PrP (hPrP) with high affinity. First models use ionic interactions between both species including two Lys-rich parts (aa 23 to 27 and 95 to 110) of the hPrP. As a result the C-terminus can be deleted without preventing the binding between hPrP and Aβ [5], [6].

We show first MAS NMR experiments on species-specifically uniformly labeled samples, with N-terminal hPrP (aa 23 to 144).

References
Conformational ensembles of (disordered) proteins with DNP-NMR

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DNP overcomes the inherently low sensitivity of magnetic resonance methods by transferring high polarization of unpaired electrons to nuclei. Low temperature NMR spectra usually suffer from severe line broadenings due to freezing out different conformations \cite{Tycko2013}. While this is usually accounted for as an unwanted side-effect of DNP-NMR, these inhomogeneously broadened lines also contain valuable information about conformational ensembles of (disordered) proteins.

We have investigated amyloid binding protein “β-wrapin AS69” \cite{Mirecka2014} which is designed to specifically bind alpha-synuclein (α-syn) in the free form as well as bound form to α-syn fibrils by DNP-NMR. Low temperature studies allowed us to probe the effect of binding on the conformational ensembles of β-wrapin AS69 from different NMR line widths in free and bound state.

Besides uniform \textsuperscript{13}C,\textsuperscript{15}N isotope labelling, sparse selective labelling with 2-\textsuperscript{13}C glucose \cite{Hong1999} is employed for α-syn to quantify the conformational distribution for both monomeric and fibrillar form in the frozen state. We demonstrate the high sensitivity obtained with this specific \textsuperscript{13}C labelling protocol for fibrillar sample in comparison with fully \textsuperscript{13}C labelled samples.

\cite{Tycko2013} Tycko R. NMR at Low and Ultralow Temperatures. Accounts of chemical research 2013;46:1923-32.
Structural investigations of a functional amyloid important for long-term memory

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Orb2 is a functional amyloid essential for long-term memory in Drosophila melanogaster. Aggregation of Orb2 switches it from a repressor to an activator of mRNA polyadenylation. This activation allows synapse-specific protein expression. The N-terminus of Orb2 has a glutamine-rich (Q-rich), low complexity sequence reminiscent of huntingtin exon-1 and asparagine and glutamine rich sequences found in yeast prion proteins. The amphiphilic sequence in Orb2 isoform A that precedes the Q-rich sequence has been shown to be important for aggregation in cell culture and long-term memory in vivo.

Using a combined solid-state NMR and EPR approach, we identified the location of Orb2A’s N-terminal amyloid fibril core and found that it adopts an in-register parallel β structure. These data show why the sequence preceding the Q-rich region is so important for amyloid formation. We also present solid-state NMR data comparing fibrils formed by the two Orb2 isoforms A and B. The comparison of Orb2 with other Q-rich or polyQ fibril forming proteins reveals a surprising amount of differences that give clues to why Orb2A is a functional amyloid while other proteins can form toxic fibrils.

Figure 1. The Orb2 protein is a functional amyloid that is a key regulator of synapse-specific protein expression and long-term memory in Drosophila. Solid-state NMR spectroscopy was used to identify and structurally characterize the amyloid core of this protein.
In oxygenic photosynthesis, sophisticated regulation mechanisms have evolved to enable the splitting of water while preventing the photosynthetic machinery from photodamage under high light. Photo adaptation involves membrane phase transitions and supramolecular re-arrangements of light-harvesting proteins (LHCII), which are abundant in photosynthetic membranes, as well as conformational switching and subtle changes in pigment interactions on a molecular and atomistic scale. We present preliminary NMR data recorded in situ of \( ^{13}\text{C}^{15}\text{N} \) *Chlamydomonas reinhardtii* (Cr.) thylakoid membranes using polarization transfer to probe rigid and mobile parts by spectral editing with CP and INEPT in \(^{13}\text{C}\) MAS NMR, and T\(_1\) relaxation spectroscopy to follow protein and lipid dynamics in situ. A comparison of wild type and photoprotective Cr. mutants (npq2) suggests altered protein and lipid dynamics in the npq2 membranes, demonstrating the use of in situ NMR for structure-functional screening of photosynthetic mutants. LHCII protein complexes are abundant in the thylakoid membranes, which may open possibilities to investigate their structure and function in situ.
**Aβ 1-42 fibrillar structure studied by high resolution solid state MAS NMR**

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The progress of Alzheimer’s disease is believed to be related to the formation of amyloid beta (Aβ) aggregates in the human brain. Although differing only slightly in the amino acid sequence, compared to Aβ 1-40, Aβ 1-42 is more prone to aggregation, causing a higher toxicity [1]. Here we present the preliminary site specific resonance assignment of one conformation of Aβ 1-42 fibrils, obtained by solid state magic angle spinning (MAS) NMR [2][3]. Interestingly, specific differences to previously described fibrillar Aβ 1-42 secondary structure can be found.

References:

Homogeneity Factor: A Simple Analysis of $^1$H ssNMR Linewidth at Various MAS Rates

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A simple factor, homogeneity factor $k$, is proposed to estimate the $^1$H NMR linewidth for each $^1$H resonance at arbitrary MAS rate $\nu_r$. The factor $k$ is defined by

$$k = \nu_r \frac{\Delta^{\text{homo}}}{\Delta^{\text{inhomo}}} = \nu_r \frac{\pi T_2(\nu_r)}{\Delta^{\text{inhomo}}},$$

where $\Delta^{\text{homo}}$ and $\Delta^{\text{inhomo}}$ are the linewidth contribution from homogeneous and inhomogeneous broadening, respectively, and $T_2$ is transversal relaxation time measured by spin echo measurements. Assuming $T_2$ is inversely proportional to $\nu_r$, $k$ is MAS rate independent. The $k$ gives quantitative measurements of effect of MAS rate to $^1$H NMR linewidth, and hence signal intensity through $\Delta = \Delta^{\text{inhomo}}(1 + k/\nu_r)$ and $1/\Delta$, respectively. If the MAS rate is comparable to $k$, there are equivalent contributions from homogeneous and inhomogeneous broadening in $^1$H NMR linewidth. If the MAS rate is $m$ times larger than $k$, the homogeneous contribution is $m$ times smaller than the inhomogeneous contribution. The value of $\Delta^{\text{inhomo}}$ is not obvious, but can be given by either the linewidth of homonuclear decoupled $^1$H NMR or $\Delta = \Delta^{\text{inhomo}} - \pi T_2$ at a certain MAS rate. The $^1$H NMR spectra of L-Histidine.HCl.H$_2$O at 110 and 60 kHz MAS rates together with experimentally obtained $k$-factors are shown in Figure 1. While the improvements at 110 kHz for peaks with small $k$ are limited, linewidths are significantly narrowed for peaks with large $k$. While the $k$-values calculated from literature are 5-10 kHz for 10% back $^1$H exchanged protein, the $k$-values increases to 30-100 kHz for 100% back exchanged protein and to 300 kHz for 100% proton protein, indicating the requirements MAS rate much faster than 100 kHz for these samples.

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\textbf{Figure 1.} $^1$H NMR spectra of L-Histidine.HCl.H$_2$O at 110 kHz (solid) and 60 kHz (dotted) and $k$-factors. $\Delta^{\text{inhomo}}$ is taken from wPMLG measurements.
Towards atomic-resolution structure of functional amyloids named hydrophobin rodlets by solid-state NMR

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Hydrophobins (class I) are ubiquitous fungal proteins that are functional amyloids called rodlets\(^1\). These small proteins self-assemble into rodlets forming an amphipathic monolayer at hydrophilic: hydrophobic interface. Rodlets play many roles in functional fungal biology such as facilitating the spore formation and dispersal, as well as mediating host attachment and function. Although monomeric structure (β-barrel topology) of many hydrophobins has been extensively studied by liquid-state NMR, the structure of hydrophobin rodlets is still unknown. While the rodlets share many structural characteristics with disease-associated amyloid fibrils, they further pack laterally to form amphipathic layers. Detailed structural information is crucial for understanding the mechanism of rodlet assembly, designing new hydrophobin-based materials, and developing new anti-fungal drugs.

Structural characterization of amyloids is challenging due to the inherent heterogeneity of the samples resulting in broad peaks in any NMR spectra\(^2\). We employed proton-detected solid-state NMR under fast MAS to determine the rodlet structure using 100% proton-back-exchanged [\(^2\)D, \(^15\)N, \(^13\)C]-labeled EAS\(_{115}\) hydrophobin from Neurospora crassa. Using a set of tailored experiments based on efficient dipolar-based transfers, we assigned backbone resonances and characterized secondary structure. Conformational changes (increase of β-sheet content in the C-terminus) are observed in the rodlet form compared to the monomeric structure. Secondly, we obtained side-chain shifts using J-based isotropic mixing (MOCCA)\(^3\) and hydrophilic-surface information using water-edited sequences. Later, we obtained long-range distance restraints information using \(^15\)N- and \(^13\)C-edited RFDR-based 4D experiments. Intermolecular restraints were obtained by using a mixed-labeled sample and 4D experiments. We will present an initial atomic-resolution model based on these ssNMR observations.

Figure 1: (A) TEM image of in vitro EAS\(_{115}\) hydrophobin rodlets of Neurospora crassa. (B) Monomeric structure of EAS\(_{115}\) protein determined by liquid-state NMR. (C) 2D \(^1\)H-\(^15\)N-correlation spectrum of 100% back exchanged \(^2\)D, \(^13\)C, \(^15\)N-labeled EAS\(_{115}\) rodlets recorded at a MAS spinning speed of 56 kHz and 800 MHz. \(^1\)H Larmor frequency. The heterogeneity of the sample is clearly visible in the spectrum with a proton linewidth of 0.5 ppm. In contrast, microcrystalline protein has a proton linewidth of 0.1 ppm under similar experimental conditions.

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Lipid-protein assemblies regulate apoptotic pore formation via dynamical and structural alterations – a solid state NMR study.

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Mitochondria have a significant importance for eukaryotic cells by providing the majority of a cell’s ATP supplies. Additionally, they play a key role in the early stages of regulated cell death – apoptosis – where cytochrome c is released into the cytosol. This first step of the intrinsic apoptotic pathway is regulated by members of the Bcl-2 protein family interacting with the mitochondrial outer membrane (MOM) and modulating its permeability. For the longest time MOM forming lipids have been seen merely as structural building blocks without any mechanistically role within the intrinsic apoptotic pathway. This conception changed recently after evidence for the direct involvement in apoptotic events of oxidized phospholipids (OxPls) formed under intracellular stress has been provided. Since the presence of OxPls strongly impacts the equilibrium of pro-apoptotic Bax protein towards its membrane bound state (Fig. 1), we investigate their role in the Bax induced step of membrane pore formation and the undergoing structural changes of Bax protein during this event.

We therefore created cell-free MOM mimicking liposome systems which resemble the cellular situation prior to apoptosis and upon various apoptosis triggering degrees of oxidative stress. By using ¹H, ¹³C and ³¹P solid state MAS NMR spectroscopy accompanied with differential scanning calorimetry (DSC) we could obtain insights into the hydrophilic interface region of the membranes as well as their hydrophobic fatty acid regions. After implementation of OxPls into our liposome systems both methods revealed severe dynamical changes in model membranes.

Previously, published DSC data suggested that the incorporation of Bax into our model membranes countered the effects of lipid oxidation to a certain extent – namely the increased permeability and decreased melting temperature. For the first time we could confirm these macroscopic observations on a molecular level by using ³¹P CP-MAS NMR spectroscopy.

Figure 1: Working model of MOM pore formation, regulated by Bcl-2 proteins. A: The Bax equilibrium is heavily shifted towards the cytosolic state in a healthy cell. B: Upon lipid oxidation (triggered apoptosis) the equilibrium is shifted towards the membrane bound state of Bax protein, which leads to BH3-only protein assisted MOM pore formation after saturation of anti-apoptotic Bcl-2 protein.
Tailored 1H-detected ssNMR approaches for the study of large (membrane) protein complexes

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One of the formidable challenges in NMR-based structural biology is to study membrane protein complexes (MPCs), preferably in their native environment. An example is the β-barrel assembly machinery (BAM) which is involved in the insertion of proteins into the outer membrane (see, e.g. 2,3). This complex consists of the core component BamA (89 kDa) and 4 other accessory components BamB, BamC, BamD and BamE leading to a 200kDa membrane protein complex. Recently, several crystal structures of BAM–(sub) complexes have been reported3,4,5. Yet, the organization of the entire machine and its function in cell membrane remains to be determined.

Significant progress has been made in using fast magic-angle spinning in combination with deuteration strategies to obtain high resolution 1H-detected spectra of membrane proteins6,7,8. In our contribution, we show how these advances can be combined with tailored labeling and sample preparation approaches to probe large MPC’s such as BAM in high-resolution 1H ssNMR.

Optimization of Dynamic Nuclear Polarization solid-state NMR for oriented and non-oriented membranes

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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. The protocols, sample geometries, cooling and microwave irradiation have been optimized for such measurements and novel membrane-anchored biradicals developed, investigated in physico-chemical 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. Whereas under MAS conditions signal enhancements are up to 50-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 its 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.


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Structure of fully protonated proteins by proton-detected MAS NMR

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Protein structure determination by proton detected magic-angle spinning (MAS) NMR has focused on highly deuterated samples, in which only a small number of protons are introduced and observation of signals from side-chains is extremely limited. Here we show that in fully protonated proteins at 100 kHz MAS and above, spectral resolution is high enough to assign amide and side-chain protons of all residue types, and to reliably measure a dense network of 1H-1H proximities that define a protein structure. The high data quality allows the correct identification of internuclear distance restraints encoded in 3D spectra with automated data analysis, resulting in accurate, unbiased and fast structure determinations.

Additionally, we find that narrower proton resonances, longer coherence lifetimes and improved magnetization transfer offset the reduced sample size at 100 kHz spinning and above. Less than two weeks of experiment time and a single 0.5 mg sample were used for acquisition of all data for backbone and side-chain resonance assignment and for unsupervised structure determination. We show how the technique paves the way for structure analysis of a wide range of proteins, and present examples ranging from large assemblies to membrane proteins in lipid bilayers.

Figure 1. Distance measurements using 3D 1H-1H RFDR spectra. A model protein (A-C) was used as a test case for the de novo structure determination of a dimeric 130 residue protein assembled in a 2.5 MDa particle.
Abstracts for posters in session "Biomacromolecules"
Recognition of dimethylarginine by the SMN Tudor domain involves conformational selection

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Tudor-domains are an important family of proteins that recognize N-methylated side chains of arginine or lysine in proteins involved in the regulation of gene expression. A subset of Tudor domains, such as the one of the Survival of Motor Neurons (SMN) protein, have a preference for the recognition of symmetrically N-dimethylated arginine (sDMA), a post-translationally modified amino acid that is central for regulating RNA processing in eukaryotic cells. The ligand-binding sites of these proteins exhibit a characteristic aromatic cage containing several aromatic side chains that employ π–cation interactions for the recognition of sDMA.

Here we have combined NMR and computational methods to dissect the thermodynamic and kinetic features of the recognition of methylated arginines by the SMN Tudor domain. Using NMR exchange line-broadening and quantum chemical calculations, we can show that sDMA isomerizes rapidly in solution between the anti-syn and anti-anti conformations of its N-methyl groups. Although the anti-anti conformation is present in <1% in solution, the SMN Tudor domain conformationally selects this minor conformation in the recognition process.

Quantum mechanical calculations indicate that the aromatic cage in SMN leads to a stronger cation–π interaction within the active site, and thus results in significant selectivity for anti-anti sDMA vs. the anti-syn conformation. We have identified the contributions of specific aromatic side chains in the aromatic cage that tune the energetics and thus discriminate between the two conformations. Our findings reconcile structural differences observed between the anti-anti sDMA-binding of the basic SMN Tudor domain and the recognition of an anti-syn sDMA conformation by other Tudor domain family members.

Figure 1. The structure of symmetric N-dimethyl arginine (sDMA) in A) the anti-anti and C) the anti-syn conformation; B) NMR structure of the survival motor neuron (SMN) with sDMA bound in the anti-anti conformation (PDB ID: 4A4E), and D) X-ray structure of the SND1 with the anti-syn conformation of sDMA (PDB ID: 3OMC).
Enzymatic hydrolysis of plant proteins monitored by NMR spectroscopy and Chemometrics

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Plant proteins constitute an underutilized source of proteins in foods. A part of the reason for this is their low water solubility. One approach to improve the solubility and thereby the process characteristics is to modify the proteins by enzymatic hydrolysis.

In the present study a number of industry grade plant protein sources (rapeseed, wheat gluten, corn gluten, potato, pea and soy) have been exposed to enzymatic hydrolysis in a lab-scale setting and the progress of the hydrolysis have been monitored by at-line analysis of supernatants using ¹H liquid-state NMR spectroscopy. Before hydrolysis all protein sources were characterized by ¹³C solid-state MAS NMR to characterize the composition (lipids, carbohydrates, proteins) in the samples.

Subsequent multivariate data analysis resulted in time profiles displaying the amount of soluble protein as a function of time and significant differences in hydrolysis efficiencies were observed. Part of these differences was related to the amount of non-protein compounds in the protein sources.

Principal Component Analysis (PCA) of the ¹H NMR spectra provides an overview of the performance of the enzyme using different substrates.

Altogether this approach represents a strong approach for testing substrates as well as enzymes.
Nucleotide and DNA binding to a DnaB helicase studied by solid-state NMR

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DnaB helicases are bacterial, ATP-driven enzymes that unwind double-stranded DNA into their single-stranded analogues as a key step during the DNA replication process[1]. The detailed mechanism of this process differs from organism to organism and in many cases atomic-resolution structural data are not accessible since the formed complexes of the helicase with nucleotide and/or DNA escape crystallization and therefore X-ray diffraction as an essential structure determination technique.

In these cases, solid-state NMR is besides electron microscopy (EM) a convenient choice since the proteins can be studied as a sediment formed in the Magic-Angle Spinning (MAS) rotor[2]. In this contribution, we study the helicase-nucleotide and helicase-DNA binding of the dodecameric DnaB helicase from Helicobacter pylori[3]. In a first step, the complex between DnaB, MgCl₂ and the non-hydrolysable ATP-analogue Adenylyl-imidophosphate (AMPPNP) is characterized by 2D and 3D solid-state NMR experiments. Chemical-shift perturbations (CSPs) were used to monitor the AMPPNP binding and to identify the binding interaction interfaces, as well as to determine overall structural changes in the helicase.

In a second step, a polythymine stretch consisting of 20 nucleic acids is added to the helicase-AMPPNP-MgCl₂ complex to mimic the binding of single-stranded DNA (ssDNA). The formed complex is studied by solid-state NMR experiments. ³¹P CPMAS-NMR experiments are performed additionally for proving the binding of ssDNA, as well as the ATP-analogue to the helicase.

HpDnaB

Literature
Attachment of Norovirus to Histo Blood Group Antigens: A cooperative multi-step process

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Human noroviruses recognize histo blood group antigens (HBGAs) as cellular attachment factors. Recently, it has been discovered that norovirus infection can be significantly enhanced by HGBA binding.1 Yet the attachment process, and how it promotes host-cell entry is only poorly understood. So far, independent and equivalent multiple binding sites were held responsible for viral attachment.

Here we present studies into the binding of GII.4 Saga, GII.4 Ast6139, and GII.10 Vietnam026 norovirus protruding domains (P-dimers) and virus-like particles (VLPs) to HBGAs and GM3 at atomic resolution. Using NMR experiments and native mass spectrometry we had shown before that binding of HBGAs to norovirus P-dimers is a cooperative multi-step process revealing four instead of two HGBA binding sites per P-dimer (fig. 1).2 A crystallographic study had then localized these extra binding pockets in the case of P-dimers of the GII.10 Vietnam026 strain.3 STD NMR experiments employing VLPs show the presence of at least six distinct and cooperatively coupled HGBA binding sites are present on the surface of GII.4 or GII.10 VLPs. It appears that assembly of viral coat proteins into VLPs adds an extra dimension of complexity to the recognition of HBGAs.

These findings should have implications for understanding the infection process at atomic level and for the design of novel entry inhibitors.

Structural effects of copper-binding to a lytic polysaccharide monooxygenase

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Enzymes known as lytic polysaccharide monooxygenases (LPMOs) are abundantly present in biomass-degrading microbes and are currently classified as auxiliary activity auxiliary activity (AA) families 9 (AA9, formerly GH61), 10 (AA10, formerly CBM33), 11 (AA11) and 13 (AA13) (see www.cazy.org). LPMOs have been shown to catalyze the cleavage of glycosidic bonds in polysaccharides through hydroxylation of either carbon within the scissile bond, thus they are of great importance in biorefining. This mode of action requires an external electron donor, molecular oxygen, and the binding of a copper ion to the LPMO. Whereas x-ray crystallographic studies have been carried out on Cu(I)-LPMOs, solution NMR studies have been solely performed on apo-LPMOs, because of the paramagnetic relaxation enhancement (PRE) brought about by Cu(II).

We have used NMR structural and relaxation studies to show that the core structure of an AA10-family apo-LPMO is a compact and rigid β-sandwich with a flat substrate-binding surface that includes the protein N-terminus. Furthermore, 2D NMR titration experiments (¹⁵N-HSQC, ¹³C-aromatic HSQC and ¹³C-detected COCA) exploiting the PRE indicated that binding of Cu(II) to the LPMO has a marginal structural effect, manifested in minor rearrangements for histidines coordinatating the metal ion. Thus showing that apo-LPMOs can be reliably used for NMR structural investigations.

Figure 1. Left: The outer sphere of 12 Å radius represents the region in which ¹H signals are expected to be affected by Cu(II) PRE, while the inner sphere of 6 Å radius represents the expected effect on ¹³C signals. Right: Residues with vanished ¹⁵N-HSQC signals upon addition of 50 µM Cu(II) to 1 mM ¹⁵N-labeled LPMO are colored blue on the structure.
Characterization of the ligand specificity of an intramolecular *trans*-sialidase from the human gut microbiota: an STD NMR study

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The gastrointestinal mucus layer is colonized by a dense community of microbes catabolizing carbohydrates during their growth within the gut. The composition of the gut microbiota has a major impact on health and intestinal mucus also plays a critical role in maintaining its balance. However, the underlying mucus-bacteria interaction in the gut remain largely unknown. Our focus is on the study of an intramolecular *trans*-sialidase (IT-sialidase) [1] recently characterized from the gut symbiont *Ruminococcus gnavus*, a species studied for its ability to utilize mucin glycans [2]. *R. gnavus* IT-sialidase cleaves α2-3 linked sialic acids from glycoproteins within intestinal mucus. It is a modular enzyme constituted of a sialic acid specific Carbohydrate Binding Module (CBM40) and a catalytic domain (GH33) [1].

In this work, the *selectivity, affinity* and *binding mode* of a set of sialic acid-containing oligosaccharides against 1) CBM40 and 2) inactive GH33 are investigated by Saturation Transfer Difference (STD) NMR. We show that, although the sialic acid moiety is the main recognition element in the interaction with CBM40, sialic acid does not bind as a monosaccharide, and at least an adjacent galactose moiety is required for recognition. Both, α2-3 and α2-6 linked disaccharides and trisaccharides, are recognized by CBM40 and bind with comparable affinity and binding mode, as shown by binding epitope mapping.

In contrast, the inactive GH33 is selective with strong affinity for α2-3 sialyl-lactose. Sialic acid is the main recognition element, as observed for CBM40. Weak binding of inactive GH33 to α2-6 sialyl-lactose has also been detected and characterized, but our preliminary data suggest that this may occur via a distinct, non-specific, binding site.

This work shows that STD-NMR at 800 MHz is an optimal and versatile technique to study protein-carbohydrate interactions and it is particularly well adapted to the investigation of weak and flexible interactions, as reported for CBM40. This approach allowed us to obtain an atomic level 3D view of the recognition processes in solution. Elucidating the structural basis for the interactions between IT-sialidase domains and sialylated structures is important to enhance our understanding of the mechanisms by which mucin degrading bacteria adapt to the mucosal environment in health and disease.


Figure 1. Binding epitope of α2-3 sialyl-lactose with the inactive catalytic domain GH33. The colours represent a scale of relative saturation values after normalization, obtained from STD initial slopes at 800MHz (Very strong, strong, medium, weak contact).
Acetylation-dependent bromodomain interactions studied on reconstituted nucleosomes

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The bromodomain and extra-terminal (BET) family are multi-functional chromatin effector proteins that are conserved from yeast to human and have a conserved domain architecture that features two N-terminal bromodomains separated by a linker of ~110 amino acids. The bromodomains of Brdt, the testis-specific member of the BET family that is essential for spermatogenesis, show a preference for multiply-acetylated histone peptides1. The first bromodomain of Brdt (BD1) preferentially binds histone H4 tail peptides acetylated at lysines 5 and 8, whilst the second bromodomain (BD2) had highest affinity for histone H3 tail peptides acetylated at lysines 18 and 23.

To get a more comprehensive picture of the multivalent interaction with chromatin we examine the tandem bromodomains of Brdt. Comparison of NMR spectra recorded on the tandem domain show that BD1 and BD2 tumble independent of each other and are connected by a flexible linker. Titration of acetylated histone peptides confirm the binding preference and known binding sites of the bromodomains1. To follow the interaction in the context of the histones, we utilize native chemical ligation to reconstitute nucleosomes with unmodified or specifically acetylated histone tails. We then follow the interaction by monitoring chemical shift changes in methyl-TROSY spectra of 2H / 13C1H ILV labeled bromodomains. We find that Brdt interacts solely through BD1 and the interaction is enhanced by a direct interaction with DNA. BD2 does not interact with the nucleosome and it remains flexible in the context of the double construct when BD1 is attached. The absence of observable interaction of BD2 is not due to inaccessibility of the acetylated H3 tail, since BD1 interacts (weakly) with the corresponding nucleosomes.

Our study highlights the importance of studying chromatin reader’s interactions with nucleosomes rather than isolated peptides. While the ‘divide and conquer’ approach gives useful information on specificities and molecular details of the histone binding pockets, there are important additional features that are crucial for the interaction with histones.

Structural and functional characterization of the complexes between the transactivation domain of p65 and CBP/TFIIH
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p65 is a member of the NF-κB family, that plays a key role in the immune response and cell survival. p65 contains a C-terminal transcriptional activation domain (TAD) responsible for initiating transcription of genes regulated by NF-κB[1]. The interaction between p65_{TAD} and the general transcription factor TFIIH would be essential for the role of NF-κB in HIV infection[2]. In addition, the Creb-binding protein (CBP)/p300 is known to interact with p65, leading to an enhanced transcriptional activity.

Despite playing a crucial role in transcription, no high-resolution structure of the TA1 subdomain (residues 521-551) of p65 bound to a transcriptional regulatory protein was available. In this context, we characterized the interaction of p65_{TA1} with two target proteins: the pleckstrin homology (PH) domain of the Tb1 subunit of TFIIH (Tb1_{PH}) and the KIX domain of CBP (CBP_{KIX}). The NMR studies reveal that p65_{TA1} transitions from a partially helical conformation in the free form to an 11-residues α-helix when bound to Tb1_{PH} and CBP_{KIX}. Intermolecular NOEs were used to help define the binding interface and combined with ITC studies the structure shows that both complexes are stabilized by hydrophobic and ionic interactions.

The binding interface notably involves three hydrophobic amino acids located within a characteristic ΦXXΦΦ motif found in almost all acidic TADs. In particular, Phe542 of p65 makes crucial contacts with both Tb1_{PH} and CBP_{KIX}. In addition, we show that these three residues are important to activate transcription in a yeast model system. Taken together, the results provide detail information on how the p65_{TA1} bind to two transcriptional regulatory factors and suggest the key role played by the three Φ residues in regulating a number of genes activated by NF-κB.

Figure 1: (A) NMR structure of the complex between p65_{TA1} (in green) and the Tb1 subunit of TFIIH (in blue). (B): The three hydrophobic residues from the ΦXXΦΦ motif of p65 are involved in crucial interactions in the binding pocket. (C) HADDOCK model of the complex between p65_{TA1} (in green) and CBP_{KIX} (in grey).

Photochromic Molecules and Activity Switching of a Beta-Helical Antifreeze Protein

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Antifreeze proteins depress the freezing point of water and thus enable several bacteria, insects, fish, plant and fungi to survive at subzero temperatures. This unique activity is of great interest in several medical or food technical applications such as organ storage, cryosurgery, or frozen food production. The tertiary structure of antifreeze proteins is closely correlated to their activity. All antifreeze proteins, though different in tertiary structure, contain a flat ice-binding surface for interacting with ice crystals. The activity of the proteins depends on the existence of this surface, i.e. the correct fold of the protein.

The concept of switching the activity of a protein on and off by using an external stimulus like light has been well established in biochemistry. The basic principle is the covalent linkage of a protein with a photoswitchable unit. Here we present the synthesis of several photochromic molecules with improved rigidity, water solubility, and crosslinking reaction yields. The half-life times and ratios of their two conformations have been determined by NMR- and/or UV/vis-Spectroscopy.

Initial results of crosslinking such a photochrome with an antifreeze protein are presented. The chosen protein from Lolium perenne (LpAFP) possesses a β-helical fold, in contrast to most applications to date that utilize α-helical peptides.
Redox control of the human iron-sulfur repair protein MitoNEET activity via its iron-sulfur cluster.

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Human mitoNEET (mNT), the first identified Fe-S protein of the mammalian outer mitochondrial membrane (OMM), is composed of a 32 aa membrane anchoring N-terminus and a C-terminal cytosol-exposed folded domain. This domain dimerizes and each monomer binds one [2Fe-2S] cluster through three cysteines and one histidine. The lability of the cluster greatly depends on its redox state and on pH. Recently¹, we have proposed that mNT plays a specific role in Fe-S cluster repair of the cytosolic iron regulatory protein-1 (IRP-1), a key regulator of cellular iron homeostasis in mammalian cells. Interestingly, mNT is able to transfer its cluster in vitro to human IRP-1 but also to (cyano)bacterial apoferrredoxin. Here, we deciphered the mechanism by which mNT triggers in vitro its Fe-S repair capacity using complementary approaches including NMR, UV-visible, Mössbauer and Resonance Raman spectroscopies.

We determined² the respective roles of the redox state of the mNT cluster and the presence of dioxygen both in cluster transfer and protein stability. We show that only the oxidized holo-mNT cluster triggers cluster transfer to a generic acceptor protein and that dioxygen has no effect on the cluster transfer reaction. In the absence of apo-acceptors, a large fraction of the oxidized holo-mNT form is converted back to reduced holo-mNT under low oxygen tension. By contrast, reduced holo-mNT, which holds a [2Fe-2S]¹ with a global protein fold similar to oxidized holo-mNT, is resistant in losing its cluster or in transferring it. Our findings thus demonstrate that mNT uses an iron-based redox switch mechanism to regulate the transfer of its cluster. The oxidized state is the "active state" which initiates Fe-S transfer independently of dioxygen, whereas the reduced state is a "dormant form". We propose that the redox-sensing function of mNT is a key component of the cellular adaptive response to help stress-sensitive Fe-S proteins recover from oxidative injury.

Structural Characterization of Protein Kinase ASK1 and Its Interaction with Reduced Thioredoxin

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase (MAPK) cascade, which is one of the stress-activated intracellular signaling pathways in eukaryotes. The activity of ASK1 depends on various stimuli, including oxidative stress (presence of ROS), endoplasmic reticulum (ER) stress, presence of lipopolysaccharide molecules (LPS) and cytokines (tumor necrosis factors TNFα) or influx of calcium ions. The regulation of ASK1 has a strong influence in pathogenesis of several diseases, the excessive activation of human ASK1 or failure in the control of its function are associated with cardiovascular diseases, inflammatory and infectious diseases, neurodegenerative disorders, tumorigenesis, asthma and diabetes.

The activity of ASK1 is regulated by its interaction with several proteins, the attention is focused on two physiological inhibitors, mammalian thioredoxin (TRX) and the 14-3-3 protein [1]. ASK1 in its inactive form is inhibited by complex formation with TRX and 14-3-3, however the explicit mechanism of this interaction is unclear due to the absence of structural data. Therefore, the aim of this study was the structural characterization of the TRX binding domain of human ASK1 (ASK1-TBD) and ASK1-TBD:TRX complex formation.

We have previously shown that ASK1-TBD is a compact rigid and monomeric domain with slightly asymmetric shape that under reducing conditions forms with TRX well defined and stable complex with 1:1 molar stoichiometry through a large binding interface. We have also showed that the complex formation does not involve the formation of intermolecular disulfide bonds, nor it is inducing any dramatic conformational change [2].

In this study we present structural insight of the regulation of ASK1 using Small-angle x-ray scattering (SAXS) and NMR spectroscopy studies of the ASK1-TBD alone and its complex with TRX.


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KRAS promoter oligonucleotide forms a dimeric G-quadruplex structure

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Point mutations in ras proto-oncogenes lead to uncontrolled cell proliferation and cause malignant transformations in human cells. The human KRAS proto-oncogene contains a G-rich nuclease hypersensitive element (NHE) upstream of the major transcription initiation site, which is believed to form non-B-DNA structures, specifically G-quadruplexes. A decoy strategy, using stable KRAS promoter analogues, has been devised in order to sequester the transcription factor MAZ and prevent it from activating KRAS transcription. Xudo and Pedersen et al. found that a 32-nt fragment of the KRAS promoter, designated as 32R-3n, folds into a G-quadruplex structure. Furthermore, twisted intercalating nucleic acid (TINA) modified 32R-3n reduces tumor growth in mice carrying a pancreatic cancer xenograft.

We have determined the folding topology of 32R-3n using NMR, CD and UV spectroscopy. Experiments with truncated oligonucleotides have shown that 32R-3n readily dimerizes and forms two individual dimeric G-quadruplex units. The G-quadruplex units formed at 5'- and 3'-ends of 32R-3n (5q and 3q, respectively) have been extensively characterized. 5q folds into an antiparallel dimeric fold-back head-to-head topology with three G-quartets and edge-type loops. 3q also adopts a dimeric fold-back head-to-head topology with edge-type loops, however, its core consists of two G-quartets flanked on one side by two G:C base pairs. Additionally, two inter-strand G:G base pairs are formed in the loop region. Interestingly, some structural elements within the loop region and adjacent G-quartet of 3q are melted at higher temperatures.

The GGGAGG MAZ binding sequence is located in the loop region of the 3q G-quadruplex unit. NMR data shows that the 3q loop region within 32R-3n is dynamic and involved in a conformational equilibrium, which could be important for its availability for binding to MAZ. This could allow for a sequence based recognition of the G-rich motif. Additionally, the two stable G-quadruplex structures could serve as a stability enhancer and protect both ends of 32R-3n. While the dimeric G-quadruplex structures are not possible within a single G-rich strand of genomic KRAS NHE, molecular crowding conditions could favor a monomeric topology including additional nucleotides flanking the 5'- or 3'-ends of the 32R-3n sequence.
Interactions of a fungal lytic polysaccharide monooxygenase with β-glucan substrates and cellubiose dehydrogenase

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Lytic polysaccharide monooxygenases (LPMOs) are key components of enzymatic biomass (e.g. chitin and cellulose) degradation processes. LPMOs catalyze a reaction cycle that requires copper, molecular oxygen and an electron donor, such as cellubiose dehydrogenase (CDH). Docking models have suggested that residues on the surface of the LPMO, near the copper site, interact with the cytochrome domain of CDH during electron transfer. However, experimental data that could clarify interactions of AA9 LPMOs with their substrates and CDH are lacking.

In this study, we have used NMR and ITC we to study the interactions between a fungal LPMO and three soluble substrates and CDH. In addition, we have analyzed the structure and dynamic features of the LPMO in solution. The results reveal potential adaptations of the LPMO surface to varying substrates and unambiguously show that both the substrate and CDH bind to a patch that is focused around the copper-site. In addition to providing insights into enzyme-substrate interactions in LPMOs, the present observations shed new light on possible mechanisms for electron supply during LPMO action.

**Figure 1.** Left: The outer sphere of 12 Å radius represents the region in which $^1$H signals are expected to be affected by Cu(II) PRE, while the inner sphere of 6 Å radius represents the expected effect on 13C signals. Right: Residues with vanished $^{15}$N-HSQC signals upon addition of 50 μM Cu(II) to 1 mM $^{15}$N-labeled LPMO are colored blue on the structure.
Human cystatin C and HCC3 antibody interactions

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Human cystatin C (hCC) is a small protein belonging to the family of papain-like cystein proteinases. It takes part in several different processes that lead to amyloid formation, which is associated with a number of neurodegenerative diseases that affect the independence and quality of life during aging. Human cystatin C (hCC) is one of the second wave of proteins that have been found to undergo amyloidosis associated with disease among which we find specific forms of cerebral amyloid angiopathy and male fertility disorders. hCC is it is widely studied in both experimental and theoretical laboratories. Studies show that the occurrence of hCC associated diseases correlates with its dimerization, which then leads to amyloid formation.

In our study we want to determine how hCC interacts with HCC3 mouse monoclonal antibodies. The increase of knowledge about these interactions may have a great impact on further development of methods for blocking hCC dimerization. The interactions were registered by NMR techniques. The ¹H, ¹³C and ¹⁵N assignments of hCC have been determined using standard NMR protocols. From this assignment, we will attempt to follow titrations of hCC with the antibody using HSQC spectra of the protein to map the HCC3 antibody binding site.
Screening of membrane mimetics and NMR backbone assignment of isolated VSD of human Kv2.1 channel

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Voltage-gated potassium channels (Kv) play crucial role in functioning of cardiovascular, nervous and muscular systems of multicellular organisms. Despite their great importance, aspects of structural organization and mechanisms of voltage-dependent activation of Kv channels are insufficiently studied. Four symmetrically arranged $\alpha$-subunits comprise the main functional group of a Kv channel. Each of four $\alpha$-subunits contains six transmembrane helices that make up voltage-sensing domain (VSD, S1-S4 helices) and pore domain (PD, S5-S6 helices).

Here we present a study of isolated VSD of human Kv2.1 channel, which is the main potassium channel in human brain. Also, it takes part in glucose-dependent insulin secretion in pancreatic $\beta$-cells and in the process of apoptosis as both initiator and propagator. These particular features make Kv2.1 interesting as a potential drug target. It is known that blockers of Kv2.1 channels can enhance insulin secretion and reduce damage caused by a stroke or ischemia. Development of subtype-specific drugs requires atomic resolution structure of the individual domains making up the channel.

The milligram quantities of totally labeled $^{15}\text{N}$- and $^{13}\text{C}/^{15}\text{N}$-VSD samples were produced by cell-free expression using S30 extracts of \textit{E.coli} cells. To find optimal conditions for NMR study the following membrane-mimicking media were screened: micelles of zwitterionic and anionic detergents (FOS\textsubscript{10}, DPC (FOS\textsubscript{12}), FOS\textsubscript{14}, LDAO, LPPG, DC\textsubscript{7}PC); mixed DPC/LDAO micelles at 1:1, 1:2, 2:1 ratios; and DHPC/DMPC, Chaps/DMPC, Chaps/DMPG bicelles. The pH of the samples was varied in the 5.0 – 7.0 range. The best quality spectra were obtained for VSD solubilized in 1:1 mixture of DPC/LDAO detergents at pH 5.0. The VSD/DPC/LDAO sample was stable for longer than one-week period. The VSD samples in DC\textsubscript{7}PC micelles and bicelles were unstable.

Almost 50\% of VSD backbone assignment was obtained in the DPC/LDAO mixture using a set of 3D triple-resonance NMR experiments. The observed line-broadening for the subset of $^1\text{H}-^{15}\text{N}$ resonances revealed the presence of $\mu$s-ms motions in the four helical VSD bundle.

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Study of G-quadruplex in 5'-untranslated region of the mRNA of the human NRAS proto-oncogene

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Naturally occurring RNA guanine-quadruplex (G-quadruplex) sequences have a large diversity of loop lengths and arrangements, nevertheless, all possess remarkably thermodynamically stable structures, and this appears to facilitate their role as robust, but mechanically challenging, regulatory elements for translation. Being devoid of the competitive constraints from the complementary strand (as in the case of DNA) and majorly cytoplasmic location in the cell, RNA G-quadruplexes may be more relevant in the context of structure based gene regulation. Additionally, they are regarded not just as molecular switches in regulating gene expression, they also seem to hold great promise in therapy, both as therapeutic targets as well as therapeutic agents themselves, and can act as tunable devices depending on cellular conditions.

The aim of our work is to obtain information about RNA G-quadruplex structure in NRAS mRNA using NMR, CD and UV spectroscopies. NRAS proto-oncogene encodes for protein called N-Ras, which is involved in regulating cell division through a process known as signal transduction. Mutations of this gene are present in malignant melanoma, the most dangerous form of skin cancer, and lead to over-activation of gene. RNA G-quadruplex forming sequence is present in 5'-untranslated region of NRAS mRNA and is known that stops translation (1). Modified RNA G-quadruplexes were prepared with different G-tract and loop lengths in order to stabilize the two most probable structures.

Reference:
Mapping Interactions of Filamin and Migfilin: Mechanism of Inside-Out Signaling Integrins

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Integrin is a transmembrane heterodimer receptor that facilitates the connection of a cell to its environment. Integrin receptors are able to bind to both the extracellular environment as well as the internal cytoskeleton of the cell. Filamin A and Talin 1 are negative and positive regulators of integrin respectively. They exert their regulatory effects by binding to the tail of beta integrins. Migfilin is a negative regulator of filamin A activity and binds to filamin A at the same site where integrin beta tail binds. Throughout the recent years, these integrin tail regulators have been the interest of research and in this study we attempt to characterize some of the binding interactions of these proteins. Filamin A immunoglobulin domain 21 (IgFLNa21) and talin1 F3 domain were expressed and purified. 3D NMR experiments were carried out with IgFLNa21 for heteronuclear single quantum coherence (HSQC) spectrum assignment. The secondary structure of IgFLNa21 was predicted using the values of random coil and IgFLNa21 alpha carbon and beta carbon values. HSQC titrations were carried out for IgFLNa21 and talin1 F3 with migfilin derived peptides and integrin beta-2 peptide respectively. The titrations revealed strong and weak binding affinity of migfilin and integrin beta-2 peptides for their proteins respectively. Collectively, these results set in the foundation for further studies of the two proteins.
New Approach for SDSL of Long Natural RNAs Exemplified with Hepatitis C Virus RNA Internal Ribosome Entry Site

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Nanoscale distance measurements by pulse dipolar EPR spectroscopy are increasingly applied for gaining new insights into the structure and dynamics of complex biopolymers. EPR detection requires SDSL of biomolecules, which is therefore an essential integral part of these studies. Recently, we developed promising approach to SDSL of RNAs, which is based on the complementary-addressed reaction between target RNA residue and a derivative of oligodeoxyribonucleotide [1]. Contrary to other methods, novel approach is generally applicable to RNAs of arbitrary size. In this work we apply this approach to SDSL of Hepatitis C Virus (HCV) RNA Internal Ribosome Entry Site (IRES) consisting of up to 350 nucleotides and having a complicated spatial structure, and thereby for the first time clearly demonstrate the SDSL of long structured RNA [2]. Nitrooxide spin labels were attached at two definite nucleotide positions of HCV IRES domain II, as was confirmed by room-temperature continuous wave EPR. Furthermore, double spin labeling of HCV RNA IRES allowed application of pulsed DEER and obtaining reasonable spin-spin distance distribution, which agrees well with the results of MD calculations. Thus, novel complementary-addressed SDSL approach in conjunction with EPR and MD allows structural studies of long natural RNAs with nanometer resolution and can be applied to systems of biological and biomedical significance.

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Figure 1. Distance measurements on dsRNA. (a) Background-corrected Q-band DEER/PELDOR time trace (exp) and DeerAnalysis fitting (fit); (b) obtained distance distribution using Tikhonov regularization parameter 1000 (DEER exp) and calculated MD distribution (MD calc); (c) Typical conformations of spin labels corresponding to the selected ranges of distances (highlighted by colored bars in (b) and pointed out by corresponding arrows). Red circles indicate the NO group of the label, for clarity. Spin label attached to C83 (top) is shown in blue, spin label attached to A73 (middle) is shown in green.
NMR interaction studies of Aβ1-42 with pyroglutamated Aβpy3-42

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Amyloid peptides, mainly Aβ1-40 and Aβ1-42, are the main actors of Alzheimer’s disease (AD). They are the major constituents of the extracellular plaques found in brains of AD patients and are present as extremely toxic soluble oligomers (Aβo), in the early stage of the fibrilization process (Nussbaum, 2012). The N-terminus truncated Aβ peptides show an higher aggregation propensity than full-length peptides and it has been proposed that they initiate amyloid plaque formation (rNussbaum, 2012). The 15-20% of the total truncated Aβ peptide bears a pyroglutamate residue at the N-terminus (here Aβpy3-42).

In order to investigate the interaction between Aβ1-42 and Aβpy3-42 we designed and performed a series of NMR experiments: 1) NMR analysis of Aβ1-42 self assembling as a function of concentration; 2) titration experiments performed at constant concentration of 15N Aβ1-42 in the presence of increasing amounts of Aβpy3-42; 3) titration experiments performed at constant total peptide concentration (15N Aβ1-42 + Aβpy3-42) varying, at each point, the concentration of each peptide. Aggregation kinetics was also followed for all the samples by NMR. The results allow to elucidate the complex mechanism of the proposed interaction.

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Structure elucidation of capsular polysaccharides from the Streptococcus pneumoniae serotypes within serogroup 7

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The cell-surface capsular polysaccharides of Streptococcus pneumoniae are to a large degree responsible for the virulence of the disease as it masks the pathogen from recognition by the host immune system.

More than 90 serotypes exist and while many of them have been characterized in the last few decades, some are still unknown structures and there are still new serotypes being identified in patients. As a result of this, the structures of these polysaccharides are of great interest as they can increase the understanding of the cross-reactivity of the different serotypes. Herein the focus have mainly been on the capsular polysaccharides belonging to serogroup 7 in the Danish classification system. An example of a repeating unit for one of these polysaccharides can be seen in figure 1.

We have elucidated several polysaccharide structures from the serogroup 7 series by the extensive use of high quality well-established 2D NMR experiments obtained at high field utilizing cryogenically cooled probes to perform detailed structural analysis. By careful analysis of the full NMR data sets it is possible distinguish between the very similar groups found in the capsular polysaccharides. This includes using proton, carbon and phosphor NMR spectroscopy as well as NMR prediction software to fully assign the structures of these polysaccharides.

Figure 1. Example of repeating unit structure
Automatic resonance assignment of intrinsically disordered proteins using advanced methods of amino acid recognition

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NMR is the primary method of studying intrinsically disordered proteins (IDPs) [1]. However, resonance assignment is in this case a more demanding task than for folded proteins. The particularly narrow chemical shift range (caused by the fast molecular motion of the protein and high incidence of sequential repeats) often makes the routinely-used 3D spectra insufficient. On the other hand, the low relaxation rates (also caused by the fast molecular motion) make spectra of high dimensionality feasible. The TSAR program [2] performs automatic resonance assignment basing on 2D cross-sections of such high-dimensional spectra, obtained with sparse multidimensional Fourier transform (SMFT) [3].

Here we present a new version of the TSAR program [4], that is able to utilize some advanced methods of amino acid recognition, facilitating mapping of the cross-sections chains onto the protein primary sequence. In particular, we demonstrate how the automatic procedure can be improved by incorporating information from amino acid-selective experiments [5] and information on chemical shifts typical for IDPs [6]. The approach was tested in simulations on 16 disordered proteins and experimentally on α-synuclein, with remarkably good results.

Systematic Incorporation of C2’-Modified Analogs into a DNA G-Quadruplex

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Compared to other DNA secondary structures the G-quadruplex (G4) exhibits an impressive structural variability. Its core is formed by at least two stacked tetrads composed of four guanines arranged in a plane and connected via Hoogsteen hydrogen bonds. It is this basic scaffold that can already vary in terms of molecularity, polarity of stacked tetrads, direction of G-tracks or type of cation coordinated in the central channel. Additionally, insertions like bulges, loops and termini extend its accessible structural diversity.

Depending on particular conditions a quadruplex-forming sequence can adopt various topologies. However, the reliable prediction of the favored conformation is often difficult due to the complex superposition of different driving forces. We systematically incorporated modified nucleotide analogs to induce small structural perturbations and analyzed effects via NMR spectroscopy. The native DNA sequence folds into a (3+1)-hybrid G4 consisting of three parallel and one antiparallel G-tract [1]. Both types of glycosidic torsion angles are present, therefore anti-favoring 2’-fluoro-2-deoxyribo-(FG) and riboguanosine (rG) were used as perturbing dG analogs.

Substitution of residues in anti-conformation conserves the native structure in most cases. However, their impact on adjacent nucleotides differ depending on position and sugar pucker of the analog. On the other hand, the incorporation of rG or FG for syn-conformers can easily induce a reversal of all glycosidic torsion angles within a tetrad while maintaining the global fold [2]. As a result of this unexpected alternative to a complete refolding, a new type of G4 featuring only all-anti and all-syn G-tracks is observed.

References

Changing the Tetrad Polarity in a Parallel Monomolecular Quadruplex

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DNA can adopt a variety of secondary structures. Thus, guanine-rich sequences are known to fold into quadruplex structures consisting of G-tetrads instead of base pairs. The four guanine bases in these tetrads are connected via Hoogsteen hydrogen bonds. Quadruplexes show great diversity in strand orientation and base configuration depending upon sequence and conditions. In parallel structures guanine bases prefer the \textit{anti} configuration. However, studies on tetramolecular quadruplexes have shown that suitable base modification may result in \textit{syn} guanines with an associated change in tetrad polarity\textsuperscript{1}.

Introduction of 8-bromoguanosine into the 5'-terminal tetrad of the well-defined \textit{c-myc} quadruplex\textsuperscript{2} derived from the promoter-sequence of the \textit{c-myc} oncogene yields two coexisting species. Through two-dimensional NMR experiments the first species was shown to adopt the same fold as the native sequence with all guanine bases being in an \textit{anti} configuration. The second species could be identified as a novel monomolecular quadruplex with parallel G-tracts but with an all-\textit{syn} tetrad as a result of a 5'-tetrad flip. EASY-ROESY-experiments reveal both species to be in slow exchange at 50 °C. A decrease in temperature shifts the equilibrium towards the native fold.

\textbf{Figure 1:} H8/H6-H1' NOESY spectral region of \textit{c-myc} quadruplex with 8-bromoguanosine at three 5'-terminal positions reveals two possible folds; green and grey rectangles represent \textit{anti} and \textit{syn} guanosine nucleotides, respectively.
Functional amyloid fibrils controlling cell death investigated by solid-state NMR

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Protein misfolding into amyloid fibrils is intimately associated with a number of neurogenerative diseases, including Alzheimer’s, Parkinson’s and type II diabetes. It has recently been discovered that several proteins fold into amyloid fibrils to play a fundamental role in biological processes; these fibrils are therefore termed functional amyloids. We have shown recently that a functional amyloid is involved in signal transmission in fungal cell-death [1] and we could further establish an evolutionary relation between the fungal and mammalian cell-death control [2]. To understand amyloid functional formation and signal transmission, it is essential to determine the atomic structural details. We have therefore set up the production of isotope labeled fungal functional amyloids [3] and our solid-state NMR data reveal well-ordered amyloid fibrils of two distinct functional amyloids. Using different solid-state NMR approaches, we can identify rigid amyloid core regions and flexible parts in the functional amyloid fibrils.

The ABC exporter MsbA studies by MAS-NMR

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MsbA is a 584 amino acid E. coli ABC exporter, functional as a homodimer of 130 kDa. In vivo and in vitro data suggest its physiological function as lipid A flippase (Doerrler & Raetz, FASEB J 2002) but binding and transport of hydrophobic drugs has also been shown (Eckford & Sharom, JBC 2008). Its structure resembles Sav1866 and has been shown to have overlapping substrate specificities with Pgp and LmrA. MsbA serves as a model ABC exporter on which questions relevant to all ABC transporters can be studied. These involve elucidating the catalytic cycle, its link to substrate transport and details of substrate recognition and translocation. We have established sample preparation procedures by which proteoliposomes containing isotope-labelled MsbA suitable for solid-state NMR can be prepared (Kaur et al. Biol Chem 2015). The application of time-resolved solid-state NMR provided new insight into the catalytic activity of MsbA and related ABC exporters. We present for kinase activity in a prokaryotic E. coli ABC transporter MsbA using solid state NMR. Exploring the possibility of MAS-NMR for probing the structure and dynamics of the full-length transporter within proteoliposomes, we show in detail analysis of the coupled, kinase and ATPase, reactions occurring in reconstituted MsbA.
Optimising pulse sequence design for high-dimensionality and high-resolution protein experiments

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The recent advances in non-uniform sampling and novel methods of spectral reconstruction\cite{Stanek2012} in NMR have enabled the acquisition of spectra of both high dimensionality and outstanding resolution in individual dimensions\cite{Nowakowski2015}. Performing such experiments using pulse sequences that are simple extensions of lower dimensionality techniques offsets these advantages by lower sensitivity, limiting their applicability. Here we show that the sensitivity-enhancement procedures based upon the idea of coherence-order selective coherence transfer\cite{Palmer1991} can be successfully applied to transfers between two indirect dimensions recovering a large part of the sensitivity lost by the increase in dimensionality. At the same time, the high resolution can be used to utilize two magnetisation transfer pathways (for example forwards/backwards) in some experiments, with their co-addition yielding improved sensitivity. The utility of the two optimisation strategies is illustrated using 3 and 4D HN-NH type protein backbone assignment experiments and N, N-edited NOESY performed on a sample of the ubiquitin protein.

References


Diversity of G-quadruplexes formed by DNA oligonucleotide with ALS and FTD related GGGGCC repeat

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Guanine rich nucleic acids can in the presence of cations fold into non-canonical four stranded structures called G-quadruplexes. A prolonged expansion of GGGGCC repeat within the non-coding region of C9orf72 gene has been identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), which are devastating neurodegenerative disorders. Formation of unusual structures within the expanded GGGGCC repeat, including DNA and RNA G-quadruplexes and R-loops was proposed to drive ALS and FTD pathogenesis. Initial screening of DNA oligonucleotides containing four GGGGCC units indicated their folding into multiple G-quadruplexes in the presence of K⁺ ions. Oligonucleotide d[(G₄C₂)₃G₄] displayed the most favorable ¹H NMR spectra adopting two predominant antiparallel G-quadruplex structures with some guanine residues in syn conformation. To push the equilibrium towards a single structure, we designed the sequence d[(G₄C₂)₃GGBrGG] with a single dG to 8Br-dG substitution at position that was expected to adopt a syn conformation in only one of the predicted topologies. Surprisingly, dG to 8Br-dG substitution did not select between the two predominant structures indicating G21 adopts a syn conformation in both forms. However, ratio between the two structures could be influenced by folding conditions. Two extreme conditions were identified at which either form 1 or form 2 was populated in sufficient excess to enable its structural characterization with NMR. Oligonucleotide d[(G₄C₂)₃GGBrGG] folds into two very stable unimolecular structures with similar topologies composed of four G-quartets and antiparallel orientation of strands. G-quartets in both forms are connected by three lateral (l) loops which have a clockwise (+l+l+l) progression in form 1 and anticlockwise (-l-l-l) progression in form 2.

Figure 1. Folding of d[(G₄C₂)₃GGBrGG] into two distinct topologies.
A high intake of berries is considered healthy, and epidemiological studies have shown health benefits of polyphenols in regard to cardiovascular disease, diabetes mellitus and cancer. These beneficial effects are often ascribed to their antioxidant properties. However, there is no evidence that a high intake of pure antioxidants promotes health, and the mechanism behind their bioactivity remains unclear. Thus, the aim of this study was to examine how interactions between berry components and cell membranes could potentially contribute to explain the health benefits of berries. Berry samples from a large variety of berries were collected and samples prepared through dual-phase extraction procedure in which the methanol-water phase was used in this study.

Different NMR methods were applied to characterize the perturbations of the phospholipid headgroup of cell membranes by measuring the $^{31}$P chemical shift. In a DPC (dodecylphosphocholine) micelle model system, addition of wild bilberry extract resulted in changes in the $^{31}$P chemical shift of the DPC micelles, which was not found for the other berry types. Mechanically aligned samples of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) with the addition of berry extracts were studied with oriented 31P Solid-State NMR in a flat-coil NMR probe, which also revealed wild bilberry extract as the most significant modulator. Further experiments involving static and MAS $^{31}$P NMR experiments with POPC multilamellar vesicles (MLVs) also revealed major changes with wild bilberry extract. It is difficult to explain these major changes by addition of wild bilberry extract to changes of the axial rotational motional averaging of the lipids, but more likely it is due to alterations of the vesicle composition in the samples. Supporting the NMR findings, dynamic biophysical measurements of the membrane fluidity in liposomes and jurkat cells showed that wild bilberry was one of the most potent berry extracts in decreasing membrane fluidity at both the surface level and in the hydrophobic core of the membrane. Overall, wild bilberry extract has a strong perturbation of cell membranes, which suggests that intake of wild bilberry could potentially have the most health beneficial effect compared to other berry types. Thus, more studies are needed to investigate the mechanism behind the bioactivity of wild bilberry and its health effects.
Exploiting hydrophobicity for efficient production of transmembrane helices for structure determination by NMR spectroscopy

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The high biological and pharmaceutical relevance of membrane proteins is currently poorly translated into the PDB repository. One important type of membrane protein is the single-pass transmembrane receptors (SPTMRs), of which approximately 1300 have been identified in the human genome. Despite the known importance of the single-pass transmembrane domains (TMDs) of SPTMRs, less than 2% of these domains have currently been structurally characterized [1].

One of the major obstacles for initiating structural studies of membrane proteins by NMR spectroscopy is the generation of high amounts of isotope-labeled protein. We have exploited the hydrophobic nature of the membrane proteins to develop a simple and efficient production scheme for isotope-labeled single-pass TMDs with or without intrinsically disordered regions [2]. We have evaluated the applicability and limitations of the strategy using seven TMD variants that differ in their overall hydrophobicity and length, and show a recovery for suitable variants of >70%-90%. The production scheme has been successfully applied to obtain NMR samples of sufficient quality to solve the structure of the TMD of the human prolactin receptor (hPRLR) in DHPC micelles [3]. The developed production scheme is cost-efficient and easy to implement, and has the potential to facilitate an increase in the number of structures of single-pass TMDs, which are difficult to solve by other means.

References


The Growth hormone Receptor (GHR) is a member of the Class I Cytokine Receptor family. As single pass transmembrane receptors it comprises three different domains, an extracellular domain (ECD), a cell membrane spanning helix constituting the transmembrane domain (TMD) and an intracellular domain (ICD), Figure 1. The TMD is not only responsible for anchoring the receptor in the lipid bilayer, but also to drive and mediate homodimerization. Growth hormone (GH) binds extracellularly to the GHR forming a 1:2 complex activating Janus Kinases 2 (JAK2) bound to the ICDs. Despite that these receptors have been studied for decades it remains an enigma how the signal of the extracellular binding event is translated into the cell. It has been suggested that the TMDs rearrange upon receptor activation, but neither is the TMD dimer conformation of the inactive nor active state in the membrane known.

We sought to investigate the structure and membrane interaction of the GHR TMD and ICD using NMR. Using our new method for TMD purification (1) we successfully expressed and purified the isotopically labelled GHR TMD in sufficient amounts for NMR investigations. From a broad detergent screen, DHPC detergent micelles allowed the NMR chemical shifts of the TMD protein backbone to be assigned. Consecutive secondary structure analysis of the backbone data confirmed the expected α-helical fold of the domain in DHPC micelles. Former chemical shift analysis of the isolated GHR ICD indicated that the nine residues stretching from the TMD to the JAK2 binding site are intrinsically disordered as the full GHR ICD (2). Thus the question arises how the mechanical force of a hormone induced ECD and TMD rearrangement is translated to the JAK2 binding site. However, interestingly lipid-binding sites in the GHR ICD juxtamembrane region have been proposed (2) providing the required stability for a rigid body movement. In the present work we will present data that address the dimerization of the TMD and how lipid interactions affect this process as well as receptor signalling.

Human cystatin C V57G mutant – nuclear magnetic resonance studies

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Human cystatin C (hCC), member of the superfamily of papain-like cysteine protease inhibitors, is the most widespread cystatin in mammalian body fluids. It is a small protein, involved in various diseases, including cerebral amyloid angiopathy, cerebral hemorrhage, stroke, and dementia [1]. In pathological conditions human cystatin C participates in the formation of amyloid deposits together with the amyloid β peptide (Aβ), particularly in elderly individuals and in patients suffering from Alzheimer’s disease or Down’s syndrome [2]. Under physiological conditions hCC is a monomer but attempts to crystallize allowed receiving only the dimeric form [3], formed as a result of the three-dimensional exchange domains (3D domain swapping) [4]. While the experimental structure of wild-type hCC occurs as a dimer, the monomeric crystal structure is known for two hCC variants: V57N hCC and stab1 hCC. The first hCC mutant is stable in monomeric form and crystallizes as a monomer [5]. In our group we have designed and performed biophysical investigations for two groups of hCC mutants: one located in loop L1 [5,6] (residues 56-57) and second introduced to the hydrophobic core of the molecule (residue 68) [7]. In this study we selected V57G mutant to our NMR investigations as the most promising to give a monomeric form of hCC in solution.

120 amino acid residues long hCC V57G mutant (13 kDa) was expressed in E.coli and purified in 13C,15N-double labeled form. Analysis of acquired heteronuclear NMR data sets provide to assignments of 1H, 13C, and 15N backbone resonances for 90 residues. Evaluated chemical shifts were used as input for TALOS+ and CS23D programs to extract information about 3D structure of hCC V57G protein. Further structural analysis will be presented on a poster.

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Unusual structure and dynamics of the RYMV-encoded Viral Suppressor of RNA silencing P1

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Rice is central in the diet of many people in Africa and Asia. Rapid population growth and food habit changes result in an increasing demand, while rice is particularly prone to pathogens attacks. Among these is the yellow mottle virus (RYMV: Rice Yellow Mottle Virus), that possesses a worrisome epidemiological profile, and generates crop losses from 20 to 100%. It encodes the suppressor of RNA silencing P1 (1), a multi-functional protein that allows, among other, to bypass the RNA "antiviral silencing", an essential mechanism of defense in the plant (2). The P1 protein has many cysteines and histidines but the topology of the putative zinc finger was unknown and impossible to deduce the primary sequence. Labor intensive production of truncated proteins and their NMR analysis identified two fragments that are folded and relatively stable over time in their reduced forms, which initiated their joint study by crystallography and NMR. The isolated showed a structure composed of CCCC and HCHC zinc fingers respectively, the latter representing a new type of zinc finger. NMR on the whole protein revealed a conservation of these folds in the intact protein. In addition, the linker adopts an helical fold, but allows a certain mobility of the two domains relative to each other, that was quantified by ¹⁵N relaxation and D_{HN} RDCs measurements.


(2) Pumplin N. et Voinnet O. Nature Reviews Microbiology 11, 745-60 (2013)

Figure 1. Relative independence of P1 zinc fingers as seen by RDC measurements.
A Structural and Thermodynamic Investigation of the DNA-binding Tet1-CXXC domain

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The Ten Eleven Translocation (Tet) proteins is a group of demethylases capable of initiating the process of active demethylation of cytosine bases in DNA. The removal of this epigenetic trait enables the reactivation of previously silenced genes. This implicates the Tet proteins in diverse processes from stem cell differentiation to tumorigenesis. However, while the catalytic powers are well described, the method of DNA recognition and binding is not. Tet1 contains a CXXC-style zinc-finger domain which is predicted to interact with DNA.

We have expressed the CXXC domain of human Tet1 to characterize the structural changes and thermodynamics of DNA binding. The solution structure of the Tet1-CXXC domain was solved, and the effect of DNA methylation on the binding specificity has been quantified. The insights gained from the work moves us closer to understanding the role of Tet proteins in both normal cell behavior and tumorigenesis.
Binding of Curcumin and Rosocyanine to Amyloid-beta Oligomers

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One of the well-established methods to detect boron in biological fluids is so called “the curcumin method” from 1960s. Curcumin, a dietary polyphenol, is a natural spice of a yellow color, which forms with boron a stable orthoborate complex of a characteristic pink color. It is also well known that curcumin possesses a spectrum of anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic properties and it also has preventive and therapeutic potential for neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases. Since there are considerable quantities of boron containing compounds in biological fluids, a pertinent question is whether curcumin or rosocyanine, or both have actually all these outstanding medicinal properties.

We have recently reported on the effect of curcumin on lateral diffusion of lipids in saturated and unsaturated bilayers as studied by the 1H NMR diffusometry (Filippov et al., Langmuir, 2014; Mendeleev Comm., 2016): the lateral diffusion coefficients of lipids in phospholipid bilayers are significantly reduced by addition of curcumin. We suggested that the curcumin molecule binds to the lipids, thereby increasing the size of the diffusing entity. A similar study is currently in progress with rosocyanine and phospholipid membranes.

Interestingly, a number of groups have reported on binding of curcumin and curcuminoids to amyloid fibrils of Alzheimer’s Amyloid-beta peptides, though no studies on binding of curcumin and rosocyanine to the most neurotoxic species of Amyloid-beta, oligomers and protofibrils, were yet performed, to the best of our knowledge. One of the most plausible neurotoxicity hypothesis of Amyloid-beta peptides is incorporation of small oligomers of these peptides into phospholipid membranes and formation of channels/pores. Therefore, it is an intriguing topic to be further explored: (i) whether and how curcumin and/or rosocyanine bind to oligomers of Alzheimer’s Amyloid-beta peptides, in particular to a more neurotoxic Abeta(1-42) variant, and (ii) whether and how curcumin and/or rosocyanine block Abeta channels/pores in phospholipid membranes.

In order to answer on the above questions we used ultra-fast MAS 2D and 3D 1H-13C correlation NMR spectroscopy on complexes of selectively 13C labeled both curcumin and rosocyanine with oligomers formed from 13C/15N labelled wild-type Abeta(1-42) and/or cross-linked Abeta(1-42)Cys21-Cys30. The latter peptide is solely forming toxic oligomers and, therefore, it is a very useful system for vaccine and drug development strategies (Lendel et al., Angew. Chem. Int. Ed. 2014).
Coagulation factor VIII and the von Willebrand factor
A vital interaction in the blood stream

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Coagulation factor VIII (FVIII) and von Willebrand factor (VWF) are large proteins (>2000 residues) and together they form a stable complex found in circulation in the blood stream. Upon vascular injury of a blood vessel, both FVIII and VWF are intricately involved in formation of the blood clot and arrest of bleeding, making them essential in haemostasis.

If FVIII is not bound to VWF, it is rapidly degraded. As FVIII is vital in haemostasis, too little or no FVIII at all will make formation of a blood clot inefficient causing prolonged duration of bleeding. The inability to form the VWF:FVIII complex can be caused by mutations in either VWF or FVIII. If the mutations are found in FVIII, the bleeding disorder is called Haemophilia A; if they are found in VWF, the bleeding disorder is known as von Willebrand Disease. Either way, prolonged bleeding is in both diseases caused by the lack of FVIII due to the inability to form a stable VWF:FVIII complex.

Though these two bleeding disorders combined affect more than 1% of the population, not much is known about the structural and molecular features governing formation and stabilisation of the VWF:FVIII complex. To further our understanding of the complex we have used nuclear magnetic resonance (NMR) spectroscopy and isothermal titration calorimetry (ITC).

Two different constructs of the minimal domain of VWF capable of binding FVIII, the D’ domain, were devised: one construct with the native N-terminus, and one with a non-native N-terminus. This allowed us to assess the importance of this very specific area in the binding to FVIII. By NMR spectroscopy, the two constructs of VWF D’ were found to be structurally and dynamically very similar despite their sequential differences. However, in ITC experiments, only the construct with the native N-terminus bound FVIII. Further NMR experiments on the VWF D’:FVIII complex (~170kDa) have revealed that primarily the N-terminal subdomain of D’, the TIL’, is affected upon complex formation with FVIII (figure 1). This leads us to a binding mechanism involving the N-terminal TIL’ subdomain, but omitting the C-terminal subdomain of D’, the E’. Furthermore, the native N-terminal residues were found to be essential for mediating the binding to FVIII.

**Figure 1.** The effect on VWF D’ upon binding to FVIII. 
Left: Overlay of $^{13}$C-$^2$H heteronuclear multiple quantum correlation spectra of U-2H, $^{13}$C, $^{15}$N $^{13}$C-L-L-V VWF. Grey spectrum shows free VWF D’; orange spectrum shows VWF D’ in complex with unlabeled FVIII. Several peaks are seen to disappear upon complex formation. Right: The structure of VWF D’ (pdb 2MHP) with the N- and C-termini indicated. The peaks that are unaffected by complex formation are shown in orange sticks. The major area affected by binding to FVIII is the N-terminal TIL’ domain, indicated by the blue circle.
6 Abstracts for posters in session "Biomolecular polarization and relaxation"
Flavin-linker-Trp model compound for cryptochrome: spin-dynamics and MFE on photochemical reactions

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The remarkable ability of many animals to sense the Earth’s magnetic field and to use its direction for navigation has received significant attention in the last few years.¹ It has been proposed that a light-dependent quantum effect in cryptochrome photoreceptor proteins allows for such an iron-free spin-chemical compass. In cryptochromes and photolyases a striking pattern of three highly conserved tryptophan residues in the PHR domain is interpreted as an electron transfer chain. Figure 1A shows this chain for *Escherichia coli* photolyase [PDB entry 1DNP].² Functional studies require model compounds. A fullerene-based triad model system has already been demonstrated to show magnetic field effects (MFE).³ Here we present a model compound inspired by the cryptochrome structure, a flavin-linker-tryptophan dyad, called F10T⁴ (Figure 1B and 1C). We demonstrate a magnetic-field effect on the photo-chemical yield of the flavin-tryptophan biradical in F10T and present detailed experimental and theoretical studies of its spin dynamics.

Structural Characterization of Lipid Nanoparticles using Dynamic Nuclear Polarization

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Lipid nanoparticles (LNPs) are used as drug carrier systems to achieve cellular internalization of hydrophilic bioactive molecules such as small interfering RNA (siRNA) and messenger RNA (mRNA). (V. Torchilin, Nat. Rev. Drug Discovery, 2005, 4, 145-160.) The structure of LNPs and the mechanism of encapsulation of siRNA or mRNA by LNPs is still unknown. Several propositions for LNPS structures have been made on the basis of modelling or diffraction results, including “onion” type layered structures or “raisin pudding” type structures, but there is currently no consensus.

In this work, we develop and apply dynamic nuclear polarization surface enhanced NMR spectroscopy (DNP SENS) to characterize structural features of LNPs in the presence and absence of siRNA. The LNPs used here are composed of cationic lipid (DLin-MC3-DMA), a phospholipid (DSPC), cholesterol and PEG (DMPE-PEG 2000), also sulphonated-siRNA is used to mimic mRNA. (A. Leung, I. Hafez, S. Baoukina, N. Belliveau, I. Zhigaltsev, E. Afshinmanesh, P. Tieleman, C. Hansen, M. Hope, P. Cullis, J. Phys. Chem. C, 2012, 116, 18440-18450.) We present for the first time the successful application of 1H, 31P and 13C DNP SENS to these systems. By studying the DNP enhancement (ε) as a function of polarization time in terms of a relayed spin diffusion model we are able to determine the architectures of the LNPs. In particular the various chemical components of the LNPs are well resolved in the NMR spectra and we can establish their relative positions with respect to the LNP surface. In addition, slow CP magic angle turning (CPMAT) experiments in the presence and absence of siRNA allow measurement of the chemical shift anisotropy (CSA) of the sulphonated siRNA and the lipid components of the LNP (PEG and DSPC).
Enzyme kinetics of the pentose phosphate pathway (PPP) by Dissolution Dynamic Nuclear Polarisation (D-DNP): preliminary studies

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NMR spectroscopy of substrates at low concentration or with low γ nuclei have benefitted from the recent introduction of hyperpolarized techniques, which aim at enhancing the nuclear spin polarization well beyond the Boltzmann equilibrium, and lead to considerably enhanced sensitivity. Dissolution Dynamic Nuclear Polarization (D-DNP) has proven a very efficient hyperpolarization technique, providing dramatic, up to four or five orders of magnitude, enhancement. It has been applied to a very broad variety of molecules and nuclear spins. As a result, many applications have emerged, among which the study of fast time-varying processes is of particular interest. Several groups have recently demonstrated the possibility to probe metabolic processes both in solution (“in vitro”) and in cell suspensions or living tissues. To date, only a limited number of in vitro D-DNP NMR studies aiming at the investigation of protein-substrate interactions or enzyme kinetics have been performed. These very promising results open new avenues for the investigation of fast kinetic processes, where short experimental times prevent product re-association or enzyme inhibition by reaction products that may skew quantitative measurements.

For instance, glycolysis or the pentose phosphate pathway (PPP) represent crucial metabolic pathways. Therefore kinetic studies of the individual enzymes contributing to the metabolism of glucose are of primary interest for the detailed investigation of these metabolic chains.

In a previous round of investigation with D-DNP, we focused on the kinetic study of Glucose phosphorylation by Hexokinase, which represents the first metabolic step, common to glycolysis and the PPP. Glucose-6-Phosphate (G6P) buildup curves were observed. Experiments analyzed by a simple kinetic model, taking into account the inhibiting action of G6P, through which the relevant kinetic parameters could be extracted. [1]

We have pursued our investigations of the PPP and focused on the next step of the pathway, the dehydrogenation of the G6P performed by the glucose 6 phosphate dehydrogenase (G6PDH). Kinetic experiments using DNP-polarized, uniformly labelled 2H6, 13C7 where both Hexokinase and G6PDH are present in an NMR tube were performed on a 400 MHz spectrometer. The production of the δ-6 Phosphogluconolactone, the reaction product of G6PDH was observed, and analyzed. Various technical and methodological issues raised by these experiments will be discussed.

References

Abstracts for posters in session "Computation and processing"
Developing force fields for the accurate simulation of both ordered and disordered protein states

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A large fraction of eukaryotic proteins are partially or completely disordered under physiological conditions. NMR has emerged as a powerful method for characterizing the residual structure and conformational dynamics of intrinsically disordered proteins (IDPs) at atomic resolution. If sufficiently accurate models are available, Molecular Dynamics (MD) simulations can provide a powerful complimentary tool for studying IDPs and interpreting NMR measurements in these systems.

In this investigation we present an extensive benchmark study to systematically assess the ability of commonly used molecular dynamics force fields to reproduce NMR, SAXS, and FRET data for a number of ordered and disordered proteins. We found that, while the properties of folded proteins are generally well described in simulation, large discrepancies exist between simulation and experiment for disordered proteins. We developed a new water model, TIP4P-D, that better balances electrostatic and dispersion interactions, resulting in significantly improved accuracy in the description of disordered states, but slightly degraded results for ordered proteins. Guided by experimental measurements from folded proteins, fast-folding proteins, weakly structured peptides, and disordered proteins, we are further optimizing force fields to more accurately simulate proteins across the order-to-disorder spectrum.

Figure 1. Systems used to assess and refine the accuracy of molecular dynamics force fields in this investigation.
Spin-Scenario: a flexible library for realistic MR simulation

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Introduction: Numerical simulation of spin dynamics has become a powerful tool for a variety of MR studies, including pulse sequence design and optimization, experimental validation, educational purposes and many others. In this work, we introduce a new package named Spin-Scenario, which is aimed at providing a flexible, intuitive and fast simulation framework that may find applications in both MR imaging and spectroscopy. As the name states, users are able to realize their creative ideas in a manner that like the scenarist, who creates a scenario script, and the spin actors will act accordingly.

Methods: The C++ framework consists of three key layers: computing engine layer, pulse sequence layer and scenario script layer. The kernel calculations inside the computing engine are based on Liouville-von Neumann equation to make it essentially compatible with different MR applications, and moreover, parallel computing techniques including CPU (openMP) and GPU (ArrayFire) acceleration are utilized to satisfy the potential huge spin ensemble. The pulse sequence layer is accomplished via introducing the sequence blocks concept. Basically, a pulse sequence can be always broken down into two-level blocks. Level 1 blocks are atomic blocks of RF pulses, gradients, delays and signal acquisition modules, and level 2 (glue level) blocks are serial or parallel combination of arbitrary components from both levels. The pulse sequence inherently uses tree representation, which serves as low-level interface. The scenario script layer is designed to enable a high degree of user flexibility. As a powerful, embeddable and fast scripting language, Lua has been widely used in many industrial applications, especially games [1]. We extend the library with Lua bindings, which provides a straightforward scenario scripting environment in a natural manner.

Results: Fig. 1 shows a script snippet of gradient echo (GRE) imaging and its result based on the MIDA head model [2], which contains over 22 million spins in a $480 \times 480 \times 480$ cube (0.5 mm spatial resolution). The running time for each phase step was $\sim 70$ seconds with a Tesla K40 card.

Discussion: In this abstract, we demonstrated that the library is able to perform flexible, straightforward and fast MRI simulation. Since we adopt the Liouville equation in the computing engine, it has the potential of performing more complex MRI experiments compared with Bloch equation based simulators. The package is undergoing for format conversion for execution on real hardware and performing more test examples, and will be released on https://github.com/juncy/spin-scenario.

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Acquisition and processing high-resolution 5D NMR spectrum using Sparse Fast Fourier transform

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Rapid development of non-uniform sampled (NUS) NMR experiments enables an acquisition of 4-7 dimensional spectra with high resolution. Processing of such experiments can be challenging because algorithms for spectrum reconstruction from NUS data require heavy computational power and data storage. Recently proposed sparse fast Fourier transform (SFFT) is a non-iterative method for signal collection and processing, which allows reconstructing large size and dimensionality spectrum from extremely sparse data (~0.01-3%)\(^1\). SFFT algorithm combines the projections (based on Fourier projection-slice theorem) and NUS NMR. The “voting” part of the SFFT algorithm uses projections for identification of essential intensities in the spectrum. The remaining frequency points are assumed to be exactly zero and omitted from calculation and data storage. Intensities of non-zero frequencies are obtained with high fidelity by solving system of linear equations. Since in a sparse spectrum the number of both measured and reconstructed points is small, SFFT allows to process high quality NMR spectra of any size and dimensionality.

Here we demonstrate an application of SFFT algorithm for high resolution 4D methyl NOESY and 5D HACA COHN spectrum of Azurin with 64 complex time points in each indirect dimension. For the later spectrum, we recorded 29 unique discrete line projections and additional 5% of randomly sampled NUS points. In total, the data set included 1896 (0.01%) time domain points. Then SFFT algorithm identified 57k out of 1.7*10\(^{11}\) (0.00003%) non-zero points in the frequency domain. Values of intensities of these points were estimated and used for peak picking. The detected cross-peaks perfectly match the expected amount of signals in Azurin. The final data set of sparse 5D spectrum requires less then 10Mb of storage space that is only 0.00003% of its full size.

Our findings show that SFFT is well suited for high-resolution 4-5D spectra for backbone assignment and structure calculation.

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Computational prediction of liquid-state DNP enhancements

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Interest in Overhauser dynamic nuclear polarization (ODNP) has been revived in the last decade due to the ability of the technique to enhance NMR signal intensities in liquids. Although the spin-spin interactions responsible for the ODNP effect have been known for a long time [1], prediction of the enhancement prior to the experiment requires detailed knowledge of the magnitudes of these interactions and their fluctuations in time. In the case of $^1$H nuclear spins, for instance, the polarization enhancement is dominated by the dipole-dipole interaction with the electron spin [1]. The purely geometrical nature of the dipolar interaction makes possible the prediction of proton DNP using analytical models of diffusing spherical molecules [2]. Such analytical modeling is impossible when the scalar (Fermi contact) coupling contributes to the ODNP effect [3].

A computational methodology for predicting the ODNP enhancement due to the dipolar interaction was recently developed and applied to the nitroxide radical TEMPOL in simple liquids like toluene [4], water [5], acetone and DMSO [6]. Here, the methodology is extended such that it treats both the dipolar and scalar interactions [7,8], thus providing access to ODNP of nuclei other than proton. The dynamics of the molecules in the liquid are modeled at two different resolutions: hydrodynamic (Figure 1a) and atomistic (Figure 1b). A third—quantum mechanical—scale is introduced to calculate the electron spin density at the nuclear spin position (Figure 1c). The predictions of the developed approach are shown to be in excellent agreement with experimental $^{13}$C DNP data for 5 M acetone in water [9] and pure chloroform [3,10] doped with TEMPOL. Our results demonstrate that at magnetic fields of interest to structural NMR (≥ 10 T), scalar-dominated $^{13}$C enhancements that are an order of magnitude larger than those of $^1$H nuclei can be obtained.

References
Multiscale stochastic modeling of NMR relaxation in flexible molecules: the diffusive chain model

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An "ab-initio" multiscale integrated computational approach tailored to the calculation of nuclear magnetic resonance (NMR) relaxation data of flexible molecules is here reported. The method is based on the definition, parametrization and solution of a Smoluchowski stochastic equation defined for a set of relevant molecular coordinates. Such a set is split into the molecular global tumbling coupled to an appropriate set of internal degrees of freedom. In particular, here we focus on internal flexibility described in terms of torsion angles under the paradigm of what we call the diffusive chain model (DCM) [1], schematized in Figure 1A.

The multiscale protocol consists in the following steps: i) system complexity reduction heuristically done by employing short molecular dynamics trajectories; ii) quantum mechanical (or hybrid quantum/classical) evaluation of the magnetic tensors; iii) estimation of the free energy function over the relevant coordinates employing enhanced classical mechanics sampling techniques; iv) calculation of the friction tensor using an hydrodynamics approach.

Once the stochastic equation has been fully parametrized, the long times dynamics can be accessed by solving the Smoluchowski equation or performing a Brownian dynamics simulation integrating the equivalent damped Langevin equation. Basic theoretical, and computational aspects are presented, with examples taken from recently published studies [1, 2, 3].


2D lineshape reference deconvolution for elimination of signal tilting

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A method for eliminating the effects of field inhomogeneity in high resolution 2D spectra is presented. Imperfection of the B0 field appears as elongating signals along principal diagonal for those kinds of experiments, where chemical shift evolution is detected along both dimensions [1]. The method we propose here is based on reference deconvolution technique [2], in which the correction function is determined from a reference signal and then applied for treatment of the whole spectrum. We applied our method to spectra obtained using the recently developed DIAG experiment [3] F1-decoupled using nemoZS [3] or PSYCHE [4]. Significant resolution enhancement is reached after eliminating of signal tilting, which can be crucial for analysis of spectra, containing strongly overlapped multiplets (see fig. 1).

Figure 1. Comparison of the original and treated 500 MHz 1H PSYCHE-DIAG spectra of a sugar mixture (raffinose, maltotriose and melezitoze) in D2O solution.

Transient Effects in Nuclear Spin Noise Spectroscopy

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The work presented here sheds light on a novel effect which has been discovered during our continued research in the field of spin noise NMR. While conducting spin noise experiments, it has been noticed that the line shapes in 1D magnitude or power spectra is dependent not only on the setup of the spectrometer (cable length, etc.) but also on the acquisition time (Figure 1). This is surprising because a spin noise signal is in theory time-independent. Since then considerable effort has been put into the understanding and quantification of this phenomenon. Because the processing of the recorded data is not entirely straight forward, due to the lead-in points caused by the digital signal processor and some idiosyncrasies of Bruker’s “convd2a” routine, one prerequisite was the adaption of the in-house developed processing toolkit “MxNMR”. This is a custom program for use in the TopSpin environment designed with flexibility and speed in mind. New experimental setups and processing schemes were implemented to elucidate the origins of these experimental observations, which could be tracked down to the transmit-receive switch in the pre-amplifier.

Furthermore, the latest results on spin noise gradient echoes and the interference of radiation damping and spin noise are presented.

![Figure 1: Left spectrum: First 1k data points of the noise data. Right spectrum: Last 1k data points. 8k data points recorded in total.](image)

Total lineshape analysis of NMR spectra by simulated annealing

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The global optimization by the method of simulated annealing is proposed for the total lineshape analysis of high-resolution NMR spectra. The algorithm has been verified on the test tasks of simulated strongly coupled four-spin systems [1], and was successfully used for analysis of experimental NMR spectra of L-proline. In our opinion, the simulated annealing is superior to the existing algorithms [2, 3], especially, in case of analysis of highly complicated NMR spectra, for which classical procedures often end in a local minimum. Essentially, that our method does not require any estimation of J-couplings initial values, which makes it practically applicable for automated analysis of NMR spectra.

Figure 1. Analysis of 600 MHz ¹H NMR spectra of L-proline in CD₃OD. The solution is obtained after a procedure of steadily decreasing of temperature, starting from “hot” initial conditions with widely spread parameters.

Auxiliary Matrix Formalism for Computation of Chained Integrals in Magnetic Resonance

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Auxiliary matrix exponential method [1, 2] is used to derive simple and numerically efficient general expressions for the following, historically rather cumbersome, and hard to compute, theoretical methods: (1) average Hamiltonian theory following interaction representation transformations; (2) Bloch-Redfield-Wangsness (BRW) theory of nuclear and electron relaxation; (3) gradient ascent pulse engineering version of quantum optimal control theory. In the context of spin dynamics, the auxiliary matrix exponential method is more efficient than methods based on matrix factorizations and also exhibits more favourable complexity scaling with dimension of the Hamiltonian matrix (Figure 1).

As an example, in the case of BRW spin relaxation theory the relaxation superoperator contains a chained integral which can be evaluated by an auxiliary exponential relationship [3].

\[
\int_0^T e^{-\mathbf{H}_0 t} Q e^{i(\mathbf{H}_0 + i\lambda_1)t} dt = \mathbf{A}^T \mathbf{B},
\]

\[
\exp\left[ \begin{pmatrix} \mathbf{A} & \mathbf{B} \\ 0 & \mathbf{C} \end{pmatrix} t \right] = \begin{pmatrix} i\mathbf{H}_0 & \mathbf{Q} \\ 0 & i\mathbf{H}_0 - \lambda_1 \end{pmatrix}^T.
\]

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Figure 1: (a) Wall clock time taken by the $^{14}$N rotating frame transformation to the specified order, applied to the spin system of methylaziridine. (b) Wall clock time comparison between the matrix-valued numerical quadrature method for the calculation of the main integral in relaxation superoperator of Bloch-Redfield-Wangsness (BRW) spin relaxation theory and the auxiliary matrix method presented in this paper.


Quantum optimal control is the task of taking a quantum system from one state to another to a specified accuracy with minimal expenditure of time and energy. Optimal solutions are found by numerically maximising “fidelity” - overlap between current and desired state of the system.

Particularly successful in the area of magnetic resonance, the quadratic convergence of the GRAPE (Gradient Assisted Pulse Engineering) family of quantum optimal control algorithms [1, 2] is widely employed to give optimal pulses for experiments such as state transfer. We demonstrate in this communication that the Hessian of the GRAPE fidelity functional is unusually cheap, having the same asymptotic complexity scaling as the functional itself [3].

This leads to the possibility of using very efficient numerical optimization techniques. In particular, the Newton-Raphson method, requiring a Hessian calculation, is shown in this work to require fewer system trajectory evaluations than any other algorithm in the GRAPE family. This communication describes algebraic and numerical implementation aspects: matrix exponential recycling; Hessian regularisation and conditioning; parallelism of propagator derivatives used in constructing the gradient and Hessian; utilisation of Krylov propagation.

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Figure 1: Convergence profiles for the state transfer within the $^1H$-$^{13}C$-$^{19}F$ three-spin system for the BFGS quasi-Newton method and the Newton-Raphson method using TRM or RFO Hessian regularisation techniques.


Experimental ESR Pulse Optimisation using Feedback Control and an Arbitrary Waveform Generator

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Microwave pulses with variable amplitude and phase can be designed with an arbitrary waveform generator (AWG) to achieve higher excitation bandwidth, and hence higher sensitivity, than hard pulses [1, 2]. These methods require a transfer matrix, pre-calculated to allow for distortions a waveform will suffer being sent through electronic equipment.

This communication will report on the results of optimising an echo integral or curve matching (Figure 1), through a set of python scripts forming the communication between a numerical optimiser in Matlab and a Bruker SpinJet AWG. A modified simplex method [3], with a guaranteed convergence properties and a level of parallel computation, is implemented in a direct measurement feedback loop on an actual instrument.

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Figure 1: Diagram of curve matching from an echo signal (signals shown inset with the same arbitrary scales) - (left) after 1 optimisation iterate, (right) after 50 iterates. The direct measurement feedback loop is based on a broadband echo experiment using 2 optimised CHIRPS [2]. The Fourier transform of the echo signal is matched to a reference from a field sweep experiment.


Implementing Optimal Control Pulses in ESR using an Arbitrary Microwave Waveform Generator

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Microwave pulses with variable amplitude and phase can be designed, using optimal control theory, to achieve higher excitation bandwidth, and hence higher sensitivity, than hard pulses. The main problems in directly applying optimal control techniques [1] (designed originally for NMR) to EPR spectroscopy are the time-scales and the spectral widths involved.

Bruker SpinJet, an arbitrary waveform generator (AWG), makes it possible to use a discretised pulse shape to realise those optimal control solutions. However, waveforms produced by an AWG inevitably become distorted in transit between the AWG and the sample. A number of solutions have been proposed for creating a transfer matrix to transform the proposed input waveform to that seen by the sample [2, 3, 4].

In this communication we propose forming a transfer matrix from measurements at the transmission monitor, after the AWG. This transfer matrix is then used within a cost function for quasi-Newton optimisation (tolerant to static noise), then simulated with gradient ascent pulse engineering (GRAPE) [1]. Typical optimal control pulses involve sharp transients and it is expected that those would generate pronounced waveform distortions. To reduce this effect, we propose the use of smoothing Tikhonov regularisation functions within the optimisation.

The numerical solutions produced by the cost functional described above would not be optimal in the strict sense; further waveform distortions are expected to be sample- and resonator-specific. The proposition here is to use the GRAPE solution as a starting point, close enough to a local minimiser, from which a modified simplex method, running in a direct measurement feedback loop on an actual instrument, would not struggle to find a minimiser proper.

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Many-body CE DNP simulations to study biradical efficiency

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Cross effect DNP (CE DNP) is currently the most efficient DNP mechanism, resulting in high nuclear polarization levels. Large signal enhancements are seen after short irradiation times - typically on the order of minutes. To date, enhancements as high as ~ 400 have been obtained [1] with the use of MAS and biradical molecules, with strongly coupled electrons. Following our previous work on the solid effect (SE DNP) [2], we took a similar approach for CE DNP. We focused on the static case of CE DNP - the studies of which are limited, and the understanding of which is far from complete. In addition, static DNP experiments avoid the use of expensive hardware, and lower the experimental complexity.

We show that using adiabatic elimination the polarization dynamics of CE DNP can be projected onto the Zeeman subspace of the density operator. The resulting effective dynamics are incoherent, hence classical in nature, which allowed us to simulate large spin ensembles using kinetic Monte Carlo algorithms. In addition, the analytical form of the effective dynamics allows a clear interpretation of the physics underlying CE DNP. We thus illustrate improvements in build-up time and steady-state polarization levels that can be expected; an example is shown in figure 1, where we show the evolution of the polarization of protons in time using colour and dot size, for a cuboid lattice of dimension 5x6x5. The inset figure shows the mean polarization build-up of the nuclei and demonstrates the efficiency of CE DNP over SE DNP. Our overall findings point to optimisation capabilities of our approach, and the possibility it holds as an aid in bi-radical design and testing.

Figure 1: More optimal conditions are chosen to give a rapid build-up of polarization; 90 % nuclear polarization is reached after 15 seconds, the build-up time constant is approximately ~ 5s. Extrapolation indicates a level of 95 % polarization will be reached at steady-state. The inset compares build-up for CE DNP (black line), to the case of very weak electron dipolar coupling (blue line), where the SE DNP mechanism dominates.

Abstracts for posters in session "Disordered proteins"
Parkinson’s disease is the second most common neurodegenerative disorder and the characteristic α-Synuclein (α-Syn) fibrillar aggregates, which are, also known as Lewy bodies, are found in the neurons of substantia nigra (1). α-Syn is a small (140 residues), natively unfolded presynaptic protein of about 14kDa (2). It acquires α-helical secondary structure upon binding to lipid vesicles (3) or the characteristic cross β-sheet conformation in case of the amyloid-like fibrils (4). Accumulation of unfolded or misfolded proteins leads to the increased levels of chaperones and foldases in endoplasmic reticulum (ER) (5), which is a salient pathological feature associated with various neurodegenerative disorders (6). Chronic ER stress, inducing cell death has been reported during the overexpression of wild type (WT) (7, 8) and mutant α-Syn (9). Elevated levels of Protein disulfide isomerase (PDI), a multifunctional stress protein abundant in ER, has been reported in the brain of patients with PD and is found in Lewy bodies (10). Cheng et al. (11) for the first time reported interaction studies between PDI and its domains with α-Syn and showed α-domain of PDI to be essential and sufficient to inhibit α-Syn fibril formation. However, detailed residue specific mapping of PDI binding to α-Syn remains unexplored. Probing the PDI binding sites on α-Syn will help us understand how PDI inhibits its aggregation. In my current research work, we identify the putative PDI binding sites on α-Syn using nuclear magnetic resonance (NMR) spectroscopy and observed H50 as one of the important key residues involved in PDI binding. In order to understand the role of H50, NMR interaction studies were done for the familial mutant H50Q (12). We also investigated the aggregation of α-Syn WT and H50Q in the absence and presence of PDI using thioflavinT (ThT) fluorescence for understanding aggregation kinetics and circular dichroism (CD) for structural conversion. Further, morphological characterizations of the aggregates were done using atomic force microscopy (AFM).

In conclusion, we found that N-terminus of monomeric α-Syn showed (V3-S9 and L38-40) higher affinity for PDI ($K_d = 1-8 \mu m$) compared to V49-V52 ($K_d = 26-32 \mu m$) and residues located at the C-terminus ($K_d = 37-52 \mu m$). Previous studies have identified five β-strands within the fibril core of α-Syn comprising residues 30-110 (13, 14). Residues 49-52 are suggested to adopt beta-turn conformation during the fibril formation. Even though the binding of PDI within this region is weaker, the binding is crucial for preventing the formation of β-strand within the core region and hence the fibril formation. In case of the familial mutant H50Q, where no PDI binding is taking place surrounding Q50, fibril formation is not perturbed even though the binding at the N- and C- terminus is similar to wild type. From the above studies, it can be postulated that binding of PDI within the residues surrounding H50 along with the residues at the N- and C-terminus stabilizes the native conformation of α-Syn and does not allow it to form unstable misfolded intermediate, thus inhibiting the fibril formation.

Effects of small molecule compounds on alpha-synuclein

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A characteristic of Parkinson’s disease is the accumulation of Lewy bodies within nerve cells and the simultaneous death of nerve cells. The main component of Lewy bodies is the protein α-synuclein (αSN). Oligomeric αSN is considered to be the potential toxic species responsible for the neurotoxicity of Parkinson’s disease, this is based on several in vitro observations that amyloid oligomers are able to permeabilize membranes by pore formation [1, 2], which has also been confirmed in few in vivo studies.

Together with the pharmaceutical company Pfizer Ltd. we have carried out a screening of 746,000 small molecules for their ability to inhibit the aggregation of αSN. More than 60 compounds showed strong inhibition both towards the aggregation process but also towards the ability of the oligomer to permeabilize membranes. Top 10 compounds have been studied further using NMR to characterize the effect on alpha-synuclein monomer.

We have used NMR to get structural information on the interaction of small molecule fibrillation inhibitors and alpha-synuclein monomer to be able to understand how they inhibit fibrillation of alpha-synuclein and find out what kind of interaction is made.

References:
A conserved aromatic-residue code shapes the intrinsically disordered Unique domain of c-Src

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c-Src is a membrane bound, non receptor tyrosine kinase involved in cellular signaling pathways. Its deregulation is associated to different kinds of tumoral processes. The intrinsically disordered N-terminal region includes the SH4 and Unique domains and displays low homology between the members of Src Family Kinases (SFKs), which are otherwise highly conserved. The role of this disordered region has however remained obscure beyond its lipid binding function.

Our group had previously reported transient intramolecular contacts within the SH4-Unique region (positions 1-85), as well as interactions of both domains with specific regions of the adjacent, folded SH3 domain (85-150). In this work, we provide an insight on this complex interaction pattern using Paramagnetic Relaxation Enhancement (PRE) and Chemical Shift Perturbations (CSP).

We demonstrate that transient contacts between distant regions of the Unique domain (PRE measured from positions 27 and 59) are reciprocal in a SH4-Unique-SH3 construct. Also, PRE-detected long-range perturbations from both positions map in the same region of the SH3, in agreement with previous CSP measurements. We then show that the intra-Unique interactions also exist without the scaffolding effect of the SH3 domain.

PRE profiles of all isolated SH4-Unique F#A mutants, represented as 1D-heat maps, show significantly reduced intramolecular contacts in all cases. CSP of the F#A mutated SH3-bound constructs versus wild type also show modified interactions between SH3 and Unique domains. These observations indicate that the Unique domain, although disordered, is constitutionally restrained. A conserved pattern of aromatic residues in the Unique domains of SFKs suggests an aromatic-residue code as a mechanism restricting their conformational space.

Figure 1. Scaled SH4-Unique-SH3 cartoon. In blue (spheres), position of Phe residues in the Unique domain. In red, the alternative paramagnetic label positions used (spheres), and PRE affected regions of the SH3 domain (ribbons and mesh surface). Dashed arrows qualitatively remark distant interacting regions. Figure 2. Alignment of SH4-Unique domain sequences of four SFKs. In blue, fully or partially conserved aromatic residue positions.
Interaction studies of UNG2 with its DNA replication fork partners

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In recent years intrinsically disordered proteins (IDPs) have come into the focus of the research community. Prediction shows that up to 33 percent of all functional eukaryotic proteins have an intrinsically disordered segment longer than 25 residues. NMR allows studying these intrinsically disordered proteins or segments at atomic level.

The human uracil DNA glycosylase UNG2 specifically and efficiently removes uracil from DNA in the nucleus. The catalytic C-terminal core domain of UNG2 (85-313 AA) was crystallized both as native protein and in a complex with DNA-uracil. The 85 residue long N-terminal region was predicted to be mainly unstructured which could be confirmed by recent SAXS and NMR analysis. Despite the lack of intrinsic structure, the N-terminal region markedly changes functional properties of UNG2 compared to the catalytic domain alone, including increased preference towards single-strand DNA and localization to replication foci in S-phase. It could be shown that PCNA and RPA associate with the N-terminal region of UNG2 at specific motifs. To what degree these interactions are regulated by post-translational modifications remains, however, elusive.

Both N- and C- terminal UNG2 have now been assigned to allow detailed studies of both domains separately and as well in the full-length protein. Structural rearrangements upon interaction between RPA and N-terminal UNG2 in presence and absence of DNA have been determined at atomic level. Interactions between UNG2 and RPA have been measured either on the N-terminal UNG2 or on peptides containing the aforementioned RPA binding site with domains of RPA known for binding UNG2. Additionally the effects of post-translational modifications of UNG2 on binding to its interaction partners have been tested by peptides with these post-translational modifications. Finally, we have studied the effect on the catalytic C-terminal domain of UNG2 when RPA interacts with the unstructured domain using a full-length UNG2.

Figure 1. HSQC spectrum of N-UNG2 was subtracted from the complex N-UNG2/RPA domain. The amino acids involved in the binding (T60 - V90) are negative (green)
Sequence context influences the structure and aggregation behavior of a polyQ tract

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Expansions of polyglutamine (polyQ) tracts in nine different proteins cause a family of neurodegenerative disorders called polyQ diseases. Since polyQ tracts are potential therapeutic targets for these pathologies there is great interest in characterizing the conformations that they adopt and in understanding how their aggregation behavior is influenced by the sequences flanking them. We used solution NMR to study at single-residue resolution a 156-residue proteolytic fragment of the androgen receptor that contains a polyQ tract and causes the disease called spinobulbar muscular atrophy, also known as Kennedy disease. Our findings indicate that a Leu-rich region preceding the polyQ tract causes it to become α-helical and appears to protect the protein against aggregation, which represents a new mechanism by which sequence context can minimize the deleterious properties of these repetitive regions. Our results have implications for drug discovery for polyQ diseases because they suggest that the residues flanking these repetitive sequences may represent viable therapeutic targets.

Backbone Resonance Assignment of Tau3x Protein Using New 4D Experiments with Carbonyl-Carbonyl TOCSY

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The resonance assignment, a necessary prerequisite for any further NMR studies, in the case of intrinsically disordered proteins (IDPs) often appears to be a challenging task.

We propose novel 5 and 4 dimensional experiments, exploiting tocsy type carbonyl-carbonyl coherence transfer using MOCCA-XY16 mixing block [1,2], providing valuable CON connectivities (cf. Fig. 1) as well as easy proline residues resonance assignment. The performance of 5D experiments was tested on α-Synuclein (140 a.a.), 4D techniques allowed full backbone resonance assignment of Tau3x protein (354 a.a.) (cf. Fig 2).

![Figure 1. Assignment process using 2D cross-sections from 5D (H)NCOCONH with combination with 2D CON projection from 3D HNCO of α-synuclein.](image1)

![Figure 2. Strip plots taken from 4D (HACACO/N(CO)CONH (left) and (HACA)CO(NCO)CONH (right) of Tau3x protein. Diagonal peak positions are marked with black crosses. Strips plots for proline residues are not shown.](image2)

All spectra were nonuniformly sampled to provide high resolution in all indirectly sampled dimensions. 4D spectra were processed using Signal Separation Algorithm [3], whereas 5D spectra were processed using Sparse Multidimensional Fourier Transform (SMFT) approach [4]. All processing software used is free to use for academic users and is available on [http://nmr.cent3.uw.edu.pl/software](http://nmr.cent3.uw.edu.pl/software).

Molecular Basis of Microtubule Regulation by Microtubule-Associated Protein Tau

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Microtubules regulate cell division, cell morphology, intracellular transport and axonal stability and therefore play crucial roles in cell function (1). The structure, dynamic behavior and spatial organization of microtubules in neurons is regulated by microtubule-associated proteins (2). The microtubule-associated protein Tau promotes formation and stabilization of axonal microtubules and thus influences intracellular transport, axonal stability and cell morphology (3, 4). The adult human brain contains six isoforms of Tau, which are generated from a single gene by alternative splicing. The six isoforms are composed of either three or four repeats, with up to two N-terminal inserts, and range from 37-45 kDa. In Alzheimer’s disease the interaction of Tau with microtubules is impaired.

Despite the importance of the regulation of microtubule structure and dynamics by Tau, very little is known about the interaction of Tau and other microtubule-associated proteins with microtubules. To fill this gap, we studied the interaction of Tau with microtubules using a combination of NMR spectroscopy and mass spectrometry. We show that Tau, which is intrinsically disordered in solution, locally folds into a stable structure upon binding to microtubules (5). We further show that Tau promotes microtubule assembly by binding to protofilaments at the interface between α-β-tubulin heterodimers using small groups of evolutionary conserved residues (6). The binding sites are formed by residues that are essential for the pathological aggregation of Tau, suggesting competition between physiological interaction and pathogenic misfolding. Collectively, our study establishes a conserved mechanism of microtubule polymerization and thus regulation of axonal stability and cell morphology by microtubule-associated proteins.

References
The phosphorylation kinetics of tyrosine hydroxylase and its interaction with 14-3-3ζ protein studied by NMR

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Human tyrosine hydroxylase 1 (hTH1) is activated by phosphorylation of its N-tail regulatory domain (RD-hTH1, 169 residues) and by the interaction with regulatory 14-3-3 protein. In order to understand the nature of changes introduced by phosphorylation of residues Ser19 and Ser40, we assigned the RD-hTH1 protein. This task was complicated due to the fact, that about 70 residues at the N-tail are intrinsically disordered, while the rest is well folded and structured. This obstacle was overcome for the disordered part by usage of two 5D experiments, measured with non-uniform sampling approach followed by sparse multidimensional Fourier transform. These techniques allowed to acquire all the information necessary for backbone and side-chain assignment in less than 3 days of spectrometer time. For the assignment of structured part we had to rely only on standard set of 3D spectra.

We measured kinetic rates of the phosphorylation of Ser19 by PRAK kinase. These were derived from intensity changes of signals of several residues close to the phophorylation site. Also, we compared the HSQC spectra of non-phosphorylated, Ser40-phosphorylated and doubly phosphorylated sample to find the most affected residues. To reveal the interaction mode with 14-3-3ζ protein we titrated highly concentrated 14-3-3ζ protein to doubly phosphorylated RD-hTH1. The changes in peak intensities indicate the regions of RD-hTH1 most involved in the interaction.

References


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Interaction of intrinsically disordered proteins with the cellular hub proliferating cell nuclear antigen

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Ribonucleotide reductase (RNR) is involved in controlling the mutation rate in cells and dysregulation of RNR activity has been linked to the development of cancer. To increase our understanding of cancer development, it is vital to gain more knowledge about RNR regulation. A layer of RNR regulation in yeasts happens through its inhibition and subcellular sequestration by intrinsically disordered proteins. Some of these proteins are degraded during DNA replication, thus freeing RNR to allow dNTP synthesis. A key step in their degradation is their interaction with proliferating cell nuclear antigen (PCNA), which presents them to ubiquitination and proteasomal degradation. A subset of these IDPs contain a short linear motif known as the PCNA interacting protein-degron (PIP-degron), which mediate their PCNA interaction usually by binding to the the interdomain connecting loop (IDCL) of PCNA (Figure 1). Mutational studies have shown that the PIP-degron in the IDPs is important for their interaction in vivo but no direct interaction studies with PCNA has been conducted in vitro. To resolve if this motif in the regulatory IDPs is indeed the key site for PCNA interaction and to decompose the defining contacts, we have studied the interaction of intrinsically disordered protein with PCNA using nuclear magnetic resonance (NMR) spectroscopy and x-ray crystallography. By NMR we have pinpointed several contact points in the IDPs of which one is in the PIP-degron. We will present data that compare the properties of these sites and relate them to the transient structures in the unbound states as determined by NMR secondary chemical shifts. From the crystal structures of their complexes with PCNA we will discuss if and how these structures are preserved in the bound state and if the transient structures play any role in recognition.

Figure 1. Schematic representation of homo-trimeric PCNA (blue), bound to three PIP-degrons (red), by interacting with the IDCL of PCNA (black)
Probing Transient Functional States in the Aβ Peptide Using the Chemical Shifts Covariance Analysis

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When not self-associated into toxic soluble oligomers, amyloidogenic peptides are either unstructured or minimally structured and they access a highly heterogeneous conformational ensemble, in which multiple discrete conformers are populated. Despite the complexity in the structural ensemble accessible to amyloidogenic peptides, a key determinant of the propensity to aggregate is the transient population to a subset of functional conformations, i.e. conformers that are either aggregation competent or are incompetent but compete with aggregation competent states. However, due to the conformational ‘noise’ arising from the highly degenerate free-energy landscape typical of amyloidogenic peptides, the identification of minor populations of functional conformers relevant for aggregation remains a major experimental challenge. Here, we propose a method to identify which residues within amyloidogenic peptides are involved in functional states based on the covariance analysis of NMR chemical shifts (CHESCA).\(^1\)\(^\text{4}\) CHESCA relies on inter-residue linear chemical shift correlations measured for the peptide system of interest subject to a set of perturbations that modulate a given functional property, e.g. the aggregation propensity in the case of amyloidogenic peptides. While originally developed for globular protein, the CHESCA approach has proven useful to map allosteric networks that extend also to flexible inter-domain linkers.\(^2\) Specifically, linear chemical shift correlations are useful to identify the residues involved in transient and minimally populated, yet functionally relevant, partially folded conformers. This is because a distinct advantage of the chemical shift covariance method is that the relative order in which the perturbed states appear in the inter-residue correlations defines the functional relevance of the partially folded conformers.\(^1\)\(^\text{4}\) The general CHESCA approach will be illustrated through its application to the Aβ peptide, whose self-association into insoluble cross β-sheet structured aggregates is linked to Alzheimer’s disease.

Characterization of the Interaction Between the Disordered C-terminus of Artemis and Ligase IV

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Since the early days of structural biology, a stable structure had been thought to be necessary to the function of proteins. By the end of the 20th century, this structure-function paradigm has been challenged by the discovery of intrinsically disordered proteins (IDPs). IDPs lack the hydrophobic core that stabilizes folded protein domains and do not display a stable structure in their functional form. Intrinsically disordered proteins and regions (IDRs) are ubiquitous in eukaryotes where they play a wide range of function through highly specific interactions (with a large interaction surface) yet with weak affinity of binding because of the entropic cost of binding.

Artemis is an endo- and exonuclease involved in the non-homologous end joining (NHEJ), a process essential to the repair of double-strand break in DNA and thus in the V(D)J recombination. In addition to its large N-terminal catalytic domain, Artemis possesses a 300-residue C-terminal domain, which is predicted to be intrinsically disordered by computational analysis of the primary sequence (Figure 1a). This domain is known to interact with other members of the NHEJ recombination machinery as well as regulation factors [1].

NMR spectra of the C-terminal region of Artemis confirm its disorder nature (Figure 1b). High-dimensionality experiments (up to 5D) were recorded at 950 MHz (HN(Ca)CONH and HabCabCONH) and complemented with 13C-detected experiments recorded at 700 MHz ((Ha)CaCONCaCO and (H)C(CC-TOCSY)Ca(CO)N) to obtain more than 90 % resonance assignments of this long IDPs [2]. The characterization of the interaction between this full C-terminal domain and the DNA-binding domain of the protein Ligase IV was investigated by titration followed by 2D and 3D NMR.

References:

Figure 1: Characterization of the structural ensemble of the C-terminal domain of Artemis. a) Predicted disorder propensity (Predisorder) show a high propensity to disorder for the C-terminus. b) 1H-13N HSQC spectrum of the full C-terminal domain of Artemis.
Abstracts for posters in session "EPR"
MD and multifrequency EPR studies of the dynamics of the MTSL spin-label in the activation loop of Aurora-A kinase

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Studies of kinase activation through characterisation of their conformations and dynamics are important to enhance in understanding of molecular processes related to diseases, including cancer. In this work, classical molecular dynamics (MD) simulations, taking advantage of the modern graphic processing unit (GPU) architecture, were performed to study the dynamics of the methane-thiosulfonate spin label (MTSL) in the activation loop of Aurora-A kinase, in a very short time and with good quality of sampling. MD provided a wealth of information about the timescale of the different motional contributions to the overall dynamics of the spin label. These data were validated by multifrequency continuous-wave electron paramagnetic resonance (EPR) measurements that were used to distinguish the fast internal motion of the spin label from slow protein tumbling.

It was found that the activation loop oscillated between two conformational states separated by 7 Å and the average structures obtained from the MD trajectories were comparable with those obtained from X-ray crystallography. The theoretical EPR spectra were calculated using selected configurations of MTSL in different protein environments characterized by different polarity. Comparison between theoretical and experimental 9 and 94 GHz EPR spectra revealed that fits, obtained from magnetic parameters calculated using the a and b configurations shown in Fig. 1, successfully reproduced the experimental spectra, in agreement with that observed in the average MD structures that showed the MTSL exposed to the solvent and probing the region of the C-lobe of the protein.

Fig. 1: Comparison between experimental and simulated EPR spectra using two conformations of the MTSL in which it is exposed to the solvent (a) and interacting with a tyrosine residue in the C-lobe of the protein (b).

Computational Study of a Fourth Stable Radical in X-irradiated Sucrose

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Ionizing radiation produces radicals in solids, which are often well suited for studies by electron paramagnetic resonance (EPR) spectroscopy. Radiation-induced radicals in sugars are particularly interesting as model systems for sugar-containing macrobiomolecules. Thorough characterization of radicals enables their identification and understanding of their chemical reaction pathways. This knowledge may subsequently provide insight into the radiation chemistry of more complex sugar-containing and biologically relevant systems, e.g. DNA and RNA.

Sucrose, also known as the main component of table sugar, presents a practical interest as a versatile dosimetric system. The stable radiation-induced EPR spectrum of sucrose has a sufficiently low detection limit and a considerable linear dose response range, which makes it a viable candidate for emergency dosimetry and characterization of radiation-sterilized foodstuffs. The multicomponent nature of the spectrum has slowed down further improvement of dose assessment protocols, which in turn motivated a series of investigations to address this issue.

Recently, it was shown that four radical species are sufficient to completely explain the dosimetric spectrum of X-irradiated sucrose. Three radicals have been identified prior to this by combining electron-nuclear double resonance spectroscopy with density functional theory (DFT) calculations [1, 2]. A similar approach has been applied to the fourth species, yielding its chemical structure [3]. The exact location of the radical in the crystal still remains unknown. In this contribution, we present results of our efforts to identify this last known stable species using periodic DFT calculations. The model we propose (Fig. 1) involves abstractions of H and O atoms, and scission of multiple bonds in the fructose unit. This effectively breaks the sucrose molecule into two parts, creating a detached radical molecule, suspended by hydrogen bonds.

Improved sensitivity for W-band Gd$^{3+}$ - Gd$^{3+}$ DEER measurements with chirp pulses

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Chirp 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.\textsuperscript{1,2} The implementation of such pulses for pulsed EPR has become feasible due to availability of arbitrary waveform generators (AWG) with high enough sampling rates to support pulse shaping for pulses with tens of nanosecond durations. Implementations of these pulses for improving DEER sensitivity have been demonstrated for X and Q-band pulse EPR setups including a recent work focusing on Q-band Gd$^{3+}$ -Gd$^{3+}$ distance measurements.\textsuperscript{1} In this work we demonstrate a setup for obtaining chirp pulses on our home-built W-band spectrometer. DEER with chirp pulses was performed using two types of Gd$^{3+}$ tags differing in zero field splitting (ZFS) parameters and therefore spectral width: (i) A Gd-PyMTA ruler\textsuperscript{3} ($D_{ZFS} \sim 1150$MHz) model system with a distance of 3.4 nm (see figure). (ii) Ubiquitin, doubly labeled with Gd-DOTA ($D_{ZFS} \sim 550$MHz) with an average distance of 3.5 nm. An optimization procedure as recently described by Doll et al.\textsuperscript{4} was adapted and used to choose the best conditions for positioning of the detection frequency within the EPR spectrum, the spectral width and the position of the chirp pulse. The optimal conditions were found to depend on the width of the central transition. Additionally, the effect of different pulse types on the DEER results is investigated. A significant improvement in sensitivity was obtained for both samples. This work marks the first demonstration of the application of chirped pulses for high field EPR experiments.

Figure 1. (a) DEER traces after background subtraction for the Gd-PyMTA ruler for a rectangular pulse and chirp pulses with different lengths (see legend) (b) distance distribution (obtained with DEERAnalysis)

Chirp effects in EPR/ESEEM correlation spectroscopy

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In pulse electron paramagnetic resonance (EPR) spectroscopy typical rectangular monochromatic high power pulses of 10 ns length have a bandwidth of about 100 MHz. This restricts application of many EPR experiments for transition-metal compounds, which have spectra that can extend over several gigahertz and plays an important role in two-dimensional (2D) or three-dimensional correlation experiments, where high sensitivity is required. In the ultra-wideband (UWB) excitation regime (excitation bandwidth of 500 MHz and more), reached with a fast arbitrary waveform generator (AWG) [1], frequency-swept or “chirp” pulses can generate electron spin echoes whose direct digitization and subsequent Fourier transformation provides an EPR dimension without explicit variation of the magnetic field or of an interpulse delay. That way we were recently able to measure FT-EPR correlated electron spin echo envelope modulation (ESEEM) and HYSCORE from paramagnetic copper centers in crystals [2]. Compared to spectra obtained with rectangular pulses, the EPR/ESEEM correlation spectra obtained with chirp pulses exhibited additional peaks. Such peaks may arise because during a passage pulse coherence is generated on transitions which share a level with a transition that has already been passed [3]. Using our home-written Spin Dynamics Analysis (SPIDYAN) software [4] and SpinDynamica [5] we could assign the additional peak for a nucleus with spin 1/2 to a particular coherence transfer pathway during passage of the two allowed and two forbidden electron spin transitions. Such assignment also predicts different correlation patterns for the strong and weak coupling cases.

Gd(III)-Nitroxide Distance Measurements on an Oligoproline Construct using RIDME

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Figure 1: Echo detected field sweep, primary RIDME data and background corrected form factors (from left to right) measured at 40 K with different mixing times on the orthogonal spin pair Nitroxide - Gd(III)DOTA in an oligoproline peptide sample (expected distance $\sim 3$ nm).

We present distance measurements in frozen solutions of oligoproline peptides in a PPII helix conformation based on the relaxation induced dipolar modulation enhancement (RIDME) effect \cite{1}. The peptides are modified with two spectroscopically orthogonal spin labels (nitroxide and Gd(III)DOTA). The measurements were based on the dead-time free 5p-RIDME pulse sequence \cite{2}, recently applied to Gd-Gd spin pairs \cite{3}. The interlabel distances are between 3 to 4 nm, as has been determined by DEER distance measurements \cite{4}. The samples were prepared in deuterated solvent to reduce background decay. The orthogonality of the spin pair offers the possibility to tune the ratio of longitudinal relaxation in a range that is favourable for RIDME efficiency. We studied the change of the modulation amplitude with mixing time and temperature. We thank the group of Prof. H. Wennemers for providing the oligoproline samples. This work was supported by SNF grant 200020\_157034.

References


The activation and functionalization of the C-H bonds of alkanes has been extensively studied in the last decades and remain a main challenge in contemporary catalysis. However, the high reactivity comes at the expense of using large stabilizing ligands that limit the reaction scope and conditions, and thereby their application into catalytic processes. One approach that circumvents this problem is surface organometallic chemistry, which provides through site isolation on a surface, a way to access highly electrophilic and coordinatively unsaturated early transition-metal complexes supported on high surface oxide supports. In particular, this approach allows access to unsaturated metal hydrides that display unprecedented reactivities, such as the low temperature activation and catalytic conversion of alkanes. Besides Ti$^{4+}$ hydrides, which have been evidenced by solid-state NMR, electron paramagnetic resonance (EPR) spectroscopy points towards the presence of titanium (III) species, but it is not clear what are the bound ligands of the latter species. Computational studies suggest that Ti$^{3+}$ hydrides should be more active than the corresponding Ti$^{4+}$ analogues in ethylene polymerization or alkane hydrogenolysis, but no direct evidences for these species exists.

Here we show that hyperfine sublevel correlation (HYSCORE) spectroscopy, a pulsed 2D EPR experiment, combined with density functional theory (DFT) calculations is a very good method for probing the structure, the bonding and the environment of Ti$^{3+}$ sites. In this work for synthesis of Ti(III) hydrides a TiNp$_4$ precursor was obtained by reaction of Ti(OEt)$_4$ with LiNp. To get a silica-supported species, this precursor was reacted with SiO$_2$-700 and finally in an attempt to get a hydride it was treated with H$_2$ gas. The g values obtained from analysis of continuous-wave (CW) EPR spectra are similar to other known Ti(III) complexes. In the HYSCORE spectra, besides strong proton hyperfine couplings, two peaks appear with the nuclear Zeeman frequencies of $^6$Li and $^7$Li. The intensity ratio of the two signals agrees with the natural abundance of the two lithium isotopes (7.5 and 92.5%). Other features observed in the HYSCORE spectrum can be ascribed to the interaction with a nitrogen nucleus. The proximity of lithium and nitrogen to the Ti(III) centers can be explained by reaction of LiNp with nitrogen which produces lithium nitride (Li$_3$N) that remains in the sample as an impurity and appears to preferentially coordinate to Ti(III). To remove Li$_3$N, the precursor was sublimated and recrystallized at high vacuum. However, in the HYSCORE spectra nitrogen interaction with the Ti(III) center was still visible. For the treatment in H$_2$ atmosphere we used Hydrogen 5.0 gas, which contains less than 5 ppm of N$_2$ impurities. The residual nitrogen may explain the appearance of nitrogen signals in HYSCORE spectra. This assumption was confirmed by observing a larger $^{14}$N signal in a sample prepared with controlled addition of nitrogen. Simulation of the strong proton hyperfine couplings in the HYSCORE spectra revealed a Ti-H distance that is in disagreement with the assumption of a direct Ti-H bond.

After reacting the surface species with $^{13}$C isotope labeled ethylene, the coordinated nascent polymer chain could be detected via large $^{13}$C hyperfine couplings.
EPR of structural phase transitions in formate metal-organic frameworks

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Lately, novel porous materials called metal-organic frameworks (MOFs) emerged and immediately attracted attention of the scientific community. These crystalline compounds are unique due to the high degree of porosity which can be utilized for gas adsorption related applications. Additionally, many MOFs containing paramagnetic transition-metal ions exhibit peculiar magnetic properties. The organic part in several of such compounds consists of polar molecules which below a certain phase transition temperature order into a ferroelectric-type phase, making these materials single-phase hybrid multiferroics.

We use continuous-wave (CW) and pulse EPR as well as pulse ENDOR spectroscopic techniques to investigate and characterize structural phase transitions in manganese and copper doped MOFs with general chemical formula [A][Zn(HCOO)₃]ₙ (where n = 1, 2 and A⁺⁺ is molecular cation such as NH₄⁺, (CH₃)₂NH₂⁺ or NH₃(CH₂)₄NH₃²⁺) (Figure 1). The temperature dependent CW EPR spectra reveal that the local paramagnetic ion-probes are indeed sensitive to the local structural changes occurring at the phase transitions. Spectral simulations are used to obtain the g, hyperfine A and fine structure D tensors and temperature dependencies of their components allowing to characterize the observed phase transitions. Pulse EPR and ENDOR measurements are performed to study structure of the frameworks in the low temperature phase. The experimentally obtained structural information is supported by the density functional theory calculations.

Figure 1. High temperature phase of [(CH₃)₂NH₂][Zn(HCOO)₃] MOF.
Dy(III)-Induced Relaxation Enhancement of Organic Radicals: Beyond the
Lowest Kramers Doublet

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Determination of the distances between Dy(III) complexes and organic radicals from the longitudinal relaxation enhancement (RE) has been studied in detail and was shown to work on model bio-molecules with known structure [1-3]. Importantly, the technique does not require a separate measurement of Dy(III) relaxation times. However, direct comparison of the RE-based distances to the reference Gd(III)-nitroxide distance measurements by double electron-electron resonance (DEER) reveals about 20% discrepancy, the major part of which is systematic [2,3]. Here we demonstrate that this systematic discrepancy has two contributions: the first one is due to the relatively poor estimate of the g-tensor principal values for Dy(III), and the second one is due to the assumption that only the lowest Kramers doublet of the Dy(III) spin-orbit manifold plays a role in the RE effect.

Direct calculations of the RE due to all partially populated spin states of the Dy-DOTA complex with improved g-tensor principal values nearly perfectly remove the systematic contribution to the mismatch between RE and DEER data leaving only a smaller-amplitude arbitrary scatter that can be tolerated for many purposes. An experimental calibration or the RE technique can be performed in cases when Dy(III) complexes other than Dy-DOTA are used for distance determination. This work was supported by SNF grant 200020_157034.

Gd(III) – Gd(III) RIDME distance measurements under conditions of small zero-field splitting

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Methods based on pulse electron paramagnetic resonance allow measuring the electron-electron dipolar coupling between two paramagnetic tags; this interaction can report on distances in the 2.0 – 8.0 nm range. In the most popular technique, Double Electron-Electron Resonance (DEER or PELDOR), one of the coupled spins is observed while the other one is flipped by a microwave pulse at a second microwave frequency (so-called pump pulse). This produces a modulation of the electron spin echo intensity of the observed electron spins, which contains the dipolar coupling frequencies. Relaxation-Induced Dipolar Modulation Enhancement (RIDME) is a single frequency technique in which the role of the pump pulse in DEER is replaced by longitudinal relaxation of the electron spins coupled to the observed one through the dipolar interaction. Because in RIDME the fraction of spins giving rise to the modulation of the spin echo intensity does not depend on the excitation profile of the pump pulse, RIDME leads to a larger modulation amplitude [1]. Here we explore the utility of the dead time-free five pulse RIDME [2] for Gd(III) – Gd(III) distance measurements at W-band (94.9 GHz, ≈3.5 T) between Gd(III) tags featuring a small zero field splitting (ZFS). Two systems are considered: (i) a rigid model compound with an inter-spin distance of 2.3 nm, and (ii) two mutants of a homodimeric protein labeled with a Gd(III) chelate, with an inter-spin distance of around 6 nm. Experiments on the first sample show that RIDME helps to partially overcome the complications arising from the failure of the weak coupling approximation, which affect DEER on systems characterized by short inter-spin distances between Gd(III) tags having a narrow central transition. Experiments on the second sample, which is characterized by a longer inter-spin distance, point out issues that are related to the appearance of harmonics of the dipolar interaction frequency in the RIDME traces for \( S > 1/2 \) spin systems, as well as to uncertainties in the background subtraction. In both cases the sensitivity of RIDME was found to be better than DEER. The effects of the experimental parameters on the RIDME traces are discussed.

New Gd(III) and Mn(II) tags via C–S conjugation for DEER distance measurements in-vitro and in-cell

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Pulse electron-paramagnetic resonance (EPR), particularly double electron-electron resonance (DEER), via site-directed spin-labeling has emerged in the past decade as a powerful technique for exploring protein structure in frozen solutions. In this study, we present a new Gd(III) and Mn(II) chelating tag, 4PhSO₂-PyMTA, for Gd(III)-Gd(III) and Mn(II)–Mn(II) distance measurements, based on the use of a stable C-S conjugation to the protein. The reaction of phenylsulfonated pyridine derivatives and protein thiols features high reactivity towards cysteine and a stable, rigid and short thioether linker, which is valuable in distance measurements. Here we report the results of DEER distance measurement at W-band (94.9 GHz) on three mutants of human ubiquitin and one mutant of E. coli PPiB bearing two cysteines which were doubly labeled with Gd(III) or Mn(II)-4PhSO₂-PyMTA, showing the expected narrow distance distribution when the labeling site is rigid (Fig. 1 a,b). We also explored the utility of 4PhSO₂-PyMTA as a tag for in-cell DEER measurements.

We delivered a Gd(III)-4PhSO₂-PyMTA labelled ubiquitin mutant into human cervical cancer (HeLa) cells through electroporation and hypo-osmotic shock respectively and showed that DEER measurements are feasible under in-cell reductive conditions. We show that DEER measurements of Gd(III)-4PhSO₂-PyMTA labelled ubiquitin with an in-cell concentration of ~15 μM via electroporation (7h) and ~8 μM via hypo-osmotic shock (7h) are feasible (Fig.1 c,d). In contrast to Gd(III) the binding constant of Mn(II) was found to be too low for in-cell measurements.

![Figure 1](image-url)
Monitoring of bisphenol A degradation by MnO$_2$/peracetic acid system with spin trap ESR

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Advanced oxidation processes, based on the formation of hydroxyl radical, are known to be effective in the degradation of various xenoestrogens. However, the search for safer and faster methods continues. Recently, it was demonstrated that MnO$_2$/peracetic acid (PAA) system is effective in degradation of phenol due to the high rate of OH and various C-centered radicals formation. Here we have applied MnO$_2$/PAA system for the degradation of Bisphenol A (BPA), which is a phenol derivative with known xenoestrogenic properties. The degradation of BPA was monitored with spin trapping ESR with 4-hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide (FDMPO) and spectrophotometry. DFT calculations were used to determine the preferable reaction path and to predict possible intermediate radical and non-radical products of BPA degradation. All DFT calculations were performed at B3LYP/6-31G(d,p), MO6X/6-311G(d,p)) in gas phase and in water as a solvent using the PCM model.

Our results indicate that MnO$_2$/PAA system is more effective in the degradation of BPA than Fenton reaction. Spin trap experiments have shown that short contact times (up to 10 min) of PAA with MnO$_2$ lead to the formation of both C-centered and OH radicals, whereas longer contact times lead to the formation of almost exclusively OH radicals. Moreover, our results suggest that in BPA/MnO$_2$/PAA system Bisphenol A interacts preferably with C-centered radicals, not OH radical. Thus, the radicals mainly responsible for effective degradation of BPA would be C-centered radicals. It could lead to the formation of potentially less xenoestrogenic intermediate compounds than the products of BPA degradation by Fenton reaction (which result from the interaction of BPA with OH radicals only).

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Magneto-Structural Correlation of New Dihalo-Bridged Copper Dimers

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Four new dihalo-bridged copper(II) dimers: [CuL(μ-X)]2, where L = N-(L-alanine methyl ester)-N'-(2-pyridin-2-yl)methyl)oxalamide or L = N-(L-valin methyl ester)-N'-(2-pyridin-2-yl)methyl)oxalamide and ion X = Cl or Br, have been synthesized and their crystal structures have been determined. The complexes consist of dimeric units, in which copper(II) ions are double bridged by Cl or Br ions. Single crystals and powder samples of the compounds have been investigated by X-band ESR (microwave frequency ν ≈ 9.6 GHz), in the wide temperature range. For crystals, beside temperature, angular dependences of g-factor and linewidths have also been studied. The obtained results will be discussed in terms of the copper ions coordination and interaction between copper ions. ESR results will be compared with the susceptibility and torque magnetometry results. The magneto-structural correlations will be established and comparison with similar compounds will be presented [1].


This work has been fully supported by Croatian Science Foundation under the project (HrZZ - UIP-2014-09-9775).
ESR signals in quartz from the present river bed sediments and in the possible source rocks

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ESR is one of method for the dating of samples in Quaternary [1]. ESR signal in quartz have recently been used as indicators to study the transportation of sediments on the surface for the provenance of aeolian dust [2], [3], as isotopes has been used. ESR signal intensities of quartz have been shown to be useful to distinguish the sediment provenance [4]. Sediment provenance could give important information on mountain uplift, river contention, and crustal movement.

We collected five river bed sediments and pyroclastic flow deposits, metamudstone with sandstone, and three granites which are possible sources of river bed sediments. The sample were crushed and sieved to 500 μm - 1 mm grain in size. The samples were treated with hydrogen peroxide, hydrochloric acid, hydrofluoric acid and heavy liquid separation. Thereafter, extracted quartz grains were crushed again to 120-250μm. All samples were irradiated by gamma ray to a dose of 2.5kGy. Samples were measured to observe the ESR signals using an ESR spectrometer (JES-X320; X-band JEOL RESONANCE Inc.), at an operating amplitude of field modulation 0.1 mT at 100 kHz.

In the present study, the ESR measurement was employed to investigate the possibilities of identifying sediment provenance quantitatively. The mixture ratios of the present river bed sediments could be calculated from the ESR signal intensities of the possible source rocks.

Reference
Use of peracetic acid homolysis for the degradation of atrazine: spin trap ESR and DFT calculations

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Atrazine is a widely used herbicide despite the fact that it was shown to be an endocrine-disrupting chemical. It is used to control grass and broadleaf weeds in many countries, and it is often found contaminating water supplies. Although such methods as granular activated carbon, ozone oxidation, reverse osmosis or ion exchange have been used for removal of atrazine from drinking water, there is still a need for easy, high-performance and safe method for the degradation of this compound in drinking water and groundwaters.

In this work we have examined the mechanism of atrazine degradation by peracetic acid (PAA) homolysis catalyzed by MnO2. We have applied ESR spin trapping with 4-hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide (FDMPO) spin trap and UV-Vis spectroscopy, traditionally used to monitor degradation reactions of organic compounds. DFT calculations have been used to identify the energetically preferred reaction paths as well as the intermediate radical and non-radical products. The DFT calculations were performed at B3LYP/6-31G(d,p) and MO6X/6-311+G(d,p) levels of theory in Gaussian 09.

Atrazine degradation under MnO2/PAA homolysis has led to additional reaction paths (with CH3-, CH3COO- and CH3CO- radicals) to those observed under the most widely used advanced oxidation processes such as the Fenton reaction (with OH- radical only). Monitoring of the reaction with UV-Vis spectroscopy was not very effective, as the absorption of atrazine and its products strongly overlapped, thus no changes were observed in the first 60 minutes of the reaction. On the other hand, ESR spin trapping showed that in the first 10 minutes of the reaction the active degradation of atrazine occurred. Also, in the presence of atrazine only FDMPO/OH- radical adducts were present, in contrast to reference MnO2/PAA system where FDMPO/OH- (70% of the total ESR spectrum) and FDMPO/CH3COO- (30% of the total ESR spectrum) radical adducts were observed. These results suggest that C-centered radicals play an important role in the atrazine degradation.

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Advanced EPR spectroscopy at high fields/frequencies sheds light on the radical transfer in E. coli ribonucleotide reductase

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Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleotides to deoxyribonucleotides in all living organisms. The E. coli RNR uses a catalytic cycle that involves a long-range proton-coupled electron transfer (PCET) from a tyrosyl radical (Y122•) in subunit β to a cysteine (C439) in the active site of subunit α, which subsequently initiates nucleotide reduction. This oxidation occurs over 35 Å and involves a specific pathway of redox active amino acids (Y122 ↔ [W48?] ↔ Y356 in β ↔ Y731 ↔ Y730 ↔ C439 in α).1 The mechanisms of PCET steps at the interface of the αβ complex remain puzzling due to lack of structural information in this region. Recently, DFT calculations on 3-aminotyrosyl radical (NH2Y731•-α) trapped during PCET in E. coli RNR, suggested that R411-α, a residue close to the αβ interface, interacts with NH2Y731• and accounts in part for its perturbed EPR parameters.2 To examine its role, we have further modified NH2Y731-α with a R411A substitution. NH2Y731•/R411A was generated and investigated by multi-frequency (34, 94 and 263 GHz) EPR, ENDOR and DEER spectroscopies.3 The data indicate a large conformational change of NH2Y731•/R411A relative to the single mutant. Particularly, the inter spin distance of NH2Y731•/R411A in one αβ pair to Y122• in the second αβ pair decreases by 3 Å in the presence of R411A mutation.3 This is the first experimental evidence for the flexibility of this residue in the active enzyme. The flexibility of these this pathway residue might be the key to drive the RT chemistry at the subunit interface. Therefore, we further investigate the interaction between these two essential residues via combination of EPR experiments at 263 GHz with rapid-freeze quench EPR experiments at 94 GHz. These experiments provided the first direct evidence of the communication between Y731 and Y356 during PCET.

10 Abstracts for posters in session "Emerging techniques"
Fluid flow dynamics in MAS systems
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In the present work we focus on the turbine system and the radial bearing of high performance Magic Angle Spinning (MAS) probes with 0.7mm and 1.3mm-rotor diameter at spinning rates up to 100kHHz. We confine our analysis mainly to the fluid flow properties of the MAS system. Therefore, Computational Fluid Dynamics (CFD) simulations and fluid measurements of the turbine and the radial bearings have been performed (see figure below). It can be seen that the MAS turbines are operated close to the speed of sound of the drive fluid (see figure below). CFD simulations and measurement results show relatively low efficiency (about 20%) compared to standard turbo machines. However, in particular, MAS turbines are mainly optimized for speed and stability instead of efficiency. We have compared MAS systems for rotor diameter of 0.7mm to 7mm converted to dimensionless values with classical turbomachinery systems showing that the operation parameters (rotor diameter, inlet mass flow, spinning rate) are in the favorable range (see [1] for an overview of the 1.3mm turbine results at room temperature).

CFD simulations of the radial bearings have been compared with basic theoretical values. Frictional losses generated inside the radial bearings result in undesired heat-up of the rotor. The rotor surface temperature distribution computed by CFD simulations shows a large temperature gradient over the rotor.

We have conducted a comprehensive simulation study of the NMR turbine and bearing system at room temperature for different turbine diameters and geometries. This study is currently extended to low temperature probe configurations (100K) that are employed for DNP experiments. For this application drive gas consumption and turbine efficiency is of significantly higher importance than for room temperature. Therefore fluid flow optimization is essential in this case.

Streamlines of drive flow through MAS turbine (left); Mach number contours in a cut through 1.3mm turbine (middle) and 0.7mm turbine (right).

Among the NMR active nuclei used in protein spectroscopy, $^{15}$N has the lowest gyromagnetic ratio and associated slower transverse relaxation rate resulting in ultra narrow line widths. However, the low intrinsic sensitivity is a major disadvantage of $^{15}$N detection and has deterred NMR method development in this direction. It was recently shown that $^{15}$N detection with TROSY selection could be used for large molecular weight systems at higher magnetic fields to obtain resolution with reasonable sensitivity. Taking advantage of the narrower line width we have developed a suite of 3D experiments to aid resonance assignments of Intrinsically Disordered Proteins (IDPs) and Intrinsically Disordered Regions (IDRs).

IDPs and IDR are proteins or sections of proteins that lack stable and well-defined secondary structures. It is now well established that IDPs/IDRs play an important role in key cellular processes and harbor several sites capable of post translation modifications, which orchestrate transcription, translation, self-assembly and signaling cascades enabling rapid cellular response. The lack of well-defined structure and the associated flexibility make NMR as the only method to study these proteins at high-resolution. One of the main challenges in studying these IDPs by NMR is the narrow chemical shift dispersion in the $^1$H dimension (~1ppm) and $^{13}$C dimension (~20ppm). Comparing to this these proteins have a reasonable dispersion in the $^{15}$N dimension (~30ppm). IDPs are often riddled with Proline residues, and Proline rich segments, which present a challenge for traditional $^1$H, detected backbone resonance assignment strategy.

We present a suite of $^{15}$N detected NMR experiments for the resonance assignments of IDPs and IDR. These experiments take advantage of the narrower lines and relatively large chemical shift dispersion in the nitrogen dimension of disordered proteins compared to that of proton and carbon. We have used these experiments to complete 100% backbone resonance assignments of the unstructured regulatory domain of the transcription factor Nuclear Factor of Activated T cells (NFAT). The 925-residue transcription factor NFAT is a key regulator of T-cell activation. Upon translocation to the nucleus it activates transcription of interleukin-1b and stimulates the T-cell response. The N-terminal regulatory domain (~400 amino acids) is primarily unstructured has been difficult to characterize. It contains a transactivation domain, an array of phosphorylation sites and segments important for binding the calcium-dependent phosphatase calcineurin.

The talk will describe a suite of $^{15}$N detected 2D/3D experiments for resonance assignment of IDPs/IDRs and will discuss the merits and limitations of these $^{15}$N detected experiments compared to other established methods for IDPs. In addition, strategies to utilize $^{15}$N detection to tackle higher molecular weight systems will also be presented.
Optimum control-based Hahn echo sequences and pattern pulses: Individually and cooperatively optimized pulses

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Rectangular pulses are the workhorse of magnetic resonance spectroscopy, however their performance is experimentally limited. Optimal control provides tools to improve pulse performance and robustness, by tuning of hundreds or thousands of pulse parameters at feasible computational costs[1]. Individually optimized pulses have been applied to NMR as well as EPR systems[2].

It was shown previously that concurrent optimization of pairs of pulses rather than individual pulses offers significant gains in pulse performance over the latter[3]. The Hahn echo building block is part of many pulsed magnetic resonance experiments and was thus optimized cooperatively by us. The performance of cooperative optimum control echo sequences exceeds the one obtained with individually optimized pulses of similar amplitude and pulse duration. In order to improve the optimization process, we investigated a novel method to circumvent said obstacles, which ensures convergence with a small number of random starting sequences.

In addition we examined robust inversion pulses for electrically detected magnetic resonance multipulse experiments in collaboration with the group of M. S. Brandt. The setup contains strongly screened spin ensembles which interfere with the desired signal in multipulse experiments[4]. We optimized pattern pulses to suppress said ensembles[5]. Again, the problem was not well behaved and optimal control alone was unsuccessful in finding the global minimum. We combined heuristic search algorithms as demonstrated by Zahedinejad, Schirmer and Anders with optimum control to achieve a satisfying solution[6-7].


Sub-mHz Precision Measurement of J-Couplings via Zero- to Ultralow-Field Nuclear Magnetic Resonance


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Zero- to ultralow-field NMR is an alternative magnetic resonance modality where measurements are performed in the absence of an applied magnetic field, such that spin dynamics are determined primarily by “local” spin-spin interactions, rather than couplings to laboratory-frame fields [1]. In this field regime, relaxation due to magnetic field inhomogeneity and/or chemical shift anisotropy is negligible, leading in many cases to long coherence times, which allow for the acquisition of high resolution J-spectra. Resonance widths are routinely narrower than 1 Hz, even in anisotropic samples [2], and linewidths on the order of 10 mHz have been observed for benzene-1,13C1 [3]. By implementing an efficient numerical fitting program for ZULF NMR spectra, we have been able to extract J-couplings with sub-mHz precision. For example, we have measured the 6-bond 1H–1H coupling in neat toluene-α-13C1 to be −0.57512 ± 0.00027 Hz.

We have also been able to observe the primary isotope effect on the N–H coupling in a solution of 14NH3Cl and 15NH3Cl (see Fig. 1). For 14NH3+, the absolute value of the reduced coupling constant is |K14N1H| = 6.0372 ± 0.0019 × 1020 NA−2 m−3, and for 15NH3+, it is |K15N1H| = 6.0286 ± 0.0007 × 1020 NA−2 m−3, yielding a difference of 0.0086 ± 0.0020 × 1020 NA−2 m−3 (95% confidence).

We will further discuss how analysis of systematic errors (e.g. temperature gradients, field drifts, etc.) allows us to better control experimental procedures going forward as we aim for ever-higher precision. Finally, we will describe how high-precision ZULF NMR spectroscopy may be useful both for chemical analysis and for laboratory searches for exotic spin-dependent interactions that could result from physics beyond the Standard Model.

References:
NV-nuclear polarization transfer using refocused spin-locking

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Nitrogen vacancy (NV) centers in diamonds are naturally occurring point defects with an electronic spin ¹. These NV spins can be optically polarized and detected, and exhibit exceptionally long coherence times even at ambient conditions. Coherent polarization transfer from an NV spin to its surrounding nuclei is of interest for various applications, such as: quantum information storage, NV based NMR & MRI, and bulk nuclear polarization (DNP). This can be achieved at low fields, together with dynamical decoupling of the NV spin from its environment, via a spin-lock (SL) sequence with amplitude $\Omega_{SL}$ matching the nuclear frequency $\omega_{nucleus}$, as given by the Hartmann-Hahn condition.

In this work we propose a refocused SL sequence, where periodic $\pi$ pulses are applied during the SL. This can be used to tune the polarization transfer condition, allowing for example the use of high power SL for polarization transfer at very low fields or for polarization transfer to low $\gamma$ nuclei, where the SL sequence is inefficient.

![Figure 1](image-url)
Electron Nuclear Cross Polarization (eNCP) in ENDOR Spectroscopy

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Electron nuclear cross polarization (eNCP) is a method that we developed to transfer electronic polarization to nuclear spins in a more efficient way with respect to conventional sequences. It requires concomitant microwave (MW) and radiofrequency (rf) irradiation under the fulfillment of specific conditions of rf field strengths and irradiation offsets. Recently, the validity of the method was demonstrated in combination with Electron Nuclear Double Resonance (ENDOR) spectroscopy, using both model system and proteins.

In analogy to CP in NMR, the polarization transfer in eNCP is achieved by locking the electron spin magnetization with MW irradiation in the \((x, y)\) plane, perpendicular to the external magnetic field. During this process, the electron spin can be described in a tilted frame, where the quantization axis is along an effective field resulting from the combination of hyperfine coupling \(A\) and MW field strength \(e\). The simultaneous rf irradiation at the frequency \(\omega_{rf}\) at an offset \(\Delta\omega_n = \omega_n - \omega_{rf}\) (being \(\omega_n\) the nuclear Larmor frequency) tunes the energy of the states in the tilted frame and induces the polarization transfer if one of the four matching condition is fulfilled.

A malonic acid single crystal, having a well defined strong hf coupling, was used as model system. The CP-ENDOR spectra, obtained working at W-band (94 GHz) at \(T = 30\), display a polarized pattern, where the relative intensities of the two lines match the theoretical predictions. The same experiments were carried out on a protein, the \(\beta 2\) subunit of \(E.\ coli\) ribonucleotide reductase (RNR), which contains an essential tyrosyl radical. Notably, a larger signal-to-noise (S/N) ratio (factor of about 3.5) was observed for CP-ENDOR with respect to the standard Davies-ENDOR technique, although both experiments were individually optimized for best performance. This demonstrates that CP-ENDOR provides an attractive and efficient new method to record ENDOR spectra on biological samples.

Figure 1: Schematic representation of the CP-ENDOR experiment in the case of an electron spin \(S = 1/2\) coupled with a nuclear spin \(I = 1/2\) with hyperfine coupling \(A < 0\), as in the malonic acid case. The predicted spectrum matches the experimental results.

References
Arrays of IC-assisted 3D-microcoils for wideband NMR spectroscopy

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Wideband NMR detectors have recently gained increasing attention in the NMR community as a tool to perform multinuclei NMR experiments on a larger variety of nuclei compared to multi-tuned coils. It has been shown in [1] that by using dedicated, miniaturized detection coils, the sensitivity which is achievable in such broadband NMR experiments is comparable to or even higher than with tuned detection coils. Additionally, the broadband NMR setup allows to straightforward conduct heteronuclear NMR experiments both in coupled and decoupled mode with virtually any combination of nuclei inside the bandwidth of the broadband detector [1]. In our proposed talk we will present measured data from a prototype NMR detector array for high-throughput wideband NMR spectroscopy according to Fig. 1a. The system consists of two linear four-coil arrays and two dedicated CMOS ASICs each containing four broadband low-noise receivers. Compared to our previously presented system [2], the prototype displays two important improvements: Due to an improved manufacturing process the 3D-microcoils provide a filling factor of 100% and the improved noise performance of the receiver electronics removes the need for a tuning capacitor thereby enabling a true wideband operation from approximately 1 MHz to 1 GHz, cf. Fig. 1b.

Figure 1: (a) Center: Rendering of the presented IC-assisted array of μNMR coils. Left zoom-in: Micrograph of the CMOS ASIC containing for low noise broadband NMR frontends. Right zoom-in: Micrographs of the 3D-microcoils used as NMR detectors. (b) Simulated (parasitics extracted) gain and noise performance of the receivers.

References


Efficient Monitoring of Photo-Induced Reactions by NMR

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It is challenging observing photochemically-induced chemical reactions using NMR. On one hand the NMR spectrum provides an ideal fingerprint of the product formed, on the other hand a substantial background signal stemming from non-irradiated regions of the sample exists. Here, we present a strategy how photo-induced chemical conversions can be followed in situ by NMR at high efficiency minimizing background effects.

When NMR tubes (ID ≈ 0.34 cm) are used to monitor photochemical reactions, special care needs to be taken on the optical properties of the studied system. If the extinction coefficient is too high, the bulk volume inside the NMR tube is only marginally irradiated. We have used an "inner-tubing" system (Figure) providing tailor made thin liquid layers (≈ 0.05 mm) adjustable to the optical density of the sample solution. The thus produced exclusion of the “dark” bulk volume in the middle of the NMR tube allowed recording NMR spectra being virtually background-free.

We applied this approach for studying photo-triggered polymerizations and complexes displaying photo-induced ligand rearrangements. Moreover we performed CIDNP measurements with this setup.

*Figure.* Thin layer sample with inner tube moved down (left) and inner tube moved up (right). Sample volume = 200 μL.
Mechanically induced $^{23}$Na DQF MA response as a rheo-molecular biomarker in body fluids

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Many body fluids are complex in nature, driven by body physiology. Changes in their in situ rheology are usually associated with the changes in their molecular composition. It is hypothesized that this molecular-mechanical link might be indicative of the clinical picture in health and disease. We report in this work that rheo-MQ $^{23}$Na NMR probes molecular structures formed at the microscopic (histological) length scales in the drug Hylaritin V (1% hyaluronic acid injectable cross-linked gel) currently used in osteoarthritis (OA) treatment and in a 3% solution of hyaluronic acid – a major component of synovial fluid in joints.

We report that the formation of these specific molecular structures is governed by shear and occurs only in the presence of cross-links between hyaluronic acid chains or intermolecular chain entanglements. Bulk rheometry indicates that either fluid is viscoelastic and exhibits solid-like behavior when flows. We hypothesize this is governed by the necessity for the fluid to act as an efficient shock absorber to prevent unwanted bone damage in synovial joints in the case of an excessive joint load.

We have measured both $^{23}$Na TQF and DQF MA signals in both fluids under physiological sodium concentration and strong $^{23}$Na DQF MA signals in both fluids upon shear were found. Remarkably, the signals were absent in the absence of shear. Furthermore, no $^{23}$Na TQF signals were detected with or without shear in both fluids. 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.

The work is of great importance in designing the strategies for in vivo $^{23}$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 $^{23}$Na DQF MA signals must always be probed even in the absence of $^{23}$Na TQF response.
Real-time broadband proton-homodecoupled CLIP/CLAP-HSQC  
for precise and automated measurement of heteronuclear one-bond coupling  
constants

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Conventional CLIP/CLAP-HSQC experiments used for measuring heteronuclear one-bond couplings (¹JₓH) have limited spectral resolution and precision due to proton-proton couplings that yield complex multiplet structures for cross peaks. Pure shift CLIP/CLAP HSQC methods utilizing interferogram-based proton decoupling (1) show an order-of-magnitude improvement in resolution, but require longer experiment time. To speed up ¹JₓH measurements, we have implemented the real-time acquisition scheme of pure shift HSQC (2) into CLIP/CLAP-HSQC sequences. This makes it possible to acquire decoupled signals in real-time, in a single free-induction decay, repetitively applying a BIRD pulse sequence element and a non-selective 180° proton pulse (3).

The resultant PS CLIP/CLAP HSQC spectra contain broadband proton-homodecoupled pure in- or anti-phase doublets with improved resolution (Fig. 1), allowing direct and highly precise measurement of ¹JₓH using quick automatic peak picking during spectral analysis.

The utility of the real-time PS sequences presented here has been established for ¹JₓH and ¹DₓH measurements on a protein sample under both isotropic and anisotropic conditions. The numerous tests and comparisons with reference data performed on a small model compound also show that the real-time PS CLIP/CLAP HSQC experiments show good precision, reproducibility and robustness for the measurement of ¹JₓH and RDCs (¹DₓH).

References:

Financial support of this work by Hungarian Scientific Research Fund (OTKA) and 'Talentum Foundation' of Gedeon Richter Plc. is gratefully acknowledged.

Figure 1. Proton-coupled (black, lower) and real-time broadband proton-homodecoupled (red, upper) ¹H-¹⁵N CLIP-HSQC spectra of ¹⁵N-labeled Penicillium antifungal protein
A Zangger-Sterk based pure-shift experiment not requiring full spatial separation of coupling partners for studies of peptidomimetic oligomers

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The interpretation of proton spectra of molecules with increasing complexity is often hampered by signal overlap due to homonuclear scalar coupling. Recently the application of pure-shift techniques successfully enhanced the resolution in one- and multidimensional proton detected experiment by simplifying multiplet structures.

A major drawback of methods for broadband decoupling is their inherent sensitivity loss. In Zangger-Sterk[1] based techniques this is caused by a decrease in sample volume contributing to detectable signal. Various approaches have been presented to reduce this sensitivity penalty, such as multi-slice-excitation, nemo-Zangger-Sterk, ASAP or PSYCHE.[2-5]

Here we present an alternative approach to improve sensitivity in Zangger-Sterk decoupled spectra in combination with the Perfect-Echo experiment.[6] This enables us to increase the volume fraction that is used for signal acquisition, without compromising in spectral quality (figure 1). Even in the presence of strong coupling the Perfect-Echo-Zangger-Sterk experiment produces clean and widely artifact free spectra.

We show that this experiment is particularly suitable to study repetitive motives in oligomeric structures, where signal clustering and strong coupling render classic Zangger-Sterk decoupling inefficient.

Photochemical pump and NMR probe: exploiting magnetic coherence to obtain kinetic information on a microsecond timescale

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Various time-resolved techniques that involve fast detection methods such as UV/vis or IR spectroscopy are available to study reaction kinetics on very short timescales. However, the structural information provided by these methods is limited compared to that available with NMR.

We have recently demonstrated a time-resolved spectroscopy method in which NMR can be used as a detection technique for events on a microsecond timescale where sensitivity issues have been overcome with the use of para-hydrogen [1]. Reactions of $p$-H$_2$ and transition metal dihydride complexes were initiated in a synchronous manner with a laser source and the generated magnetic coherence was probed with an RF pulse after intervals as short as 10 μs.

A key feature of this method is that unlike typical $p$-H$_2$ experiments where only time-averaged states are observed, the generated zero quantum coherences, which oscillate according to the spin topology of the product, can also be monitored. Systems where the rate of chemical reaction is comparable to magnetic evolution are currently being investigated to obtain kinetic information from the amplitude and phase of these oscillations on a microsecond timescale.

Figure 1. (a) Scheme of the laser irradiation system into the NMR probe, (b) generation of PHIP enhanced product after photochemical reductive elimination of H$_2$ and (c) basic NMR pump–probe sequence employed in this work and the information that can be extracted depending on RF pulse angle $\theta$.

11 Abstracts for posters in session "Exotica"
Multinuclear low-field liquid- and solid-state NMR for industrial applications with focus on mobile $^{14}$N, $^{17}$O, $^{31}$P, and $^{39}$K NMR for monitoring of nutrients in animal slurry

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Results of a novel multinuclear NMR instrument with a permanent (Halbach) magnet will be presented. Recently, we introduced a high-sensitive $^{27}$Al NMR sensor for onboard-ship monitoring of aluminosilicate zeolites in marine heavy fuel oil, and, lately, this technology has been expanded to include detection of numerous other nuclei for various applications. One application is monitoring of the nutrient content (NPK) in animal slurry by $^{14}$N, $^{17}$O, $^{31}$P, and $^{39}$K NMR operating on a slurry spreader. Animal slurry is both a waste product and an important resource, and in modern farming, actual knowledge of the nutrient content is highly important in order to optimize plant growth and avoid environmental pollution. Our NMR results show good agreement with external reference measurements and demonstrate that cost-efficient NMR can be applied as an alternative to crude estimates or impractical off-site analyses. Using our new instrument, all isotopes can be detected interleaved on the same sensor using digital tune and match switching. Based on this, we will also include results on broadband NMR applications of the sensor focusing towards efficient acquisition of for example $^{14}$N solid-state resonances.

Figure 1. a) Animal slurry is applied to agricultural field. b) NanoNord digital NMR instrument (with 1.3 T Halbach magnet) for online multinuclear applications including monitoring of nutrients in animal slurry when spread to fields.
Tapered Stripline NMR: Revival of the Radiofrequency Field Gradient

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In recent years our group has introduced a stripline based RF coil as an efficient NMR detector[1,2] for small-volume (nL - µL) or flat samples. This stripline 'coil' achieves both good resolution (0.9 Hz for ²H) and high sensitivity (LOD = 10¹³ spins/(Hz)¹/₂ for ²H). Its applicability was demonstrated for the monitoring of chemical reactions,[3] the study of mass-limited samples (i.e. cerebrospinal fluid[4]) and thin films (i.e. III–V alloy semiconductors[4]).

A minor change in the geometry of the stripline, i.e. giving it a tapered shape, makes it possible to generate well-defined RF field gradients. This is because the B₁ field generated by the stripline depends on its width.

Magnetic field gradients are used in many areas of magnetic resonance, most often gradients in the external field (B₀) are used. However, B₁ gradients may provide several advantages over B₀ gradients: They are less demanding for the hardware (lower powers, no local mechanical forces, no additional amplifier) and the gradient is permanently present so that no time-consuming switching of the gradient is needed (advantageous for samples with short T₂/s).

We have fabricated a tapered stripline that produces a perfectly linear B₁ field gradient and demonstrated that it can be used to perform (one-dimensional) imaging using a two-dimensional nutation experiment.[5] This allows the combination of localized spectral and spatial information, obtaining both good spatial and spectral resolution.

We combined the tapered stripline with a microfluidic system and used it to monitor very fast reactions (10⁻² – 10⁻¹ s).[5] Reactants are mixed inside a capillary and this mixture flows with a constant rate over the taper. The nutation pulse sequence is then used to measure the chemical composition as a function of the spatial coordinate, which is, due to the constant flow, directly related to the reaction time. This method can provide extremely valuable mechanistic and kinetic information; however, it also shows that fast mixing essential.

As a third application we looked at performing diffusion measurements with the tapered stripline. The basis for diffusion NMR with B₁ gradients has been developed by Canet, Kimmich and others.[6,7] We found that by combining these methods with the tapered stripline and microfluidic setup leads to a unique setup to perform diffusion measurements, as pressures up to 300 bar can easily be achieved. This allows us to study for instance molecular dynamics in liquid or supercritical CO₂.[8]

References:
Alignment of Molecular Endofullerenes in a Nematic Liquid Crystal

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Molecular endofullerenes are complexes of a single small molecule (H₂O, H₂ or HF) encapsulated in a C₆₀ cage. The small molecule is trapped inside the cage but it still enjoys a complete rotational freedom. We observed that in a nematic liquid crystal (N-(4-Methoxybenzylidene)-4-butylaniline, MBBA) the rotation of the encapsulated molecules becomes anisotropic – the ¹H NMR lines of H₂ and H₂O are split by residual dipolar coupling (RDC) into doublets. The doublets collapse into single lines when the liquid crystal is heated to induce transition from the nematic to isotropic phase. The RDC contribution was also observed in room temperature ¹⁹F spectra of encapsulated HF molecules.

We discuss two possible mechanisms of the observed partial alignment of the endohedral molecules: a) Interaction between the encapsulated molecule and the cage induces distortion of the cage and orients the molecule relative to the cage. The liquid crystal then aligns the complex as a whole. b) The cages are distorted and aligned by interaction with the liquid crystal. The cages then present the endohedral molecules with an anisotropic confining potential thus aligning them.

The observed phenomenon could potentially allow the detection of quantum rotor induced hyperpolarization during para-to-ortho conversion of nuclear spin isomers of H₂@C₆₀ and H₂O@C₆₀.

Figure 1: Room temperature ¹H NMR (300 MHz) spectrum of H₂O@C₆₀ dissolved in MBBA nematic liquid crystal. The H₂O@C₆₀ signal is split by residual dipolar coupling of the two ¹H nuclei and can be seen as a sharp doublet (splitting 770 Hz) on unresolved MBBA background.
**MRFM detection of water in a single micropore in quartz**

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Magnetic resonance force microscopy (MRFM) has great potential for measuring small amounts of liquids and liquid containing samples (i.e. porous media, biological samples) from the microscopic (picoliters) down to the nanoscopic (femtoliters) regime (1, 2, 3).

In the presented work, water in a single pore of quartz (called fluid inclusion) was measured at a room temperature, home-build MRFM setup. The pore diameter is about 20 um (~ 4 picoliters) and contains mostly water and a small water vapor bubble. T₁ -relaxation measurements have been carried out (see figure). Furthermore Hahn echo experiments were done in the presence of the strong magnetic gradient necessary to detect signals with MRFM. The role of self-diffusion is discussed. These measurements help to understand the possibilities of MRFM for measuring liquids with high self-diffusion constants. The results serve as a model for imaging and spectroscopy of other water containing samples (i.e. biological cells).

![Graph of T₁-inversion recovery of water in a single micropore of quartz](image)

References:
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Magnetic inequivalence revisited:
the deceptively simple/complicated spectrum
of a $^{13}$C,$^{15}$N-labeled trimethyl amine

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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 13-spin system of a $^{13}$C,$^{15}$N-labeled trimethyl amine (Fig 1A,B). The protons of the three methyl groups are chemical equivalent due to the molecular symmetry, but are not magnetically equivalent as they have different $^1J_{CH}$ and $^2J_{CH}$ couplings. As a result, the $^1H$ spectrum of this compound shows additional splittings due to (small) $^4J_{HH}$ and $^2J_{CC}$ couplings.

Using subspectral analysis we show that the spectrum is composed of $a_3$, $a_b$ and $abc$ subspectra for both $^1J_{CH}$ doublet components.$^{2,3}$ Due to the small size of $^4J_{HH}/^2J_{CC}$ with respect to $^2J_{CH}$ and $^1J_{CH}$ the subspectra can be well approximated as $a_3$, $a_x$ and $a_x^2$ (Fig 1C).

The same reasoning is used to show that homo-decoupling of the $^1J_{CH}$ doublet transforms the spin system to a set of fully equivalent spins, resulting in disappearance of the additional $^4J_{HH}$ and $^2J_{CC}$ splittings (Fig 1D).

**Significance**

We believe this curious case is a textbook-worthy, instructive example of magnetic inequivalence. The spectra may be considered deceptively simple$^4$ 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.

**References**


*Figure 1.* (A) the downfield component of the $^1J_{CH}$ doublet; (B) molecular structure and relevant J-couplings; (C) approximated spectrum; (D) saturation of one $^1J_{CH}$ doublet component results in a subspectrum of fully equivalent $^1H$ spins.
Interconversion between long-lived singlet order and heteronuclear magnetisation to enable sensing applications

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Longitudinal magnetisation and singlet order can be interconverted by ad-hoc developed pulse sequences in homonuclear spin pairs. Singlet order is long lived, silent and accessible on demand and has been exploited for hyperpolarisation storage and sensing applications. For example, molecules carrying singlet order functionalities have been used as a \textit{spy} to sense ligand binding or as a \textit{probe} reporting on very slow flow and diffusion over macroscopic length scales. In all those examples a spin-1/2 pair acts as both the polarisation \textit{storage centre} and the \textit{sensor}.

Further interesting possibilities arise by separating the sensor functionality from the storage centre. For example, hyperpolarisation could be stored as long lived singlet order in a spin-1/2 pair made by low-$\gamma$ nuclei (to further extend the storage capability, as well as the spin memory) and then transferred to an heteronucleus with higher gyromagnetic ratio and hence better sensitivity. Conversely, proton polarisation generated easily and efficiently via the most recent DNP techniques could be stored in the much longer-lived singlet order of a nearby carbon or nitrogen spin pair. Finally, a spin located on a sidearm remote from the singlet pair could be made responsive to the environment (pH, temperature or the presence of a given compound) and hyperpolarisation can be deposited there at selected times or spatial locations to report about those environmental changes. In all those potential applications, it would be necessary to interconvert singlet order and heteronuclear magnetisation in an efficient manner.

In this contribution we describe new radiofrequency pulse sequences, and the theoretical framework they are derived from, that implement this interconversion with maximum theoretical efficiency.
12 Abstracts for posters in session "In-vivo and in-cell NMR"
Early detection of photoimmunotherapy-induced tumor cell death with hyperpolarized [1,4-13C2]fumarate

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Purpose

Photoimmunotherapy (PIT) is a novel therapy for cancer treatment. (1) PIT is induced by an antibody-photon absorber conjugate that, when exposed to near infrared light (NIR), induces tumor cell necrosis. Previously, we have demonstrated that NIR PIT is an oxygen dependent process (2). Early experience with NIR PIT demonstrates that while the tumor may become necrotic, its size does not immediately change and so it can be difficult to assess early treatment effectiveness. We explored the use of 13C-hyperpolarized MRS before and after NIR-PIT to better understand the early metabolic changes in this treatment. [1,4-13C2] Fumarate is a 13C labeled tracer for MRS, which can be used to identify tissue necrosis. Fumarate only distributes in the extracellular space where there is a low level of fumarate hydratase (FH). However, in necrosis, FH leaks out of the damaged cell and converts fumarate to malate which is detected on MRS. In contrast 13C pyruvate is readily transported across the membrane and converted to lactate by lactose dehydrogenase (LDH), a process that generally occurs intracellularly but can also occur extracellularly.

Results and Discussion

Real time sO2 mapping showed rapid decreases in local oxygenation suggesting oxygen consumption and conversion to singlet oxygen that mediated membrane damage during PIT. Histologic analysis confirmed immediate cell damage after PIT. Tumor growth curves showed the strong anti-tumor effect of PIT. Dynamic spectra of 13C labeled pyruvate and fumarate showed that the lactate-to-pyruvate ratio was unchanged after PIT, while the malate-to-fumarate ratio increased 5 fold in the PIT treated group. Since pyruvate is a tracer that can be easily taken up into the cell via monocarboxylate transporter, its conversion to lactate is independent of membrane damage. Fumarate, on the other hand is restricted to the extracellular space and is converted to malate at a higher rate only when FH leaks from necrotic cells, as occurs in early PIT. Thus, 13C fumarate hyperpolarized MRS demonstrates the early effects of NIR PIT-induced necrosis.

Conclusion

Using MRS after injection of hyperpolarized substrates, we demonstrate that the malate-to-fumarate ratio increased 5 fold in NIR PIT treated tumors whereas the lactate to pyruvate ratio was unchanged. The conversion of fumarate to malate supports a necrotic cell death mechanism for PIT. 13C labeled fumarate is thus, a promising tracer for the early detection of PIT mediated cell death

References


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Fast and Accurate Simulation of Spatially Localized Magnetic Resonance Spectroscopy

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Introduction: Spatially-selective RF pulses lead to spatial dependence of spin magnetization evolution. Consequently, numerical simulations of three-dimensionally localized magnetic resonance spectroscopy (MRS) are time-consuming for multispin systems because of the need to compute an ensemble of spins in three dimensional space [1-3]. A pervious study used 40×40×40 points to define the selected voxel, which needed several hours on a computer cluster for simulating a metabolite with more than four spins [3]. As numerical simulations are gaining popularity for designing and validating in vivo MRS detection methods as well as for providing quantification basis sets, improving the efficiency and accuracy of spin density matrix simulations is much needed. This abstract reports a highly accelerated and accurate method for simulating spatially localized MRS spectra.

Methods: A new numerical simulation method has been developed that transforms the conventional GAMMA [4] simulation of spatially localized multispin system into a highly efficient linear projection problem. The method has been implemented in Java and utilizes multithreading to further speed up the computation process. In this abstract, the simulation of a PRESS pulse sequence was demonstrated using 180°×180°×330 points to define the voxel, with RF bandwidths of 2200 Hz and 1200 Hz for excitation pulse and refocusing pulses, respectively. The simulated spectra were compared with phantom spectrum acquired from a GE 3 T scanner.

Results: Figure 1 shows that the short echo time (35 ms) lactate spectra simulated using the new method and the traditional three-dimensional GAMMA method are completely identical. The new method finished in 10 seconds while the traditional method took 16 hours on the same laptop computer. Figure 2 shows the fit of a short TE phantom spectrum (black color) using the simulated basis sets (red color). The estimated metabolite concentrations (relative to creatine) was in good agreement with those of the listed ingredients of the phantom (±4%) which consisted of NAA (12.5 mM), creatine (10 mM), glutamate (12.5 mM), myo inositol (7.5 mM), and lactate (5 mM). The voxel size was 3×3×3 cm³. The baseline was originated from the residual water after water suppression.

Discussion: The new method represents a dramatic improvement in efficiency and accuracy for the simulation of spatially localized MRS. Thanks to the massive increase in computational efficiency, more spatial points can now be used to represent the three dimensional voxel, leading to a more accurate simulation. To the best of our knowledge, this was the first reported simulation with spatial points exceeding 100 in each of the three dimensions, and the computation time was as short as ~10 minutes on a desktop computer for a six spin molecule. The resultant simulation agreed well with the phantom experiment as demonstrated by Fig. 2.

Conclusion: The dramatically enhanced computational efficiency makes spin density matrix simulation of multi-spin systems a convenient tool for designing and optimizing MRS pulse sequences and for computing basis sets for spectral fitting. As many experiments such as two-dimensional spectroscopy and T2 measurements demand highly extensive simulations, the proposed method is expected to greatly facilitate progress in those areas.

References:
Probing pyruvate metabolism in pancreatic β-cells using hyperpolarized MRS

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Introduction: The dysfunction of pancreatic β-cells associated with type 2 diabetes is characterized by an inadequate secretion of insulin to regulate blood glucose homeostasis. The mechanism of insulin secretion in β-cells has been extensively studied. When the blood glucose level rises, the increased supply of glycolytically produced pyruvate to the mitochondria stimulates TCA-cycle metabolism. The resulting increase of the ATP/ADP ratio is the primary trigger of insulin release. The regulation of the pyruvate dehydrogenase complex (PDC) is suggested to play an important role in this context1. It has also been shown that exchange of pyruvate with TCA cycle intermediates via anaplerotic pathways, rather than through PDC, correlates with glucose-stimulated insulin secretion2. Further characterization of these mechanisms may provide a better knowledge of the pathological processes in β-cells involved in diabetes.

Aim: The aim of this study is to characterize pyruvate metabolism in INS-1 832/13 cells, a clonal cell line with widespread use as a model for pancreatic β-cells in diabetes, using hyperpolarized (HP) [1-13C]pyruvate and [2-13C]pyruvate MRS.

Methods: The cells were harvested, resuspended in fresh media at ~50 mill cells/ml and kept at 37 °C with frequent mixing up to 15 min before the experiment. [1-13C]pyruvate or [2-13C]pyruvate was hyperpolarized in the in-house 3.5T DNP polarizer. 1 ml of the HP substrate was injected into a 10-mm NMR tube followed by injection of 2 ml cell suspension. The 13C MRS signal was acquired at 11.7T by series of 15° pulses. The data for INS-1 832/13 cells were compared with data from a N2a (neuroblastoma) cell line, known to exhibit a highly glycolytic and cancerous phenotype.

Results and Conclusion: Signals from [1-13C]lactate and [1-13C]alanine were detected in the INS-1 832/13 and N2a cell lines (Fig. 1 and 2) after [1-13C]pyruvate injection. [13C]bicarbonate was only detected in the INS-1 832/13 cells, indicating a higher flux through PDC than in the N2a cells. In contrast, the conversion of pyruvate into [1-13C]lactate was higher in the N2a cell line. Only a low signal from [1-13C]lactate was detected in the N2a cells using [2-13C]pyruvate. We conclude that HP [1-13C]pyruvate MRS can be used to study pyruvate metabolism in the pancreatic β-cells by measuring flux of pyruvate through PDC in relation to insulin secretion. Future studies will show if the conditions can be optimized to detect metabolites also from the anaplerotic pathway.

Using HR-MAS for \textit{in-vivo} NMR spectroscopy

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Originally designed for solid-state NMR, magic angle spinning (MAS) spectroscopy in high resolution (HR) recently gains more and more interest for \textit{in-vivo} studies of whole intact small-size organisms. Even though this approach is quite promising, there are several technical limitations such as live-threatening centrifugal forces or hampered oxygen and nutrient supply. In our work we investigated four popular model organisms in systems biology upon their capability to be used for \textit{in-vivo} studies with a commercial Bruker 500 MHz 4mm HR-MAS system:

1. The tropical freshwater fish \textit{Danio rerio} is widely used to study vertebrate development and to model human diseases. High centrifugal forces when spinning at frequencies > 2500 Hz are a major limitation to study complex organisms in HR-MAS. Recent advances in combined water and sideband suppression allow spinning at less than 500 Hz [1] and we applied this novel protocol to monitor the \textit{in-vivo} embryogenesis of a total of 25 embryos until hatching.

2. The small roundworm \textit{Caenorhabditis elegans} is a nematode and used for example in neurosciences and to study gene function. It was the first multi-cellular organism whose genome was completely sequenced. Its strong cuticle is quite resistant against centrifugal forces and we investigated the metabolic \textit{in-vivo} response against different stress triggers.

3. \textit{Saccharomyces cerevisiae} belongs to the domain of fungi and is not only one of the most important eukaryotic model organisms in biology but also indispensable for food industry and biofuel production. In this context the HR-MAS rotor converged to a micro-reactor allowing to monitor degradation kinetics of different sugar substrates and ethanol yield in real-time (Figure 1).

4. Finally we investigated the metabolic response of the bacterium \textit{Escherichia coli} against subinhibitory concentrations of antibiotics under anaerobic conditions (as they occur in the human colon) and showed dose-effective dependent reactions.

Our results demonstrate that HR-MAS in general holds a big potential for \textit{in-vivo} NMR with a wide range of applications if boundary conditions for each organism are carefully determined and kept.


\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{\textit{In-vivo} growth of \textit{S. cerevisiae} on 100 mM sucrose ($\Delta t = 34$ min) and selected kinetics}
\end{figure}
Quantitative Detection of PEGylated Biomacromolecules in vivo by Magnetic Resonance Spectroscopy

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The accumulation, biodistribution, and clearance profiles of therapeutic agents are key factors relevant to their efficacy. Determining these properties constitutes an ongoing experimental challenge. Many such therapeutics, including small molecules, peptides, proteins, tissue scaffolds, and drug delivery vehicles, are conjugated to poly(ethylene glycol) (PEG) as this improves their bioavailability and in vivo stability. We demonstrate here that 1H NMR spectroscopy can be used to quantify PEGylated species in vivo directly, rapidly and in real time. PEG bears a large number of spectroscopically equivalent protons exhibiting a narrow NMR line width while resonating at a 1H NMR frequency distinct from most other biochemical signals. PEG provides a robust signal allowing detection of concentrations as low as 10 µg/mL in blood. This PEG detection limit is lowered by another order of magnitude when background proton signals are minimized using 13C-enriched PEG in combination with a double quantum filter to remove 1H signals from non-13C-labeled species. More practically, we quantify the blood clearance of 13C-PEG and PEGylated-BSA (bovine serum albumin) following their intravenous injection in live rats. Given the relative insensitivity of line width to PEG size, we anticipate that the biodistribution and clearance profiles of virtually any PEGylated biomacromolecule can be routinely measured by this method.

Figure 1. 1H NMR spectra with HMQC filtration from a 50 µL aliquot of goat serum containing 1 µg/ml of 13C-PEG2k diluted to 400 µL containing 3 µM 13C-ethylene glycol. Blue line indicates suppressed water peak, green: 13C-PEG, yellow: 13C-ethylene glycol
NMR as evaluation strategy for cellular uptake of nanoparticles

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We developed a new experimental method based on ¹H-NMR relaxation to investigate the cellular uptake of nanoparticles in cells¹. At first, the cellular uptake of rhamnose-coated magnetic nanoparticles (MNPs) in human tumor (glioblastoma) cells was studied. After the incubation of the cells with different concentrations of MNPs, the transverse nuclear relaxation time was measured. A fast relaxing signal ($T_{2,FAST}$) coming from intracellular protons and a slow relaxing signal ($T_{2,SLOW}$) from extracellular protons can be distinguished. Given that the $T_2$ value is inversely proportional to the magnetic particle concentration, $T_{2,FAST}$ and $T_{2,SLOW}$ are indirect measurements of the amount of MNPs in the intracellular and in the extracellular compartment, respectively. The relaxation data as a function of the MNPs concentration were fitted with a dose-response analytical curve² and an uptake law for our MNPs was extracted. Furthermore, the weight of each relaxation component gives a quantitative estimation of the amount of protons in each biological environment (i.e., intra- and extracellular), allowing to follow the cellular swelling, directly connected to the cellular stress due to the injection of an exogenous agent in the biological system.

Finally, the proposed experimental protocol was successfully tested with gold nanoparticles and antitumor drug as well on two different cell lines. Such method can provide a new way to investigate in a quantitative way the general problem of cellular uptake for a variety of bio-compatible nanostructures and drugs.

**References**


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**Figure 1:** (left) Fast and slow transverse relaxation rates for T98G cells as a function of iron concentration ($C_{[Fe]}$). Fits were performed using a logistic function. The inset show a typical decay curve where the bi-exponential behaviour is visible. (right) Normalized relative weights of $T_2$ fast and slow components as a function of $C_{[Fe]}$. The bands correspond to the compartments volumes estimated from optical microscopy measurements.
Abstracts for posters in session "Large biomolecular complexes"
Efficient and stable reconstitution of the ABC transporter BmrA for solid-state NMR studies

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Here we report about the first steps of the structural investigations into the bacterial ATP-binding cassette transporter BmrA, which includes protein overproduction, stable isotope labelling, reconstitution into a lipid environment and the first two-dimensional solid-state NMR spectra.

ABC transporters are ubiquitous membrane proteins that provide passage to a wide variety of substrates across biological membranes. Several members of this protein family are involved in human diseases like adrenoleukodystrophy, cystic fibrosis and multidrug-resistance of cancer. In order to modulate their transport ability for the purpose of increasing therapeutic efficiency, a detailed structural understanding is necessary at an atomic level. BmrA from *B. subtilis* is a homologue of the human P-glycoprotein that is involved in multidrug resistance. The homodimeric drug exporter of 130 kDa was chosen as a model system because it can be overproduced in large quantities with stable isotope labelling. The aim is to investigate structural changes during substrate binding and translocation by solid-state NMR techniques. We show that the reconstitution of this protein in lipids from *B. subtilis* at a lipid-protein ratio of 0.5 w/w allows an optimal protein insertion into lipid bilayer as well as it complies with the two central NMR requirements: high signal-to-noise in the spectra and sample stability over a time period of months. The obtained spectra point to a well-folded protein and a highly homogenous preparation, as witnessed by the narrow resonance lines and the signal dispersion typical of the expected secondary structure distribution of the membrane protein. It shows the way towards studies of the different conformational states of the transporter in its export cycle, as well as towards interactions with substrates via chemical-shift fingerprints and sequential resonance assignments.

**Figure 1.** (A) ¹³C-¹³C two-dimensional solid-state NMR correlation experiment of the protein BmrA at 5 °C (20ms DARR mixing period, 48 scans) (B) Extract of the alanine Ca-CÎ± correlation, secondary structure regions for β-sheet (red), α-helical (black) and coil (green) from alanine signals are indicated. Solid lines correspond to the mean value with the standard deviation and dashed lines to the mean value with 2 times the standard deviation (C) One-dimensional trace from the two-dimensional experiment at 52.1 ppm, the peak width at half-height is 0.5 ppm.
**Structural basis of detection and signaling of DNA single-strand breaks by human PARP-1.**

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The highly abundant nuclear enzyme poly(ADP-ribose)polymerase-1 (PARP-1) is a key eukaryotic stress-sensor that detects DNA single-strand breaks (SSBs), the most frequent form of genomic damage; on binding an SSB it responds with an immediate burst of poly(ADP-ribose) synthesis that signals for assembly of DNA repair factors. PARP inhibitors hold great promise in cancer therapy as they can kill BRCA-deficient tumor cells selectively. However, the mechanism underlying PARP-1’s function has long been obscure; inherent dynamics of SSBs and of PARP-1’s modular six-domain architecture hindered structural studies. By combining solution NMR results with crystal structures of PARP domains on DNA double-strand breaks, we established in molecular detail how recognition and activation by SSBs occur in this key enzyme. A simulated annealing approach yielded a solution structure for PARP-1’s N-terminal F1 and F2 zinc-finger domains bound to a DNA dumbbell harbouring a single-nucleotide gap, showing how F1F2 bends and twists DNA at an SSB, creating a conformation inaccessible to undamaged DNA (A). A combination of chemical shift fingerprinting, 15N relaxation experiments and mutational analysis showed how assembly of further domains (B) creates a composite interface for interaction with the catalytic domain, acting as an allosteric switch. Overall, these results allowed us to propose a mechanistic model for PARP-1 recruitment and activation that may aid understanding of the mechanism of PARP inhibition (Eustermann et al., Mol. Cell, 2015, 60, 742-754).

![Figure 1](image.png)

**Figure 1.** A) Hybrid NMR/X-ray structure of the F1 and F2 domains of human PARP-1 bound to a DNA SSB; B) Model showing DNA-dependent assembly of further domains of PARP-1 that leads to catalytic activation.

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Amyloids are distinct insoluble proteinaceous fibers, traditionally associated with many chronic human neurodegenerative diseases \(^1\) and display a unique repetitive intermolecular cross-beta sheet motif \(^2\). However, not all amyloids are pathogenic; in contrast to disease-associated amyloids, functional amyloids are the product of coordinated and regulated cellular processes which avoids cell damage and death \(^3\). Functional amyloids were first discovered in microbes, their presence and relevance in higher organisms including humans has been identified \(^4\). Functional amyloids are indispensable for important physiological functions in the cell and provide insight into the mechanisms of protein homeostasis, folding, and misfolding. A study on biofilms from different habitats established that up to 40% of the bacteria present within these communities carry amyloid-like fibrils on their surface. They represent virulence traits important for biofilm formation, interaction with host proteins and promoting survival in a wide range of conditions as a result they are more infectious and difficult to treat. 80% of all infections are related to bacterial biofilms. Determining unknown structures and unravelling the structure-activity relationship of such fibrils will help to develop better therapies against antibiotic-resistant infectious bacteria in biofilms and amyloid formation in general.

14 Abstracts for posters in session "Metabolomics and small molecules"
**1H NMR Metabolomics of *Haliotis Diversicolor* Responses to Triphenyltin Chloride Exposure**

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Triphenyltin (TPT) is a worldwide pollutant that exists widely in marine ecosystems. Despite its legislatively limited release in most countries, TPT contamination is still a problem in many areas, and its persistence results in a cumulative contamination of the aquatic environment. In this study, we investigated the influence of triphenyltin chloride (TPTCl) on *Haliotis diversicolor* using 1H NMR spectroscopy together with pattern recognition methods.

The abalone hepatopancreas was collected after 28 days’ exposure to TPTCl at environmental concentration of 100 ng (Sn)/L, then its extract was analyzed by using NMR spectroscopy. The dominant metabolites in abalone hepatopancreas were found to comprise amino acids, organic osmolytes, nucleotides and energy metabolism-related compounds. Following spectral preprocessing, the orthogonal partial least squares discriminant analysis (OPLS-DA) was performed on the NMR spectral data from male and female groups. The results show that sex had a significant impact on the abalone metabolome in hepatopancreas tissue with both p values less than 0.001 calculated using analysis of variance testing of cross-validated predictive residuals.

To get an insight into the types of metabolites responsible for the class separation, OPLS-DA was conducted again with the corresponding NMR data from different pair-wise groups. Males and females were shown to respond differently to environmental stress (Fig. 1). In details, in males most metabolites (including glutamine, glutamate isoleucine, leucine, valine, phenylalanine, pantothenate, lysine, methionine, tyrosine, proline, threonine, arginine, lactate, galactose, homarine) were down-regulated except betaine. In females, the levels of glutamine, glutamate isoleucine, leucine, valine, phenylalanine, betaine, adenosine, uracil, alanine, AMP were significantly increased, only the levels of β-alanine, choline and taurine were decreased. The gender-specific metabolic responses indicated that TPT induced disturbances in osmotic regulation and energy metabolism via different metabolic pathways in male and female abalones.

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Quantification of cholesterol solubilized in human dietary micelles using quantitative $^{13}$C NMR

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Solubility of cholesterol in bile salt (BS) micelles is important to understand the availability of cholesterol for absorption in the intestinal epithelium and to develop strategies to decrease cholesterol intake from the intestinal lumen. The relation between the development of atherosclerosis and high levels of cholesterol in the blood is well established. In this work we quantify the effect of BS variability on the amount of cholesterol solubilized using a method to quantitatively follow the solubilization of [4-$^{13}$C]-cholesterol in BS micelles by $^{13}$C NMR (Figure 1) (1). The effect of some known hypocholesterolemic agents usually found in the diet is also evaluated, as well as some insight regarding the mechanisms involved. The results show that, depending on the bile salt composition, the average value of sterol per micelle is equal to or lower than one. The amount of cholesterol solubilized in the BS micelles is essentially equal to its total concentration until the solubility limit is reached. This indicates that the maximum cholesterol solubility in the BS micellar solution is the result of saturation of the aqueous phase and depends on the partition coefficient of cholesterol between the aqueous phase and the micellar pseudophase. The effect on cholesterol maximum solubility for several food ingredients usually encountered in the diet was characterized using methodology developed recently by us (1).

This method allows the simultaneous quantification of both cholesterol and food ingredient solubilized in the BS micelles even in the presence of larger aggregates, therefore avoiding their physical separation with possible impacts on the overall equilibrium. The phytosterols stigmasterol and stigmastanol decreased significantly cholesterol solubility with a reduction in the total amount of sterol solubilized, most pronounced for stigmasterol. Those results point towards coprecipitation being the major cause for the decrease in cholesterol solubilization by the BS micelles. The presence of tocopherol and oleic acid leads to a small decrease in the amount of cholesterol solubilized while palmitic acid slightly increases the solubility of cholesterol. Those dietary food ingredients are completely solubilized by the BS micelles indicating that the effects on cholesterol solubility are due to changes in the properties of the mixed micelles.

The DWET sequence: a new tool to suppress Radiation Damping for highly accurate quantitative ¹H NMR

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Radiation Damping (RD) severely hampers the accurate quantitative analysis of concentrated samples by ¹H NMR(¹). Since the early days of NMR, different approaches have been developed to cope with this phenomenon, either via pulse sequences(²) or thanks to hardware improvements(³). However, while these methods effectively reduce RD, the resulting peak shapes do not allow a proper deconvolution, which is required to perform quantitative analysis with a 0.1% accuracy. As RD is directly proportional to the number of spins located in the sensitive volume, another approach would be to dilute the sample, unfortunately this strategy introduces additional sources of error.

In this context, a new pulse sequence was designed to suppress RD. This method, named DWET (Double-WET) is based on the WET⁴ pulse sequence and on Outer Volume Saturation (OVS)⁵ methods. It combines two selective frequency-swept RF pulses applied together with excitation gradients and followed by a spoiler gradient. The sequence is followed by a non-selective hard pulse to allow the acquisition of a high resolution spectrum by ¹H NMR. With this approach, the only detected magnetization comes from a little slice of the whole sensitive volume, whose size can be customized by modifying the pulse sequence parameters. This method has already been successfully applied to a mixture of highly concentrated DMCPS and cyclooctane and allows ¹H NMR quantitative analysis with a high accuracy (0.1%)⁶.

It appears, however, that the basic segment must be repeated six times to effectively suppress the outer volume magnetization, particularly on the edges of the sensitive region where a residual signal remains. As a consequence, the duration of the saturation pulse train period becomes quite significant (~130 ms), resulting in a lower saturation efficiency if the T1 of the measured compounds is too short (e.g. when paramagnetic relaxing agents are added to the sample). In order to reduce the duration of the pulse train, we suggest two variants of this pulse sequence: (i) the use of double frequency swept pulses to simultaneously excite the two saturated regions and thus divide by two the number of selective RF pulses, hence reducing the pulse train duration at 90 ms (ii) the use of asymmetric RF pulses to increase the saturation efficiency on the edge of the sample, thus reducing the number of basic segment repetitions to reach a pulse train duration of 45 ms. This work opens many application perspectives for the quantitative analysis of concentrated samples, and could be easily incorporated in quantitative 2D NMR experiments⁷.

1) V. Krishnan and N. Murali, Prog. Nucl. Magn. Reson. Spectrosoc. 2013, 68, 41-57
Application of HR-MAS NMR spectroscopy in the study of the wooden breast syndrome in chicken

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Within the food supply chain there is globally an increasing demand for poultry meat, which can be ascribed to its attractive nutritional profile. Concomitantly, commercial chicken livestock production combats increasing challenges with high incidences of abnormalities observed in chicken breast muscles. The abnormalities are characterized by pale and bulging areas of substantial hardness in the breast muscle tissue and are referred to as wooden breast. The high incidences of wooden breast have been ascribed to efficient breeding work, which has led to progressive improvements to produce fast-growing broilers with a high proportion of breast meat [1]. However, the underlying metabolic and physiological mechanisms involved in the induction and progression of the muscle abnormalities are poorly understood. In addition, how the wooden breast impacts technological traits of the meat and its appropriateness for further processing are yet not identified. We demonstrate how the application and combination of NMR techniques including high-resolution magic angle spinning proton NMR spectroscopy and proton NMR relaxometry can gain insight into biochemical and intrinsic water biophysical changes in chicken muscle tissue defined as wooden breast.

1D $^{13}$C Spectra as Projections from Rapid HMBC-Type Experiments

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In this work we consider a combination of the two fast scanning approaches ASAP (Acceleration by Sharing Adjacent Polarization) [¹] and ALSOFAST (ALternate SOFAST) [²] and HMBC-type experiments (e.g. [³]). A comparative analysis of the different species of HMBC sequences is presented with special attention on minimizing artefacts of different origin. Be it $T_1$-Noise or the distortion of signals by undesired coupling evolution. In a first attempt the applicability of these experiments to compete with the $^{13}$C-1D experiment under the use of Ernst-angle excitation [⁴] is tested by which the $^{13}$C projection should serve as an analogue to the one-dimensional $^{13}$C spectrum. In this context speeding up the acquisition is an indispensable need. Facing ultimate resolution the potential of the synergy of the acquisition with NUS (Non Uniform Sampling) [⁵] is used. In contrast to the direct detection of $^{13}$C resonances the two-dimensional proton detected experiments have a much higher intrinsic sensitivity as the gyromagnetic ratio of protons is four times as high as the gyromagnetic ratio of $^{13}$C. Furthermore relaxation times which are crucial in the fast pulsing regime are considerably shorter for proton detected experiments compared to carbon detected ones. This becomes even more important when the molecule contains quaternary carbons. So far the acquisition of high resolved two-dimensional spectra is very time consuming which is a consequence of the way data in the indirect dimension is collected. In this work we make an attempt to explore these limits.

NMR Aerosolomics: What can we find in air aerosols?

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Aerosolomics provides complex evaluation of aerosol composition and compound concentration. It is exploiting metabolomic approach, which is applied to aerosol samples [1]. In our laboratory a novel NMR method for organic aerosol analysis was developed and tested. The method is focused on water soluble organic compounds (WSOC), which is the least examined group of organic aerosols. In NMR metabolomic approach library $^1$H spectra of individual compounds are fitted into the complex $^1$H spectrum of a mixture and are subsequently subtracted. This is allowed by the employment of ChenomX software. Since ChenomX is originally metabolomics software, WSOC are being added to its database continuously.

Collection of aerosol samples in a high volume cascade impactor together with a special preparation of the sample and use of an indirect probe increased number of identified compounds in every analysed sample. The high volume cascade impactor collects atmospheric aerosols into six stages according to the particle size. Therefore variances in WSOC composition between different particle size samples were found. In this work we present summer 2015 collection of real atmospheric aerosols from Prague – Suchdol.

Major differences were observed in the fraction of carbohydrates and sugar alcohols. Carbohydrate derivatives were found mainly in bigger particle size samples (2.24 – 4.56 µm and 4.56 – 20.00 µm). Other groups of WSOC (mono- and dicarboxylic acids, hydroxycarboxylic acids, oxocarboxylic acids, aromatic compounds or amines) were also thoroughly examined and the results are discussed in this work. The abundance of certain groups of WSOC and the presence of marker compound can indicate the origin of the aerosol particles.

References:
NMR analysis of the human saliva metabolome distinguishes demented patients from matched controls

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NMR metabolomics provides a robust analysis technique for metabolic profiling where the frequently used 1D 1H NMR and 2D experiments allow for correct assignment of resonances and quantification of metabolites. Saliva is a metabolic-sensitive biofluid, which can be easily and non-invasively collected. This availability, coupled with the simple sample handling and the robustness of NMR spectroscopy, suggest that saliva is a great candidate for NMR metabolomics studies.

Samples from the 25 year longitudinal Betula project biobank were selected from subjects that (at the time of sampling) were diagnosed with Alzheimer’s disease, dementia or developed it five years later (for evaluating the pre-diagnosis capability), together with age-, gender- and education-matched control individuals. The selected samples were analysed both in an untargeted and targeted approach. Samples were filtered to remove high molecular weight molecules (e.g. lipids, proteins) and diluted in phosphate buffer (with sodium azide, 10 % D2O and TMPS as the reference), followed by data acquisition and metabolite quantification by using the Chenomx NMR suite. Multivariate data analysis of the quantified metabolites revealed a statistically significant model, separating the demented from controls (Figure 1), but did not provide a significant model for the pre-diagnosis study.

![Figure 1. 1D 1H NMR spectra of a demented patient’s saliva sample and a matched control subject. Inset shows the score plot for the demented vs controls in an OPLS-DA model.](image-url)
Application of Real-Time Pure Shift HSQC to the analysis of Complex Mixtures containing PAHs

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Pure shift experiments are favorable for suppressing the multiplet structure caused by homonuclear scalar couplings. Recently, Kiraly et al. developed a real-time pure shift HSQC experiment[1] where a series of BIRD elements refocuses scalar couplings during detection and thus significantly improves both resolution and sensitivity compared to a standard HSQC spectrum.

In case of complex mixtures containing polycyclic aromatic hydrocarbon (PAH), the use of the RT-HSQC was shown to be successful in simplifying overcrowded spectra (Figure 1, black spectrum), by collapsing the 1H-1H multiplet structure of each resonance into a singlet (Figure 1, red spectrum in dotted square). This leads to the perspective of an automatic identification and quantification of PAHs brut extracts, independently of the Larmor frequency.

We applied RT-HSQC experiments to the analysis of tire extracts and demonstrated the presence, in some samples, of more carcinogenic PAHs than allowed by the European low[2].

Figure 1. Identification of PAHs 1 to 4 in the brut extract of a tire sample. Compounds containing “bay” hydrogens are associated with higher carcinogenicity.

Integrating HR-MAS/Solid State NMR and transcriptomic analysis of soybean upon water deficit

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In this work, we explored the feasibility of HR-MAS and solid state NMR techniques to identify metabolic changes in soybean leaves upon water deficit conditions. The metabolites identified were compared with RNAseq data and substrative library on the same condition with goal of integrated transcriptomic and fingerprinting metabolite data. Firstly, we achieved the optimal NMR parameters to HR-MAS experiment using perfect echo pulse sequence. The 1H HR-MAS spectra of leaves of soybean grown with and without drought stress revealed striking metabolites differences. A total of twenty-three metabolites were identified, and that, the impact of water deficit conditions on the metabolite profile of soybean leaves led to synthesis of amino acids isoleucine, leucine, valine, proline, glutamate, aspartate, asparagine, phenylalanine and tyrosine. The CP-MAS 13C{1H} spectra of lyophilized soybean leaves grown with and without drought stress show six chemical shifts regions. The spectra show some significant differences among the samples, mainly, due to an increase in the alkyl C/O-alkyl C ratio in leaves of soybean grown under stress. The stresses sample showed a decrease in the of methyl (δ 19) and O-alkyl groups suggests the degradation of carbohydrates of soybean leaves. 13C spectra obtained with Single Pulse Excitation show the presence of free fatty acids. The spectra did not show the triglycerides signals at signal 62 ppm (13C) and 4.2 ppm (1H) spectra. The data obtained from 1H HR-MAS and solid-state NMR revealed a correspondence profile changes detected at transcriptomic level. High expression level of genes corresponding to aspartate aminotransferase, arginine and proline, glutamate metabolism, glutamine synthase, asparagine synthase, alanine-glyoxylate aminotransferase agt2, glutamate semialdehyde dehydrogenase, samdependent methyltransferases, betaine-aldehyde dehydrogenase were highly correlated with amino acids identified in 1H HR-MAS. Therefore, our results show that the use of 1H HR-MAS and solid-state NMR integrated with transcriptomic data provided a full picture of major change in metabolic profile of water deficit in soybean. The drought enhances amino acid catabolism and represents a promising target for future studies on the signaling function of how water deficit is regulated by metabolite signals.
The potential of NMR-based metabolomics for mapping and describing postprandial processes

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NMR-based metabolomic studies in the nutrition field have merely been on fasting blood samples to investigate long-term effects, while studies on the postprandial state are more sparse. The postprandial responses to a dietary exposure are complex and during this dynamic period of metabolic activity multiple processes related to metabolism, inflammation and oxidation are affected. In fact the postprandial response has been proposed to be decisive for a food item’s effect on health (Burton-Freeman, 2010). However, many aspects of the postprandial state remain presently unexplored, and an improved biochemical description of the complex postprandial processes could pave the way for improving our understanding of the critical processes in the postprandial state and the impact on health. In a randomized controlled trial with crossover design 20 healthy subjects consumed 2 balanced diets that varied in main protein sources for 4 weeks. At the first and last day of each intervention period the subjects received a defined test meal with postprandial blood sampling. To elucidate the potential of metabolomics approaches for mapping and describing the postprandial processes different analytical techniques were applied including nuclear magnetic resonance (NMR) spectroscopy combined with mass spectrometry (MS). So far urinary data shows an effect on lipid metabolism after 4 weeks of lean-seafood intake, and we expect that postprandial analyses based on untargeted $^1$H NMR spectroscopy will add further information in the understanding of the diet-induced metabolic changes observed in urine after lean-seafood intake.
Metabolic effects of red meat consumption studied in a rat model

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Aim
The aim of the present study was to investigate the effects of consumption of red meat (beef) versus white meat (chicken) on the metabolome using a rat model.

Materials and methods
Twenty-four healthy rats were randomly assigned to 15 days of ad libitum feeding of one of four experimental diets: 1) lean chicken 2) fat chicken 3) lean beef 4) fat beef. Urine, feces, plasma and colon tissue samples were analyzed using proton (¹H) NMR-based metabolomics.

Results and discussion
Chicken intake resulted in a higher urinary excretion of acetate, anserine and 1-methylhistididine. The latter two might be useful as biomarkers of chicken consumption, which has also been shown in human studies. Beef intake increased urinary carnosine, fumarate, carnitine, trimethylamine (TMA) and trimethylamine N-oxide (TMAO) levels. TMA is produced from choline or carnitine by microbial fermentation in the colon. Oxidation of TMA by the FMO-enzymes in the liver, results in formation of TMAO, which is excreted in urine. Several studies have associated TMAO to cardiovascular disease, but the exact mechanism has not yet been accounted for.

Conclusions
This study for the first time identified specific differences in the metabolome related to intake of beef and chicken, respectively. These results might lead to a better understanding of the proposed effects of red meat consumption.

![Diagram showing the formation of TMAO from dietary carnitine.](image-url)

**Figure 1. The formation of TMAO from dietary carnitine.**
A core metabolic enzyme responsible for phosphine resistance and fundamental metabolic regulation

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Phosphine is a small redox-active gas that is used to protect global grain reserves from pest insects, but these insects are increasingly resistant to phosphine fumigation. Recently, we identified dihydrolipoamide dehydrogenase (DLDH) as the enzyme responsible for phosphine resistance and characterised in C. elegans the toxic action of phosphine and the resistance mechanisms with NMR-based metabolomics (Schlipalius et al., Science 338, 807–810 (2012)). DLDH is a core metabolic enzyme, central to metabolic regulation, and a new class of resistance factor. Polymorphisms responsible for genetic resistance cluster around the redox-active catalytic disulfide or the dimerisation interface of DLDH in insects and nematodes. DLDH participates in four key steps of core metabolism, which are affected differently by phosphine exposure in mutant and wild-type animals. The position of DLDH in the metabolic network makes it a highly likely candidate for a central regulator of metabolism. We are studying the role of DLDH in biological/clinical processes, such as lifespan determination, obesity, Alzheimer’s Disease, aerobic and anaerobic respiration. Metabolomic analysis indicates a role of DLDH in the cross-talk between branched-chain amino acid and lipid metabolism. A genome-scale metabolic model of C. elegans metabolism is being developed that will enable further characterization of this metabolic link. DLDH is an exceptional case in which a combination of systems biology methods has identified a single genetic cause of phenotypic change that is responsible for metabolic adaptation in health and disease and that can subsequently be studied with a wide range of methods ranging from structural biology, genetics, and classical biochemistry to systems biology and metabolic modelling.

Figure 1. Metabolites and pathways affected in response to phosphine, with the position of DLDH-containing enzyme complexes marked.
The Metabolome consists of thousands of Metabolites, which makes it difficult to separate them chromatographically and this culminates in the problem to identify single components. Therefore, the three-dimensional cross correlation (3DCC), first introduced by BEHNKEN ET AL., is a powerful tool for non-targeted Metabolome Analysis. [1]

3DCC combines the advantages of MS and NMR and dissects NMR spectra of a mixture into spectra of single components without fully separating the single compounds from each other. The idea of this application is using the fact of compounds occurring at the same retention time in the MS and NMR, which allows to calculate their correlation and to deconvolve the NMR-spectra mathematically to spectra of only one single compound. So far the 3DCC application was only used to separate NMR-Spectra from glycanes by using the advantage of the structural reporter group method.

This work will demonstrate an application to other compounds such as metabolites from plants. A mixture of six compounds was analysed to validate the applicability to other small molecules. Without the complete chromatographic separation, the ESI-MS spectrum and a pure $^1$H-NMR-spectrum for each individual compound have been obtained.

For many metabolites this data will be sufficient for identification either from a database or assignment of the spectroscopic data. In specific cases the application will facilitate the identification of promising candidates for isolation and further spectroscopic analysis. 3DCC considerably facilitates the search and identification of biomarkers.

Considering the increasing globalization and the growing interest in local produced products, the determination of the origin of food becomes more and more important. Today Turkey is the largest hazelnut producer with about 75% of the world production.[1] The second largest hazelnut producer is Italy with significant differences in production conditions and qualitative marketing. Therefore a unique method of determination of the products origin would be desirable.

In this study different hazelnut samples from Turkey, Italy, Georgia and Germany were analyzed by $^1$H-NMR-spectroscopy and the multivariate data analysis methods such as PCA and PLS-DA. The PCA showed a good separation of the samples. Especially the Georgian samples differed significantly from the Italian and German samples. The corresponding Loadings-Plots show the important buckets which are responsible for separation of the sample groups. In the next step a discriminant analysis was performed, which showed the total separation of the samples by their origin. It appears that the approach of using $^1$H-NMR-Spectroscopy together with PCA and PLS-DA delivers a fast and accurate determination of the geographical origin of hazelnuts.

15 Abstracts for posters in session "NMR imaging"
Anisotropy of $T_2$ in liquids entrapped in nanocavities: MRI study of biological systems

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Spin–spin relaxation in liquids or gases entrapped in ellipsoidal nanocavities (Fig.1) with different orientation ordering are theoretically investigated. The proposed model allows one to determine the relaxation times in liquids depending on the form of nanocavities and the degree of their ordering.

It was shown that the dipole–dipole interaction is determined by a single coupling constant, which depends on the form, size, and orientation of a nanocavity and on the number of nuclear spins in the cavity. The transverse relaxation rate depends on the angle between the external magnetic field and the cavity main axis. The calculation results for the local dipolar field (Fig.2) and transverse (Fig.3) relaxation time explain the angular dependencies observed in MRI experiments with biological objects: cartilage and tendon. Microstructure of these tissues can be characterized by the standard deviation of the Gaussian distribution of fibril orientations. It is shown that the standard deviation obtained at the matching of the calculation to experimental results can be used as a parameter characterizing the disorder in the biological sample.

**Figure 1.** Nanocavities with liquid or gas.

**Figure 2.** Anisotropy of the normalized square of a dipolar field. The solid black line is for perfectly oriented nanocavities. Blue dashed line, red dotted line and magenta dash-dotted line are calculated by averaging with the Gaussian distribution of cavity directions for the standard deviations of 0.2, 0.3, and 0.45, respectively. The black squares are the experimental data for a tendon, measured from the dipolar-encoded longitudinal magnetization decay curve [1]. The red circles are the data measured from the double quantum build-up curve in the initial excitation/reconversion regime [1]. The green triangles are the experimental data measured with the Carr–Purcell–Meiboom–Gill pulse sequence in a sheep Achilles tendon [1].

**Figure 3.** Angular dependence of the normalized transverse relaxation rates. The solid black line is calculated for perfectly oriented nanocavities. Blue dashed line, red dotted line and magenta dash-dotted line are calculated by averaging with the Gaussian distribution of cavity directions for the standard deviations of 0.2, 0.3, and 0.45, respectively. The green circles are the experimental data measured with the Carr–Purcell–Meiboom–Gill pulse sequence in a sheep Achilles tendon [1].

Magnetic Resonance Elastography on piglet: an alternative and rapid method to verify liver resistance, used in conjunction with the calculation of blood flow

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The program IFLOW (Intraoperative Fluorescent Liver Optimization Workup), aims to study the effect of partial hepatectomy of about 75% on piglets. This study leads to the establishment of a mathematical model (in analogy of an electrical circuit, see figure 1) used to assess liver percentage which may be resected during a hepatectomy necessary in certain pathologies.

Magnetic Resonance Elastography (MRE) is a noninvasive medical imaging technique that measures the mechanical properties (strength, hardness) of soft tissue by introducing shear waves and observing their spread in MR images.

Pathological tissues are often more rigid and harder than the surrounding healthy tissue. It is also known that the liver becomes stiffer after resection because of the increase in the intraparenchymal pressure related to blood flow.

Here we set up an experiment using the Resoundant™ elastography kit, with 40% emission amplitude of the system, coupled with MRI in order to compare the state of the propagating waves in the liver (see figure 2, a) before and after hepatectomy. The mode of propagation of these waves is then correlated with the blood flow measured by MRI. Calculating blood flow is performed in three various vessels such as the hepatic artery, celiac aorta and the portal vein using functional phase contrast imaging.

When the liver stiffness (see figure 2, b) and flow parameters are well mastered, it is possible to predict the percentage of liver that can be resected.

Pig liver is very similar to the human one. Eventually, this method would be transposable to humans and this will lead to a breakthrough for medical applications.

**Figure 1.** Analogical electrical circuit to for the establishment of the mathematical model; with Q: flow, P: Pressure, R: Resistance (FLOW due to blood viscosity), C: Capacitor (ELASTOGRAPHY).

**Figure 2.** a) Wave image on pig liver (sagittal view). b) Corresponding stiffness map with 95% confidence; the crossed out dots represents unreadable areas.
NMR Imaging as a tool for probing phase separation processes in crude oils

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Phase separation processes which take place during various chemical operations in many systems play a crucial role in industry and related technologies. One of the most severe and economically costly problems associated with phase separation is crude oil production, transportation and processing. Among different groups of hydrocarbons presented in the oil, high molecular weight paraffins (waxes) and asphaltenes are responsible for some of the problems that are encountered during transportation and processing of the crude oil. Thus asphaltenes are mostly contributing to the problems associated with oil refining and processing, while wax deposition on the walls of reservoirs and pipelines cause their blockage. Hence challenges associated with asphaltenes and wax phase behaviour are of great relevance.

In this study we present an approach for in situ measurements of asphaltenes precipitation and thermally-driven wax deposition processes in real crude oils. Despite of inherently low spatial resolution of the MRI method (typically the biggest size of the precipitated particles is about few microns), we demonstrate surprisingly high accuracy of the method which capable to track complex phase behaviour in the system. The key factor allowing MRI to be feasible is formation of spatially separated domains with a diverse composition and/or structure accompanied by interfaces. In result, the initially homogeneous system, in terms of resolution, can be visualized as the spatially separated domains with the further probing of their interfaces’ dynamics during any process that can occur in the system.

Using developed approach the flocculant-induced asphaltene precipitation processes were investigated in model asphaltene-toluene systems and real heavy crude oils [1, 2]. The process of colloidal suspension formation and two different patterns of its evolution were observed depending on both the asphaltene content and the flocculant concentration. For in situ probing of wax deposition processes a special “cold finger” cell which capable to create and maintain a prescribed thermal gradient within the cell was developed and integrated into the MRI probe. Real-time measurements of thermally-driven wax precipitation unveiled the mechanism of deposit aging.

The work is supported by Russian Science Foundation (project #15-19-00119).

References
Additive manufacturing (rapid prototyping) is a rapidly growing innovative technology that is being incorporated into a broad range of applications in diverse areas, such as industry, medicine, materials science, education, and art. This technology provides fast fabricating of polymeric 3D parts which along with polymer composites found advanced structural applications in many industries due to their favorable properties and relative ease of processing into complex integrated structures. Mechanical properties and stability of these materials strongly depend on reinforcing materials, type of polymer matrix, crosslink density distribution, samples homogeneity, presence of defects etc. Therefore many research studies are investigating polymer composites with the goal of improving their overall properties. Despite the urgency of the problem, the majority of the works were dealt with mechanical testing of polymer composites and 3D printed parts. While few works were devoted to MRI of polymer composites, no information regarding application of this method to 3D printed parts can be found in literature.

In this study we report about application of MRI method to polymer composites and 3D printed patterns. Two types of polymer composites were investigated to reveal the factors influencing on materials stability during humid aging [1]. Using MRI the role of surface finish, defects, and properties of materials was examined in providing of moisture uptake. MRI was also applied for monitoring, studying and performing output quality control of the acrylate-based polymer patterns manufactured via laser stereolithography [2]. The effects of build parameters and humid environment on sample homogeneity, distribution of crosslink density, stability and defect formation were examined. Qualitative information obtained using MRI visualization was supplemented by quantitative measurements of NMR relaxation times and 1H NMR spectra. Different types of defects in the samples were observed and classified; some defects originated from local matrix continuity failures, while other defects were found in the form of bulk layering. MRI visualization coupled with relaxometry and 1H spectroscopy of patterns during their interaction with humidity allowed tracking water distribution inside the sample and observing effects of swelling, fracturing and chemical decomposition.

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References


**Characterization of flow distributions in liquid-liquid systems by MRI**

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Ion transfer at the interface between two immiscible electrolyte solutions (ITIES) is a very important physicochemical process, while reactions that occur at the interface between two immiscible liquids are common to many natural and industrial systems [1]. For instance, solvent extraction and phase transfer catalysis rely on optimized reactions at liquid-liquid interfaces. These can be additionally considered as useful biomembranes analogs, or for experimental investigation of cell membrane transfer processes.

To perform quantitative electrochemical measurements at the interface, a nonconventional electrochemical cell is usually employed, where the interface is controlled by a small contact area between the two liquid phases [2], with flow induced through a convective flow generated by a spinning electrode. MRI is a suitable tool to study flow dynamics [3,4]. In this work we study the dynamics of two immiscible fluids (water and 1,2-dichloroethane, which is used as an organic phase) inside two-compartment electrochemical cell.

To maintain a uniform surface polarization, the interface between the aqueous and the organic phase must remain stationary and planar during electrochemistry experiments. Therefore, no velocity patterns should be observed in the organic phase. 3D velocity maps of water inside the cell were acquired for different rotational speeds (Fig. 1). Velocity fields for the liquid-liquid system were also measured, for different shapes of the interface. An optimal position for the interface, in terms of electrochemical experiments, could be determined from the obtained velocity patterns.


Fokker-Planck models of quantum effects in magnetic resonance imaging

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Many recent magnetic resonance imaging experiments involve spin states other than longitudinal or transverse magnetization. For instance, singlet state imaging requires two-spin correlations to be taken into account, hyperpolarised imaging must correctly account for multi-spin dynamics, multiple quantum imaging must take into account the dynamics of the corresponding coherences, etc. In such experiments, accurate quantum mechanical treatment of spin dynamics must coexist with accurate treatment of classical processes, such as diffusion and convection.

In this communication we report some initial results for MRI simulations using the Fokker-Planck formalism [1] which can simultaneously account for classical and quantum mechanical processes in MRI samples.

Fokker-Planck equation is superior to the more traditional Liouville - von Neumann equation formalism [2] because spatial dynamics processes (diffusion, hydrodynamics, magic angle spinning, spatially selective pulses, etc.) are represented by constant matrices that are more convenient from the programming and numerical efficiency point of view [3] than the time-dependent Hamiltonians in the Liouville - von Neumann equation formalism.

Figure 1. Fokker-Planck theory simulation of a singlet state NMR imaging experiment in the presence of one-dimensional flow and one-dimensional diffusion. The simulation includes soft radiofrequency pulses with simultaneous explicit simulation of flow, diffusion, magnetic field gradients and spin-spin coupling dynamics as prescribed by the Liouville - von Neumann equation, as well as full Redfield relaxation superoperator treatment. The flow rate is set to 5 cm/s and the diffusion constant to 3.6·10⁻⁶ m²/s. The singlet imaging sequence used is described in the recent paper from the Pileio group [4].


Reduced field of view diffusion imaging at 7T using the “blOCh” pulse design package

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Background: Nowadays ultra-high field (≥7T) MR scanners become increasingly available and better fit to clinical needs due to advanced imaging methods exploiting the higher signal-to-noise ratio, parallel transmit systems, and stronger magnetic gradients. Accompanying the benefits are challenges of stronger susceptibility based distortions, B0 and B1 inhomogeneities, and shorter T2 relaxation times. In large tip-angle radio frequency (RF) pulse design, optimal control (OC) addresses these difficulties allowing one to compute rf pulses with high fidelity and robustness.

Aim: in vivo localised diffusion weighted imaging (DWI) at ultra-high field was pursued with 2D spatially selective, parallel transmit RF pulses and a spin-echo (SE) echo-planar imaging (EPI) sequence.

Method: The 2D spatially selective RF pulses were designed using the low-memory BFGS OC algorithm implemented in the “blOCh” package4. In order to operate in a clinically feasible time the optimisation gradient precision was fixed to the first order5. Data were acquired at a 7T scanner (Siemens Medical Systems, Erlangen, Germany) with a gradient amplitude limit of 70 mT/m and maximum slew rate of 200 T/m/s. A 8/32 transmit/ receive head coil (Nova Medical, Wilmington, MA, USA) was used for excitation and reception. The 2D excitation k-space trajectory was a spiral with 70% of the maximum gradient slew rate and amplitude. Diffusion weightings of b = 0 and 1000 s/mm2 were applied in 30 directions in the SE EPI sequence (TR/TE = 3700 ms / 100 ms, T2* = 7:51 min, 4 averages). Axial slices were acquired with full and reduced field-of-view (FoV) with 200x200 mm2 (1.5 mm2 isotropic resolution) and 100x80 mm2 (1 mm2 isotropic resolution, respectively. The B0 and B1+ maps were obtained with a dual-echo GRE scan and the DREAM4 sequence using transmit channel phase encoding5, respectively.

Results and discussion: The resulting full and reduced FoV T2-weighted (w) images and coloured fractional anisotropy (FA) maps are presented in Figure 1. We demonstrated that the full and reduced FoV diffusion images have a good quality. The designed RF pulses exhibited a high fidelity not only in sense of the excitation quality, but also for outer volume suppression for all diffusion directions and diffusion scalar metrics. The low-memory quasi-Newton algorithm took local field inhomogeneities and RF power constraints into account and all computations were performed in clinically acceptable times.

Conclusion: In vivo localised DWI was realised on ultra-high field system. Localisation was attained using 2D OC parallel transmit RF pulses obtained in practical times and mitigating common complexities experienced at ultra-high fields.

The Role of Diffusion in Spatially Encoded MRI

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When the static magnetic field is inhomogeneous, hybrid imaging techniques that use $k$-space encoding in one direction and spatial encoding in the other direction can be superior to traditional imaging techniques which use $k$-space encoding in both directions. In this work, we will investigate the role of different contrast mechanisms in spatially encoded MRI. In particular, translational diffusion can lead to misleading contrast. We show how the basic pulse sequence of Rapid Acquisition by Sequential Excitation and Refocusing (RASER) needs to be adapted by using a double chirp sequence (DC-RASER) in order to obtain faithful images.

![Image](image.png)

**Figure 1.** Images obtained at 800 MHz of a phantom consisting of a piece of plastic immersed in distilled water. From left to right: effects of increasing inhomogeneity of the static field, as seen from the proton spectra of the entire phantom shown along the top. a) Spin-echo EPI, b) RASER, c) DC-RASER. The extent of the inhomogeneity can be appreciated from the proton spectra of the entire phantom depicted on the top.
The hex Phantom
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Purpose. Multidimensional diffusion MRI is used to determine the microstructure in tissue. The microscopic fractional anisotropy $^{1}$ ($\mu$FA) describes the cell shape $^{2}$ and has the potential to characterize tumors and enable grading and follow-up of treatment. When new contrasts based on microstructural imaging are used, it is of great importance to calibrate the scanner and validate that the system and data processing pipeline are set up so that $\mu$FA can be quantified accurately and precisely.

Methods. For this purpose we have designed a phantom with $\mu$FA equal to the theoretical maximum value 1. The phantom comprises a mixture of inexpensive and reasonably harmless chemicals, namely water, isooctane (gasoline), and the detergent AOT. In the temperature range 10-25 °C, this mixture forms a liquid crystal with hexagonally packed water channels in a continuous matrix of liquid isooctane, and the detergent located in the interface between the water and the isooctane. $^{3}$ The water channels have a diameter of a few nanometers and lengths approaching macroscopic dimensions. With inspiration from the hexagonal nanostructure, we propose the name “Hex Phantom”.

Results. Because of the low solubility of the water in the isooctane, water diffusion is constrained to follow the paths of the narrow channels, giving a value of $\mu$FA equal to 1. The phantom has so far been applied for measurements on a microimaging system, $^{4,5}$ see figure 1, and a clinical scanner at Hvidovre Hospital, Denmark.

Conclusions. We suggest the Hex Phantom as the “gold standard” for $\mu$FA. Whenever a protocol for measuring $\mu$FA has been modified or implemented on a new MR scanner, the Hex Phantom should be used for validation purposes.

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References
16 Abstracts for posters in session "Materials NMR"
Abstract which was submitted on the website of the conference EUROMAR 2016 on 09/02/2016

**Powder-XRD and $^{14}$N magic-angle spinning solid-state NMR spectroscopy of some metal nitrides.**

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Some metal nitrides (TiN, ZrN, InN, GaN, Ca$_3$N$_2$, Mg$_3$N$_2$, Ge$_3$N$_4$) have been studied by powder-XRD and $^{14}$N magic-angle spinning (MAS) solid-state NMR spectroscopy. For Ca$_3$N$_2$, Mg$_3$N$_2$ and Ge$_3$N$_4$, no $^{14}$N NMR signal was observed. Low-speed ($\nu_r = 2$ kHz for TiN, ZrN, and GaN; $\nu_r = 1$ kHz for InN) and “high-speed” ($\nu_r = 15$ kHz for TiN; $\nu_r = 5$ kHz for ZrN; $\nu_r = 10$ kHz for InN and GaN) MAS NMR experiments were performed. For TiN, ZrN, InN, GaN, powder-XRD was used to identify the phases present in each sample. The number of peaks observed for each sample in their $^{14}$N MAS solid-state NMR spectrum matches perfectly well with the number of nitrogen-containing phases identified by powder-XRD. The $^{14}$N MAS solid-state NMR spectra are symmetric and dominated by the quadrupolar (Q) interaction. The envelopes of the spinning sidebands manifold are Lorentzian and it is concluded that there is a distribution of the quadrupolar coupling constants $Q_{\alpha\beta}$’s arising from structural defects in the compounds studied.
Proton transport has been recognized as an essential process in many biological systems, as well as electrochemical devices including fuel cells and redox flow batteries. In the present study, we address the pressing need for solvent-free proton conducting polymer electrolytes for high-temperature PEM fuel cell applications by developing a novel all-solid polyelectrolyte membrane with self-assembled nanoscale proton-channel structure.[1]

In general, there are two fundamental approaches to the design and synthesis of an anhydrous proton-conducting membrane: The first approach is simply based on the substitution of water with another suitable proton solvent that is capable of conducting protons. PBI/Phosphoric acid composites have attracted most attention in this context.[2] However, an essential problem associated with this approach is that the phosphoric acid, as a small molecule in the PEM, may be gradually evaporated which would lead to continuous decrease in proton conductivity and hence degradation in performance. A second approach toward high temperature proton-conducting membranes points to materials with proton conductivity as an intrinsic property, i.e., functioning without any additional liquid phase. There have been numerous attempts in the past decades to develop such solid polymeric electrolytes through immobilization of heterocyclic functional groups such as imidazole, triazole, pyrazole, and benzimidazole, tethering them to oligomers or to flexible polymer side chains. However, almost without exception, all of these materials suffer from a significant lower conductivity compared to the hydrated membranes. In the present study, we developed a novel polyelectrolyte material by tethering PIL functional groups onto a polymer backbone, forming a comb-like polymer. The structure and dynamics of the materials were studied using PFG-NMR and solid-state NMR techniques. We show that this self-assembled nanostructure endows the material with exciting ‘dry’ proton conductivity at elevated temperatures, as high as 0.3 mS/cm at 120 °C, making it an attractive candidate for high-temperature PEM fuel cell applications.


Through-Space J Couplings in P-S and P-Se Heterocycles

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Weak intermolecular interactions are of great importance in areas such as polymorphism and crystal structure prediction, where the ability to understand (and, ultimately, control) these would have many applications. Here, we report the observation of both heteronuclear and homonuclear intermolecular J couplings in a series of P-S and P-Se heterocycles, where periodic density functional theory (DFT) calculations show that the interactions are mediated by the P lone pairs.

The compounds, prepared according to Scheme 1, have been characterized in the solid state by single-crystal XRD and 77Se (2 and 3) and 31P NMR spectroscopy. The 77Se NMR spectra of 2 and 3 both contain resonances with multiplicities that cannot be explained simply by considering the intramolecular 1JPSe couplings. However, an intermolecular “through space” JPSe coupling can fully explain the multiplicities. The presence of this interaction is supported by DFT calculations of the coupling deformation density (CDD), which predict an intermolecular coupling of ~350 Hz for 2, mediated by the P lone pairs (Figure 1(a)).

The DFT calculations also predicted a homonuclear 31P-31P coupling for 1 and 2, again mediated by the lone pairs of P (shown in Figure 1(b) for 2). This coupling can be observed directly for 1, where it occurs between two crystallographically-distinct P species, but the case is more complicated for 2, where there is only one crystallographically-distinct P species present. While the coupling cannot be observed directly in the 31P MAS NMR spectrum, it can be measured as ~88 Hz in a two-dimensional J-resolved spectrum. By recording the J-resolved spectrum with 77Se decoupling in t1, it can be shown that this J coupling is only observable when the magnetic equivalence of the two coupled 31P is lifted by a heteronuclear coupling to 77Se (Figure 1(c)).

References
Solid-state $^7$Li and $^{59}$Co MAS NMR study of LiCoO$_2$ prepared by a simple non-aqueous synthesis

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The LiCoO$_2$ is generally used as a cathode material. As compared to the commercially available LiCoO$_2$, simple non-aqueous synthesis method shows enhanced electrochemical properties and reduced particle sizes. The structural difference of the commercial LiCoO$_2$ and the prepared LiCoO$_2$ has been investigated by $^7$Li and $^{59}$Co MAS NMR. As using of single-pulse and Hahn-echo pulse sequences, both NMR spectra of the prepared LiCoO$_2$ are well matched with commercial LiCoO$_2$, and crystallinity of materials are also discussed.

Figure 1 $^{59}$Co MAS NMR spectra of the commercial and synthesized LiCoO$_2$. 

Poster: MN4, location: 2nd balcony
Surface structural analysis of cysteine-capped CdSe magic-sized clusters by using solid-state NMR

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Recently we developed a method to synthesize cysteine-capped CdSe magic-sized clusters (CdSe-Cys)\(^{[1]}\) from elemental Cd and Se in aqueous solution. Here, we report structural analysis of interactions between the inorganic core and the organic ligands of a CdSe-Cys, which was prepared from elemental \(^{113}\)Cd and \(^{77}\)Se and L-[\(^{15}\)N] cysteine by our method.

Figure 1 shows \(^{13}\)C CP/MAS NMR spectra of the CdSe-Cys and L-cysteine. The observation of high-frequency shift of the \(^{13}\)C signal in the CdSe-Cys as compared to that in L-cysteine can be ascribable to formation of Cd-S bond. Cd-S bond is also indicated by observation of no S-H bending/stretching peaks the FT-IR spectra of the CdSe-Cys. Further, it should be pointed out that Cd-S bond is formed in the Cd-cysteine complex used as the precursor in our synthetic method\(^{[2]}\). In the \(^{13}\)C spectra, it is also shown that the COO\(^-\) signal in the CdSe-Cys moves to higher frequency. This can be attributed to formation of ionic bonds with Na\(^+\). The \(^{15}\)N CP/MAS NMR spectrum of the CdSe-Cys shows an asymmetrical peak (Fig. 2). By fitting the peak to a sum of two Lorentzian lines, we obtained the chemical shifts at 34.8 and 42.6 ppm. For assignment, we undertook \(^{15}\)N–\(^{113}\)Cd and \(^{15}\)N–\(^{77}\)Se CP correlation experiments and found that one \(^{15}\)N peak at 34.8 ppm correlated with both of \(^{113}\)Cd and \(^{77}\)Se, while another at 42.6 ppm correlated with only \(^{113}\)Cd. Therefore we conclude that ligand-cysteine has two kinds of amino groups; one coordinates to Cd on the surface, and another forms no coordination but lies nearby the surface. Moreover, we revealed that H of CH\(_2\) of ligand-cysteine might interact with Se on the surface, from examination of the \(^1\)H peak shift of CH\(_2\) on \(^1\)H MAS NMR spectrum and the analysis result of contact time dependence of \(^1\)H–\(^{113}\)Cd and \(^1\)H–\(^{77}\)Se CP/MAS NMR.

The Structure of Sepiolite as Studied by $^1$H-$^{29}$Si Hartmann-Hahn Matching Profiles under Magic-Angle Spinning

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Sepiolite (Figure 1) is a naturally occuring, fibrous, hydrated magnesium silicate belonging to the clay mineral family and forming one of the major components of the famous indigo-based pigment Maya Blue. We have previously confirmed the assignment of the $^{29}$Si magic-angle spinning (MAS) spectrum of sepiolite made by Weir et al.\(^1\) using two-dimensional (2D) $^1$H-$^{29}$Si heteronuclear correlation (HETCOR) spectroscopy with frequency-switched Lee-Goldburg (FSLG) homonuclear decoupling\(^2\). However, this assignment relies on the attribution of $^1$H chemical shifts performed by Aramendia et al.\(^3\) In this work, we analyze the $^1$H-$^{29}$Si Hartmann-Hahn (HH) matching profiles of the different Si sites of sepiolite. Figure 1 shows that the HH profiles associated with Type 3 (center) and Type 1 (edge) Si sites are markedly different. The calculation performed on the basis of a two-step memory function approach and the Anderson-Weiss approximation\(^4\) demonstrate that the HH matching profiles are directly related to the proton dipolar local fields. The structure sensitive HH matching profile band amplitudes provide an important aid in establishing the connectivity between $^1$H and $^{29}$Si nuclei in sepiolite from dipolar couplings only.

References:

*Figure 1. Structure (top) and HH matching profiles (bottom) of sepiolite.*
Proton Magnetic Relaxation Dispersion (PMRD) profiles have been collected in the nematic phases of the low viscous mesogen 6CHBT and binary liquid crystalline mixtures composed of 4DBT and 12CB, with the aid of Fast Field Cycling NMR (10 kHz – 30 MHz). Two distinct compositions of the binary mixture i.e. 50% of 12CB (with a smectic-A phase) and 70% of 12CB (with a smectic-A$_d$ phase) have been used.\(^1\) Variable-temperature PMRD data have been analyzed by using a suitable model,\(^2\) which accounts for the influence of local organizations on the nematic phase stability. Nematic clusters of moderate size are found in the broad nematic region of 50% 12CB, whose size is almost invariant with temperature. On the other hand, local smectic clusters of relatively larger size are observed in the nematic phase of 70% 12CB. The extension of collective modes i.e., director fluctuations (DF) to higher frequencies and very weak temperature dependence of non-collective modes such as molecular reorientations, taken together remark the sustenance of short wavelength local organizations observed for 50% 12CB, and, consistent with results observed on the low viscous nematogen 6CHBT. However, DF modes limiting to relatively low frequencies, correlated to the presence of relatively long-lived local organizations as noted for 70% 12CB. The present results convey an impression that the underlying local organizations have a profound influence on the power spectrum of collective modes and hence nematic phase stability.

References:


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Solid State NMR study of layered double hydroxides intercalated with para-aminosalicylate

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Layered double hydroxides (LDH’s) are a group of inorganic material with many potential and current applications ranging from environmental remediation and catalysis to drug delivery[1]. LDHs consist of positively charged layers with anions intercalated in the interlayers for charge balance. LDH’s are generally described by the formula $M(II)_{1-x}M(III)_x(OH)_2A_n \cdot nH_2O$, where M are metals and A an anion[2], $0.33 \geq x \geq 0.16$. However, intercalation of drugs in LDHs results in many stacking faults rendering their characterization by PXRD difficult. SSNMR has proven useful to study the cation ordering in the cation layer[3-4], the mobility of the interlayer anions[5], and especial to probe the intercalated anions[6]. Para-aminosalicylate (PAS), a tuberculosis drug, was intercalated in three different LDH’s MgAl, ZnAl and CaAl. The samples were studied in detail using powdered X-ray diffraction, elemental analysis and IR in order to gain insight in to the bulk properties of the sample. 1H, 13C, and 27Al solid state NMR (SSNMR) were performed to obtain detailed knowledge about the local structure (atomic scale) of the samples. It was by the use of $^1$H-$^1$H-double quantum and $^1$H-$^{27}$Al-HETCOR possible to assign the $^1$H spectra. Intercalation of para-aminosalicylic acid in MgAl, ZnAl and CaAl LDH’s was confirmed by the use of the several techniques Including $^{13}$C CP MAS SSNMR and IR, which verified that PAS did not decompose during synthesis. $^{27}$Al SSNMR revealed large quantities of Al impurities not seen by the bulk techniques for MgAl and ZnAl LDH’s (20-41%). For the CaAl LDH’s did the Al impurities match the results from elemental analysis (35%). Thus, the current works demonstrate the advantages and need for the use SSNMR for studies of LDHs with pharmaceuticals and other larges organic anions intercalated in order to reliably validate the intercalated anions and the purity of the LDH.

Characterization of polymer morphology in composite materials by spin diffusion experiments

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Polymers form the basis of a wide variety of composite materials used in many areas such as transports or building. These materials are made of a polymer matrix which ensures the cohesion of the assembly and the transfer of forces. The mechanical properties of these materials depend on the physico-chemical characteristics of the constituents and on many microstructural parameters such as quantity, size, shape of the particles and the interface between polymer and reinforcement. This last component is generally the less concentrated compound and of mineral nature but can be also organic for some specific applications. The adhesion at the polymer/reinforcement interface is of prime importance regarding mechanical properties. This property is related to the ability of producing a fine mixture of both species during the fabrication step. This operation is even more difficult whenever the amount of polymer is considerably reduced. The final product thus depends on the surface properties of the particles and mutual affinity between constituents.

NMR spectroscopy which is widely used for the structural characterization of many molecular systems is also sensitive to the close molecular environment and to the material structuration at a length scale of a few dozen of nanometers. Such information is accessible by taking advantage of the possible spatial transport of magnetization by the spin diffusion phenomenon within the proton system. Since the work of Goldman and Shen [1], several variants of the experiment have been proposed and the methods have been essentially used for the determination of domain sizes in heterogeneous polymers, polymer blends and bloc copolymers [2,3].

In this work, we have investigated the possibility of using this approach for the characterization of composite materials containing a reduced content (less than 5%) of soft/hard polymer. It was applied for the determination of size and dimensionality of soft domains in polymer bonded high explosives (PBX). NMR results were compared to the micro-structural observation of the surface of the materials by SEM (Scanning Electron Microscopy), EDS (Energy-Dispersive X-Ray Spectroscopy) analyses and RAMAN spectroscopy.

References:

Insights into the Activity of Heterogeneous Catalysts from high-field (DNP)-NMR

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The rational development of more selective and more active heterogeneous catalysts can be undertaken in a rational way by a better understanding of the structure of the active sites. Because it can give information on the local atomic scale, Solid-State Nuclear Magnetic Resonance (SSNMR) spectroscopy is very well suited to the study of heterogeneous catalysts. However, the lack of sensitivity and resolution often limits the ability of SSNMR to characterize surface sites.

It has been shown how these limitations can be circumvented by the use of high $B_0$ field and/or Dynamic Nuclear Polarization (DNP). By using these methods, we have recently gained new insights into the activity of important heterogeneous catalysts: amorphous silica alumina (ASA) and functionalized fibrous silica nanoparticles (KCC-1).

ASA is a widely employed solid-acid catalyst. It is accepted that its Brønsted acidity arises from protons in the vicinity of tetra-coordinated aluminium sites (Al⁴⁺), whereas the penta- (Al⁵⁺) and hexa-coordinated (Al⁶⁺) aluminium sites provide Lewis acidity. Using through-space $^1$H-$^{27}$Al correlation 2D NMR experiments at high-magnetic field, we have detected Brønsted acid sites based on Al⁵⁺ sites.¹ These $^1$H-$^{27}$Al NMR experiments also show similar acidity of Al⁴⁺ and Al⁵⁺-based Brønsted acid sites to protonate ammonia.

We have also used DNP to probe the nature of active sites in functionalized KCC-1, which are promising for CO₂ capture and solid-base catalysis.² This DNP-NMR study has explained the decrease of the catalytic activity of nitridated KCC-1 when increasing the nitrogen content. More recently we have shown that in these nanoparticles, the highest enhancements for the direct DNP of isotopes other protons are achieved for radicals, which are not nitroxides.


NMR study of thermoresponsive nanoparticles of block terpolymers in water

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It is well known that some amphiphilic polymers show in aqueous solutions a lower critical solution temperature (LCST). They are soluble at lower temperatures but heating above the LCST results in phase separation which is a macroscopic manifestation of a coil-globule transition followed by aggregation and formation of so-called mesoglobules [1]. Amphiphilic block copolymers undergo self-assembly into micelle-like structures containing hydrophobic core covered by hydrophilic shell. It was shown that NMR spectroscopy can play an important role in investigations of these systems [2]. Recently we applied measurements of $^1$H NMR spectra and spin-spin relaxation times to investigate the temperature behavior of nanoparticles (NPs) of block terpolymers containing blocks of poly(ethylene oxide) (PEO), thermoresponsive poly(2-ethyl-2-oxazoline) (PEtOx) and poly(ε-caprolactone) (PCL) in aqueous solution. Due to their biocompatibility this type of copolymers has potential applications as drug nanocarriers.

Formation of micellar or globular structures results in a marked line broadening of a major part of respective polymer segments in NMR spectra. The temperature dependences of the fraction of polymer units with significantly reduced mobility can be then determined from integrated intensities in high-resolution NMR spectra [2]. NPs prepared from two Y-shape terpolymers: [PEO$_{44}$-b-PEtO$_{252}$-b-(PCL$_{87}$)$_2$] (c=0.5 and 1.5 wt%) and [PEO$_{44}$-b-PEtO$_{252}$-b-(PCL$_{131}$)$_2$] (c=0.5 wt%), and one linear system: PEO$_{44}$-b-PEtO$_{252}$-b-PCL$_{35}$ (c=0.5 and 1.5 wt%) were studied in aqueous solutions. Structural changes were characterized separately for all block types in a temperature range from 295 to 360 K. In all investigated samples a broad transition of PEtOx blocks was observed with maximum values of the $p$-fraction (fraction of units with significantly reduced mobility) around $p_{\text{max}} \approx 0.4$. The PEO blocks reach similar $p_{\text{max}}$ as PEtOx blocks. Furthermore, the temperature behavior of NPs depends on polymer concentration and especially on terpolymer architecture. At the same time we revealed by NMR measurements that the PCL blocks play important role in the temperature behavior of the NPs solutions. From measurements of spin-spin relaxation times $T_2$ of HDO, different behavior of the water molecules in NPs solutions of linear and Y-shape systems was observed. In NPs solutions of the linear terpolymer two types of water, „free” (long $T_2$ component) and „bound” (short $T_2$ component with $T_2 \approx 10$ ms) were detected at higher temperatures. For NPs solutions of Y-shape terpolymers two types of water were observed only for higher (1.5 wt%) polymer concentration.

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Since metal–air batteries require a continuous oxygen exchange from the ambient atmosphere through the cathode, limitations due to the wetting properties of the cathode and the evaporation/carbonization of the electrolyte present major challenging factors for the use of metal-air systems as secondary batteries. To overcome these issues, new electrolytes such as ionic liquids are being investigated. To systematically improve the pore structure and to provide an optimized surface area for a particular electrolyte, knowledge of the electrolyte mobility in pores and interactions with pore surfaces at the electrode is required [1]. In contrast to ordinary liquids, ionic liquids show a contradistinctive mobility behavior, in particular if motion is restricted by a porous material. An accurate understanding of such phenomena call for a multimodal investigation to adequately describe mobility and wetting of ionic liquids, where the directly accessible information of molecular displacement afforded by \(^{1}\text{H}\) PFG NMR is invaluable to gain insights on ionic liquid distribution and motion in porous electrodes.

The diffusion behavior (\(D_{\text{eff}}\)) of a series of ionic liquid was assessed by \(^{1}\text{H}\) PFG NMR and \(T_{1}\) measurements in terms of their mobility and interactions with pressed Vulcan carbon black of different porosities and cathode layers prepared via electrospinning of polyacrylonitrile solutions with subsequent carbonization. In-pore and ex-pore cations of the ionic liquid were distinguished, where exchange between these two domains led to line broadening with higher amounts of ionic liquid. For lower loads of ionic liquid, the long range mobility was found to be faster and an enhanced \(D_{\text{eff}}\) inside the pellet compared to the neat ionic liquid was monitored. Finally, the results were compared to gas adsorption measurements. From these measurements distinct behaviors of different gases (nitrogen, argon and carbon-dioxide) at the pore surface is gained. With these measurements information of the pore size distribution can be obtained.

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Experimental and numeric characterization of various PEM membranes for fuel cells

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Fuel cells are viewed as one of the most promising environmental friendly energy sources and the polymer membrane (PEMs – polymer electrolyte membranes) is their heart. Perfluorosulfonic acid ionomers (PFSI) like Nafion or are PFSI/SiO₂ are two types of polymers used which allow an easy miniaturization of fuel cells making them suitable as zero-emission energy sources for automobiles, domestic application or portable devices [1]. The water behavior in PEMs is correlated to the proton conductivity as the main parameter for characterization of PEM performances. The complex phenomenon of water dynamics in PEM is due to the presence of hydrophilic and hydrophobic domains (see Fig. 1) of the polymer matrix and to the different affinity between hydrophilic groups and water molecules [2]. A series of 2D NMR T₂–T₂ exchange maps were recorded. These are obtained for hydrated PEM Nafion 117 and for PFSI/SiO₂ membranes in function of SiO₂ content (from 0 to 10 wt) in function of exchange time. The simplified two-phase model of bulk and bound water was employed to describe the results obtained by ²H-NMR Laplace spectroscopy [2-6]. Next, we calculate the exchange velocity kₐ (free to bound water) and k₋ (bound to free water) from the integral area’s amplitudes of the extra-diagonal peaks function of exchange time. For that we use a system of coupled first order differential equation which describes the relaxation and exchange between two coupled spin systems [5]. The results are finally validated by a series of MONTE-CARLO simulation (for which we wrote a numeric program) where the inputs were ideal maps (with 3 components which can communicate directly or indirectly) as well numeric maps of channels in PEMs membranes [6].

References

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Characterization of Superabsorbent Hydrogels by Low-Field 1D and 2D $^1$H NMR Laplace Spectroscopy

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Superabsorbent hydrogels, cross-linked polymers having the ability to absorb a large amount of water from aqueous medium, can be used, in particular as intelligent materials. Such materials are able to respond to various properties of environmental changes like: solvent composition, pH or temperature [1]. In the same time other aspects of environment like the UV-radiation, during the formation of hydrogels (apriori or aposteriori), can affect the swelling behavior of such hydrogels. A series of polymer hydrogels like: Poly(acrylamide-co-acrylic acid) potassium salt-cross-linked, Poly(acrylic acid) partial salt graft-poly(ethylene oxide)-cross-linked, Poly(isobutylene - co-maleic acid) sodium salt-cross-linked were investigated by: i) one-dimensional $^1$H NMR relaxometry (CPMG pulse sequence) and saturation recovery methods and ii) two-dimensional $T_2$–$T_2$ exchange maps [2, 3] to observe the evolution of dynamic components of hydrogels after hydration. In general three dynamics components were observed for the $T_2$ and $T_1$ Laplace distributions. The effects of hydration/dehydration, action of specific environmental factors can be observed by the migration of particular component in the 1D $T_2$ ($T_1$) distributions or diagonal and in special extra diagonal peaks in the 2D $T_2$–$T_2$ exchange maps. Moreover, we found that the UV radiation even less that one hour can affect significantly the swelling properties of polymer hydrogels by destroy probably the double bonding.

References

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Reaction Monitoring of Near-Room-Temperature Synthesis in Ionic Liquids by NMR spectroscopy

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Ionic liquids are salts melting at temperatures below 100°C. Various inorganic materials can be synthesized in ionic liquids under favorable conditions, i.e. near ambient temperature. This approach enables an enormous reduction of energy usage and technical efforts compared to the commonly applied high-temperature processes. Syntheses in ionic liquids also provide great opportunities to discover completely new compounds with potentially outstanding and useful chemical and physical properties [1]. Continuous reaction monitoring is necessary for the understanding of reaction processes. Combination of liquid- and solid-state NMR spectroscopy is a valuable method for these purposes.

As an example, the reaction of red phosphorus with iodine in [BMIm]Cl · 2AlCl₃ as ionic liquid was selected [2]. ³¹P HR NMR spectroscopy was applied as an advantageous method for reaction monitoring. The reaction process and its products were investigated under varying temperature and educt composition. Depending on the ratio of red phosphorus and iodine in the starting solution and the reaction temperature, different final products appeared. Furthermore, the cation and anion of the ionic liquid were varied in the following investigations. Remarkably, the variation of the cation had no influence on the reaction, but the product formation was crucially dependent on the anion of the ionic liquid. In addition to the signals of the expected products, several NMR signals appeared which cannot be assigned to NMR signals of known product species yet. The obtained results show the complexity of the system and also the probability of halide exchange. Further studies are in progress for a better understanding of this reaction.

NMR Diffusometry Analyses of H-bonding Effect on the Diffusion of Drug Analogue inside Crosslinked Hydrogel

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Hydrogels in controlled drug delivery have received increased attention due to potential in drug eluting contact lenses and in the pharmaceutical field because of the anticipated biocompatibility of this class of materials. Currently, several studies tried to understand the relationship between the drug’s diffusion and the hydrogel matrix’s physical properties. However, a systematic study about the effect of tailored chemical structure of the gel matrix on the diffusivity of the small solute inside it is still missing due to rather complicated morphological and topological properties these hydrogel matrices possess. In our previous study, we demonstrated the important role that the crosslink density of the gel matrix (\(M_c\)) has on the diffusivity of the drug solute (\(D_{drug}\)) inside it. It was found that there is still significant interaction between gel matrices and drug solute even when the calculated mesh size is dramatically larger than the hydrodynamic size of the drug solute.

Meanwhile, as suggested by several studies, adding Hydrogen bond into the system is a common strategy used in biological systems and polymeric membranes to control the diffusion of the solute in a certain media. Hence, it would be very desirable if this strategy could also be applied into the commercial available hydrogel systems.

In this study, a series of polyacrylate-based hydrogels were synthesized via UV-initiated polymerization. The hydrogen bonding strength in these hydrogels were tailored by varying the monoacrylate copolymer in curing formula (e.g. replacing the hexylacrylate with bulky urethane-group-containing acrylate). After confirming the high double bond conversion in these networks by FTIR, they were swelling inside a drug analogue (fluorescein sodium salt) aqueous solution. Subsequently, these systems were studied by HF 1H NMR diffusometry and LF 1H NMR relaxometry. The extracted self-diffusivity of fluorescein inside these networks from PFG NMR experiments were discussed in relation to the testing temperature, H-bonding types and the ratio of rigid domain inside the system. Good correlation was found between the H-bonding strength and the diffusivity of fluorescein inside the network, and bulky side group also play an important role in these systems.

References
Nano-scale Morphological Analyses of 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 material 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 opened 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 NMR to probe the ‘nanogel’ formation during the curing procedure. A series of PEG-based polyacrylate hydrogels with different curing degrees were synthesized via UV-initiated polymerization. As suggested by previous publications using AFM and SAXS analyses, a two–phase-system should be formed during the initial curing stage, and this two-phase system would gradually disappear when the curing reaction completed. By analyzing samples with different swollen degree, the ‘nanogel’ formation is discussed with the change in the domain size and the sample’s corresponding curing degree.

\textbf{Figure 1. Build-up curve of the magnetization of mobile domain and less mobile domain in 50% curing PEG-polyacrylate gel: (left) dry sample; (right) 20% H\textsubscript{2}O swollen sample}
Synthesis and solid state NMR characterization of biofunctionalized silica-nanopores

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Understanding biological materials is a major challenge for material scientists. Many of these natural occurring materials have useful properties for employing them as smart materials.¹ Especially ion conducting materials such as biological membranes and ion channels may be used as sensors in a variety of technical applications.

However, these complex materials have to be characterized in detail to understand their structure-property relationship. A suitable way is the creation of model systems, which enable the selective optimization of their structure and thus their properties. Biologically modified silica-nanopores based on SBA-15 and MCM-41 materials employing different peptides may act as model systems for a better understanding of the structure of ion conducting materials.¹ The combination of ²⁹Si and ¹³C CP-MAS-NMR techniques provide information on the successful biological modification of the silica pore (figure 1).

For a deeper structural understanding ¹⁵N MAS-NMR seems to be the method of choice since ¹⁵N shifts are strongly influenced by their chemical environment (especially hydrogen bonds).² Nevertheless, ¹⁵N-NMR suffers from its low intrinsic sensitivity, and thus requires sensitivity enhancement techniques such as Dynamic Nuclear Polarization (DNP) to perform these experiments on peptide functionalized material containing ¹⁵N in natural abundance.³ The presentation will demonstrate the developing of proper synthesis strategies for defined peptide silica hybrid materials. This includes a high grafting density of peptides and the proof of covalent bindings. The surface structures are then characterized by a combination of solid state NMR and surface enhanced DNP.

Measurement of $^{63}$Cu-$^{31}$P J-couplings in copper iodide clusters.

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Molecular copper-iodide clusters are attracting much attention because of some of them present mechanochromic luminescent properties, i.e., they exhibit reversible modification of the emission wavelength in response to external mechanical forces (grinding, shearing, compressing or rubbing). $^1$ The origin of these luminescence mechanochromism is assumed to be closely related to the local structure of the [Cu$_n$I$_n$P$_n$] cluster cores, more particularly to Cu-Cu distances. $^{63,65}$Cu solid-state NMR, very sensitive to the Cu coordination environment in molecular solids,$^2$ is therefore particularly well suited to study the cluster modifications on a local scale. The $^{31}$P-$^{63,65}$Cu scalar couplings are also very large (greater than 1 kHz), and are a potential source of structural information. However, the complexity of the $^{31}$P J-multiplet, due to the coupling with the two copper isotopes ($^{63,65}$Cu, both spins 3/2, with 70 and 30% natural abundance, respectively), prevents an easy determination of the coupling constants when more than two or three inequivalent phosphorus sites are present.

In this contribution we report the use of the $^{31}$P-$^{63}$Cu J-HMQC experiment, recorded at different static magnetic fields on several copper(I)-based molecular compounds. By filtering out the J-coupling between $^{31}$P and $^{65}$Cu as well as the outer transitions of the $^{31}$P-$^{63}$Cu J-multiplet, a great simplification of the $^{31}$P MAS NMR spectra is obtained, with $^{31}$P-$^{63}$Cu J-coupling constants that become are readily readable on the NMR spectra. Further dipolar-based experiments, that provide information about the various species in the clusters, will also be presented.

References.

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The natural rubber segmental dynamics response at thermal treatment monitored by $^1$H DQ NMR and Laplace spectra

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Nowadays use of natural rubber (NR) materials imply the service of end product in variety of environments where complex degradations, such as oxidation, swelling, leaching, or even biodegradation under heat/cooling cycles, UV-light or other physic-chemical factors may occur [1]. In practice, the accelerate aging (i.e. under high temperature) instead of natural ageing is a largely used method for the characterization properties changes of elastomers. The investigated elastomers are based on commercially available NR SMR10 (Malaysia). Seven differently cross-linked NR samples were investigated. For ageing, all samples (three pieces for each treatment) were treated into an ECOCELL 55 stove (drying chamber). After that, the treated samples were stored one into a refrigerator (at 4 °C), one at light and one in dark, both at room temperature. It was observed an unexpected behavior after thermal treatment depending on the treatment temperature and storage conditions. The monitoring methods were: $^1$H DQ NMR and Laplace NMR spectroscopy (distribution of transverse relaxation times) [2]. For the Laplace distribution we used the Prospa analyzing software [3]. The $^1$H DQ NMR build-up curves dependent on the segmental dynamics of elastomers [4], are very sensitive to the storage conditions of aged NR. The shape of such DQ build-up curves and Laplace spectra present changes in time, up to several days after thermal treatment before an equilibrium is reached.

![Figure 1. $^1$H DQ buildup curves for NR 1 natural rubber aged for 1 hour at 60 °C, 120 °C and 200 °C.](image1)

![Figure 2. $T_2$ distributions for NR 4 natural rubber aged at 160 °C for 5 h and stored in the dark for 0, 2 and 4 days.](image2)

References


Acknowledgements

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How the method of synthesis governs the local and global structure of LDHs

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Layered double hydroxides (LDHs) find wide spread application ranging from catalysis and energy storage to environmental remediation and drug delivery. Their general chemical composition is \([M(II)_{1-x}M(III)_x(OH)]_2A_ynH_2O\), where M(II) and M(III) are a wide range of cations such as Mg(II), Ca(II), Zn(II), Al(III), and Fe(III), and A is an anion needed for charge balance. The LDH properties and hence function are controlled by the choice of metal ions and anion. Especially, the distribution of metal ions in the cation layer has a large impact on the properties, but this information is difficult to obtain by diffraction techniques due to the large number of stacking faults in the LDHs. Detailed insight into the cation distribution can be obtained by solid state NMR spectroscopy (SSNMR), where fast MAS at high field directly provides information about the distribution of cations. MgAl LDHs has been the primary focus of advanced SSNMR studies. However, ambiguities exist whether LDHs exhibit cation ordering (no Al-O-Al connectivities), as some SSNMR studies have identified cation ordering, whereas Al-O-Al defects have been clearly identified by others.

To resolve these ambiguities, we have performed a detailed study of a series of ZnAl LDHs synthesized by the two preferred routes, co-precipitation and the so-called urea method, using probes of both the bulk properties (powder X-ray diffraction, elemental analysis, TEM) and local environment, i.e., multi-nuclear \(^1H, ^27Al, \text{ and } ^{67}Zn\) SSNMR and Raman spectroscopy. The results clearly show that the presence of Al-O-Al defects is linked to the synthesis conditions and may be partially “repaired” by hydrothermal treatment. However, the preferred urea method gives large, crystalline LDHs, but SSNMR reveals only 20-40 % ZnAl LDH in the sample. Thus, to gain further insight into the formation of LDHs a time-resolved study of ZnAl LDHs formation by the urea method was performed and will be presented.


Figure 1. The ZnAl LDHs content is very sensitive to the method of synthesis.
Electrospun lipid binding proteins composite nanofibers with antibacterial properties as potential wound dressing

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One of the promising new techniques in the production of biomaterials is the electrospinning process, whereby fibers of uniform thickness down to the nanoscale can be produced from solutions of polymeric material in a high electric field. Recent investigations exploited the use of fibrous proteins such as collagen, keratin, and fibroin. We have investigated here the feasibility of electrospinning a keratin/PEO mixture containing a lipid binding protein, hosting into its internal pocket a variety of selected small functional molecules. Here a complex of a bile acid binding protein with Irgasan, a broad spectrum antimicrobial agent, was employed for the production of biomembranes with excellent antibacterial properties. Solution NMR studies were employed to investigate structure-property relations in view of the optimization of the bactericidal tissue performance.
Highly Ion-Conductive Form of Pb$_{x}$Sn$_{2-x}$F$_4$

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PbSnF$_4$ has long been known to show high anionic conductivity, and various structural studies have been done to elucidate the relationship between its structure and the conduction properties.\textsuperscript{1-4} So far, the $\alpha$ and $\beta$ PbSnF$_4$ crystalline forms have been considered as a two-dimensional super ionic conductor in which the F$^-$ ion moves in the crystallographic plane normal to the c axis. The conductivity depends on the synthesizing ratio and is largest ($\sim 3 \times 10^{-3}$ S cm$^{-1}$) at PbF$_2$:SnF$_2$=50:50.

In this study, we examine various initial ratios of PbF$_2$ and SnF$_2$ to prepare several Pb$_{x}$Sn$_{2-x}$F$_4$ samples ($x = 1.52$–$0.96$) obtained with annealing temperature of 400 °C, which is close to the melting point (ca. 390 °C) of Pb$_{x}$Sn$_{2-x}$F$_4$ with $x$~1. In contrast to the previous work that reporting the maximum conductivity achieved for $\beta$-PbSnF$_4$ at $x = 1$ ($\sim 2.2 \times 10^{-3}$ S cm$^{-1}$), the observed conductivity reaches maximum of $\sim 3.5 \times 10^{-3}$ S cm$^{-1}$ at $x$~1.2.

To examine the structural origin of the high conductivity, we applied high-resolution solid-state NMR for $^{207}$Pb, $^{119}$Sn, and $^{19}$F, synchrotron X-ray powder diffraction (SXRD), neutron diffraction (ND), and ab initio calculation. By a combined use of these methods, we show that the high conductivity is realized by a new crystalline phase, which we shall refer to as the $\beta^+$ form. It is shown that the $\beta^+$ form consists of two alternating layers; one layer resembles that of the $\beta$ form as it consists of double Pb layers flanked by a single Sn layer, while the other layer has triple Pb layers flanked by a single Sn layer. It is also shown from the $^{19}$F-$^{207}$Pb cross-polarization NMR experiment that most of F$^-$ ions, except for those lie between the double Pb layer, are mobile and thus contributing to the high ionic conductivity. Further, it is suggested, by the temperature-dependent NMR chemical shift of $^{207}$Pb, that the high conductivity in the $\beta^+$ form is supported by the elastic expansion especially of the triple Pb layers.

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Reference
EXPANDING THE NMR PALETTE: INSIGHTS ON ARTIFICIAL CHARGE SEPARATORS

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Spurred by worries over climate change, there is increasing interest in mimicking natural photosynthesis for the conversion of solar energy into fuel. The molecular structure and packing of self-assembled Zinc Salphen/NDI dyad and Perylene-based molecules, which are potential, charge separators were studied in detail in the solid state.

The computational integration of MAS NMR, TEM, Powder XRD and molecular modeling provide a powerful methodology that can be of use to investigate molecular geometry (and properties) of larger unlabeled - aggregated supramolecular systems. Systematic absence observed in the diffraction pattern and symmetry constraints from SSNMR were used to converge on a reasonable packing. DFT calculations were performed using the CASTEP module in the material studio with GIPAW wave function. Quantum mechanical calculations allow experimental $^1\text{H}$ and $^{13}\text{C}$ solid-state NMR spectra to be assigned in a quantitative manner to a specific molecular packing arrangement, starting from the chemical structure of a moderately sized molecule. Proposed packing is confirmed by selective NMR distance constraints and simulation of LGCP build up curve. To confirm the model we simulated the powder XRD pattern using Reflex module in the material studio. Observed diffraction pattern were reproduced using crystal maker from the proposed packing.

A protocol was developed in which the computational integration of MicroED, Powder XRD and SSNMR were used to propose a model for a molecule with high molecular mass, with less ambiguity (Fig 1). One of the biggest challenges with smarter crystallography is that it is limited to small molecules but here we proposed structures for molecules with higher atomic weight, which is around 1000g/mol. This methodology could be extended to understand the surface deposition on electrode surface to understand the mechanism of battery in the near future.
NITRIC OXIDE ADSORPTION IN MIL-100(Al) MOF STUDIED BY
SOLID-STATE NMR

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Gas-delivery technologies using metal organic frameworks (MOFs) are of increasing importance in many areas of science with emerging applications that include storage of nitric oxide (NO). Delivery of NO from a storage material is attractive for many in vitro and in vivo antibacterial, antithrombotic, and wound healing applications. MOFs are materials with extremely high porosities, some of them have coordinatively unsaturated metal sites (CUSs) that are available for binding of small molecules. The presence of CUSs in MOFs has a particularly pronounced effect on the NO adsorption and release characteristics. We studied MIL-100(Al) prepared by a modified synthesis and its interaction with NO by multinuclear solid-state NMR. MIL-100 is an aluminium trimesate MOF where terminal water molecules are attached to the $\mu_3$-oxo centered trimeric units $\text{Al}_3\text{O}$. This water is removed upon heating which generates CUS sites [1]. Solid-state NMR is a powerful technique probing the local geometry of the MOF, providing information on the interaction of guest molecules with the host material and on adsorption sites of NO within the MOF. The results show the presence of extra-framework $\text{Al(OH)}_3$ as evident form $^{27}\text{Al}$ and $^1\text{H}$ as well as HETCOR spectra. $^{27}\text{Al}$ area integration shows that five coordinate sites represent 50% of the material which remain open for adsorption. With increasing NO loading, a decrease of five-coordinated aluminum with a subsequent increase of six-coordinated aluminum intensity is found which hints at NO interaction with MIL-100(Al), see Fig. 1. Additionally, the $^1\text{H}$ $T_1$ is decreasing with increasing amount of NO which also supports NO interaction with the MOF because of the paramagnetism of NO.

![Figure 1: $^{27}\text{Al}$ NMR of NO loaded MIL100(Al). Color coding: black 1 NO, green 0.75 NO, purple 0.5 NO, red 0.25 NO, and light blue 0 NO per aluminum trimer.](image)

References

27Al MAS and MQMAS NMR study of ye’elimite at different magnetic fields
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Calcium sulfo-aluminate (CSA) cement is an alternative to conventional Portland cement which currently attracts significant attention in cement science, as it can be produced with lower energy consumption and lower embodied CO2 emission. The main component of CSA cement is ye’elimite (Ca4Al6SO16) which has been reported to contain eight crystallographically different Al sites, all being tetrahedrally coordinated to oxygen atoms. Very few 27Al NMR studies have been performed on ye’elimite and they have only confirmed the presence of aluminum in tetrahedral coordination. Thus, the distinct Al sites have not been identified and neither have their 27Al quadrupole coupling parameters and isotropic chemical shifts been obtained.

The 27Al quadrupole coupling parameters and isotropic chemical shifts can be determined from line shape simulations of the second-order quadrupolar line shapes observed for the 27Al (I = 5/2) central transition. However, due to significant overlap of the eight resonances in the 27Al MAS NMR spectra of ye’elimite, 27Al MQMAS NMR was utilized prior to these simulations to obtain an improved resolution of the individual sites. The F1 projections of the isotropic dimension of the triple-quantum MQMAS spectra reveal four distinct resonances (Fig. 1), indicating very similar isotropic chemical shifts and quadrupole coupling parameters for some of the eight Al sites present in ye’elimite. However, indications of resonances from all eight Al sites can be seen by a full evaluation of the 2D MQMAS spectra.

27Al MAS and MQMAS NMR spectra of synthetic ye’elimite have been obtained at five different magnetic fields (4.7, 7.1, 9.4, 14.1 and 22.3 T). Analysis of the MQMAS NMR spectra at these fields allows determination of the isotropic chemical shifts and the quadrupolar effect parameters (SOQE) from the relationship between the isotropic triple-quantum shifts and the applied magnetic field strengths. Based on these data, full simulations of the 27Al MAS spectra have been conducted using the STARS software, providing the quadrupole coupling parameters and isotropic chemical shifts for the individual Al sites in ye’elimite. These parameters are related to the specific structural Al sites in the structure of ye’elimite by density functional theory (DFT) calculations of the electric field gradients at the nuclear Al sites.

Figure 1. Contour plots of 27Al MQMAS NMR spectra of ye’elimite recorded at 4.7 T and 14.1 T. The projections onto the isotropic (F1) and anisotropic (F2) dimensions correspond to summations over the 2D spectra.
Size, Shape and Electronic Structure of an Anion Controls the Selective Binding with an Inherently Chiral Cyclohexanohemicucurbit[8]uril Host

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The current study describes a full encapsulation, which is size and shape selective, of anions by the chiral cyclohexanohemicucurbit[8]uril in 1:1 ratio in gas, solution and solid phase. Crystallographic study revealed that the 8-membered hemicucurbituril with octahedral cavity adopted the same conformation in solid state regardless of anion size (Figure 1). A complex with SbF$_6^-$ in methanol was the most stable with the association constant of $2.5 \times 10^5$ M$^{-1}$ (Figure 2). Isothermal titration calorimetry showed that complexation is exothermic and enthalpy driven, though conformational change necessary for wrapping the guest into the host is penalized by entropy. Dynamic NMR and DFT study revealed that overall complexation goes through the precomplex formation and rate limiting step is exchange of methanol solvent with a chaotropic anion. The guest exchange rate is fast in a protic solvent in ambient conditions, coalescence temperature for PF$_6^-$ and SbF$_6^-$ is 253 and 257 K respectively. Development of controlled cargo release, catalysis in confined space and other supramolecular applications can be envisioned on the base of this host.

Figure 1. a) XRD structure of 1:1 host-guest complex of cycHC[8] with TBPSbF$_6$, showing the orthorhombic cavity; orientation of b) PF$_6^-$, c) BF$_4^-$, d) ClO$_4^-$, e) IO$_4^-$, f) ReO$_4^-$ and g) CF$_3$SO$_3^-$ anions in the cycHC[8] cavity.

Figure 2. $^1$H NMR in MeOD of a) free cycHC[8], b) cycHC[8] with 0.6 eq of NaSbF$_6$ at 288K c) cycHC[8] with 10 eq of NaSbF$_6$ at 288K; d) SbF$_6^-@$cycHC[8] and free cycHC[8] formed from cycHC[8] with 0.6 eq of NaSbF$_6$ at 229K
Modeling $^{129}$Xe NMR in porous materials: Cavity properties, dynamics and unknown crystal structures

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Already the early work of Ripmeester and Davidson [1] of clathrated xenon revealed the potential of $^{129}$Xe NMR in studies of inclusion compounds. The exceptional sensitivity of the Xe shielding to its environment allows comparison between observed and calculated isotropic and anisotropic chemical shifts that enables the study of size and shape of the cavities of novel self-assembling metallosupramolecular cages [2,3] and porous organic structures [4,5]. With $^{129}$Xe potential energy and NMR property hypersurfaces, modeling can provide detailed information about temperature dependent motional effects in these systems [3,5].

While clathrates are fairly easy to produce and measure by $^{129}$Xe NMR, determination of their X-ray crystal structure may sometimes be experimentally challenging. Here we demonstrate that the combination of experimental and computational $^{129}$Xe NMR can be used to distinguish the most probable one from the numerous high-energy porous clathrate structures generated by the crystal structure prediction (CSP) [6]. Based on the comparison of modeled $^{129}$Xe NMR parameters with measured spectra, we propose structures for the two unknown clathrates [7].

References:
Multi-nuclear solid state NMR in photocatalytically active Dion-Jacobson triple-layered perovskites.

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The Dion-Jacobson layered niobium perovskite RbCa$_2$Nb$_3$O$_{10}$ possesses a variety of interesting properties, among which is the photocatalytic activity in splitting of water under UV irradiation. Substitution of Ca with Pb in the structure produces a substantial reduction in the band gap, leading to a visible light sensitization of the material. Replacement of Ca with Pb, however, has a severe negative effect on the exfoliation properties of the catalyst, which can be further used to improve the catalytic performance. Good compromise between the narrowing in band gap and exfoliation properties can be achieved through preparation of solid solutions RbCa$_{2-x}$Pb$_x$Nb$_3$O$_{10}$, specifically for an initial ratio of Ca:Pb $\geq$ 1:1.

Solid state NMR of $^{93}$Nb, $^{87}$Rb and $^{207}$Pb at 9.4 and 21.1T has been applied to study the environment of metal cations and structural transformations in a series of lead-sensitized layered perovskite solid solutions RbCa$_{2-x}$Pb$_x$Nb$_3$O$_{10}$, 0 $\leq$ x $\leq$ 2. $^{93}$Nb NMR results for the end members of the series are in good general agreement with an accepted $P4/mmm$ space group, where both Nb atoms in the structure are located on the 4-fold axis. Based on $^{87}$Rb NMR, similar conclusion was also made for Rb site in RbPb$_2$Nb$_3$O$_{10}$. In RbCa$_2$Nb$_3$O$_{10}$, however, $^{87}$Rb NMR points into a lower symmetry of the Rb site, revealing a disagreement with the proposed space group. Perhaps, the explanation of the observed discrepancy can be found in the displacement of the perovskite slabs against the separating layers of alkali metal cations. Solid state NMR spectra of $^{87}$Rb, $^{93}$Nb, and $^{207}$Pb of the solid solutions all indicate a rather homogeneous distribution of Pb and Ca throughout the lattice, with no separate phases been detected throughout the full composition of Ca and Pb.

The spectral assignments of the spectra were assisted by the first principles calculations of the chemical shift and quadrupolar tensors of studied nuclei (CASTEP, DFT/GIPAW). Good qualitative agreement was observed between the experimental and calculated parameters, and the calculations were of major assistance in interpreting the experimental data. While the quadrupolar interactions dominated both $^{87}$Rb and $^{93}$Nb, it was also possible to detect the contribution from the chemical shift anisotropy.
Encapsulation of guest molecules by a self-assembled Fe₄L₆ metallosupramolecular cage

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Water-soluble tetrahedral Fe₄L₆ cage with disulphonic acid substituents¹ (Fig. 1A) has many promising applications. For example, it can be used as a container molecule for air-sensitive molecules, like white phosphorus,² or as a trap for a greenhouse gas SF₆.³

Our ¹²⁹Xe NMR experiments show that the Fe₄L₆ cage can encapsulate xenon.⁴ The observation pave the way for exploiting metallosupramolecular cages as economical means to extract rare gases as well as ¹²⁹Xe NMR based bio-, pH and temperature sensors. The exchange rate between the encapsulated and free Xe was determined to be about 10 Hz, potentially allowing signal amplification via hyperpolarized xenon chemical exchange saturation transfer (Hyper-CEST).⁵

NMR analysis revealed also T, C₃ and S₄ diastereomers of the cage (Fig. 1B), which are playing a major role in the encapsulation of guest molecules.⁶ The encapsulation of cyclohexane changed the conformation from T to C₃, having a twisted C₃' intermediate conformation during the entering. While C₃ conformation was favored with cyclohexane (85 %), it totally disappeared in the case of Xe guest and only T (75 %) and S₄ (25 %) were present.

The stability of the cage was studied by Hyper-CEST method.⁷ The cage structure turned out to be stable at variable conditions and only strong iron chelator or high Fe(II/III) overdose could broke it down. This is an important observation for bioimaging applications.


Figure 1. (A) Synthesis of water-soluble tetrahedral Fe₄L₆ cage. (B) Three diastereomers of the cage.
Localization of aluminium sites in highly silicious ZSM-5 zeolites by solid-state NMR techniques

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The aluminium sites of calcined and steam-treated ZSM-5 zeolites were investigated by solid-state 27Al and 29Si MAS MNR methods at different magnetic fields (7.1T – 22.3T). A highly resolved picture of the distribution and the evolution of the aluminium sites after the steam-treatment can be obtained from these experiments.

Liquid hydrocarbon fuels are still one of the most important energy resources in our society, primarily due to their high energy density and quite easy transportability. For the production of consumer goods, olefins play a similar role. As an alternative to the conventional oil-based production of fuel and olefins, the methanol-to-hydrocarbons (MTH) process is one possibility. Here, peculiar aluminosilicates called zeolites act as heterogeneous catalysts for the reaction. The zeolite ZSM-5 is the archetype MTH catalyst [1]. It exhibits the MFI structure which consists of intersecting straight and sinusoidal ten-membered ring channels with a diameter of ca. 5.5 Å building up a 3D network, thereby creating negative charges which can be balanced by acidic protons. ZSM-5 has 24 crystallographically distinct tetrahedral sites which provide many different possible ways of Al substitution into the silica framework.

During the industrial operation of a ZSM-5 catalyst the material degrades mainly due to two different reasons, (1) non-selective formation of hydrocarbons and coke inside the porous structure and (2) loss of aluminum from the framework (dealumination) due to the presence of water steam at high temperatures and pressures. While coke can be burned-off effectively by a regeneration of the catalyst in oxygen containing atmosphere at elevated temperatures, dealumination is a non-reversible process, requiring finally a catalyst replacement.

The use of solid-state NMR methods, for example MAS, CP/MAS and MQMAS NMR, allows to obtain deeper insight into the species, location and distribution of Al atoms in the zeolite framework. The location and distribution of Al atoms have been recognized as important factors for the activity, selectivity and lifetime. Thus, the following questions are of particular interest: (i) In which way are the Al atoms distributed on the distinct T-sites, (ii) which effect has the distribution of Al on the catalytic behavior, (iii) can the distribution be affected by different synthesis methods, and (iv) how do the Al sites alter during a reactivation treatment like steaming. The poster will give a description of the solid-state 27Al and 29Si MAS NMR approaches that we currently work on to solve some of these important questions for the highly silicious ZSM-5 catalysts and it will show highly resolved spectra of framework and extra-framework aluminium sites.

References

INFLUENCE OF CATION & HALOGEN TYPES ON PHOTOREACTION OF CINNAMATE SALTS STUDIED BY SOLID-STATE NMR

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Solid-state reactions involve a minimum amount of molecular displacement and therefore stereo selective photoproducts are obtained according to the orientation of the reacting molecules in the crystal. Cinnamic acid a small and relatively simple molecule is therefore a good model system to improve the understanding of solid-state reactions in general. Upon exposure to UV light of \( \lambda > 260 \) nm in the solid state, the double bonds of two neighboring cinnamic acid molecules dimerize to form a cyclobutane ring; reversibly, the cyclobutane ring can be photocleaved upon exposure to UV light of \( \lambda < 260 \) nm [1, 2]. Therefore, this group of molecules has potential to be used as switching segments for instance in shape-memory polymers or embedded in porous materials. But the challenge in the exploitation of solid-state reactions is to get the desired crystal packing in a predictable and controllable manner. For this reason, an objective of supramolecular chemistry is to address this problem by controlling molecular packing through noncovalent interactions. Different cations as counterions and halogen substitution in the aromatic ring of cinnamate salts have influence on the arrangement of the anions and the reacting double bonds (e. g., distance and parallelism). Thus, the aim of this work is to investigate the influence of different alkali cations and different types of halogens on the arrangement of the anions with the goal of achieving favorable geometry for photochemical reaction, also to characterize reactant and product forms and to identify spectroscopic changes by solid-state NMR. We report on the influence of potassium and rubidium as counterion as well as the role of chlorine and bromine substitution in the aromatic ring of cinnamate salts on the photoreaction. Distinction between the reactants and their photoproducts, notably identification of phase changes and the presence of crystallographic inequivalences and disorder as well as the kinetics of photoreaction are studied. The results highlight that chlorine as aromatic ring substituent showed better crystal packing and photoreaction than bromine.

Identification and Characterization of Side Products in the Gilch Synthesis of Poly(ortho-Phenylene Vinylene)

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The class of poly(phenylene vinylene) based polymers (short: PPVs) are of particular interest as materials for organic light-emitting diodes (OLEDs). The Gilch synthesis of para-substituted PPVs (see Figure 1, left) has been studied extensively over the last decade.[1,2] A combination of experimental studies with in depth modelling studies of the reaction mechanism(s), lead to significant improvements of the yield and control of the resulting polymer properties like the molecular-weight-distribution as well as minimizing unwanted constitutional defects.[3]

Recently a new class of PPVs based on poly(ortho-phenylene vinylene)s (ortho-PPVs) were introduced.[4] Under optimized reactions conditions and by tailoring of the substitution pattern (see Figure 1, right), a new polymer material was produced, which showed longest-wavelength absorption maximum at $\lambda = 394$ nm. The maximum fluorescence intensity was found at $\lambda = 498$ nm. These properties hint at a possible application as blue-emitting polymeric materials – an area, where PPVs are currently not used, as para-PPVs usually emit from deep red to yellowish-green.[5]

After the first successful synthesis and characterization of the new ortho-PPVs,[4] we herein present a deeper look into the polymerization mechanism and explore possible reaction pathways leading to unwanted side products. By cooling the NMR tube to $-80^\circ$C and slowly heating up, we were able to follow the Gilch synthesis as the reaction progressed. We were able to first identify and subsequently characterize intermediate species, which indicate reaction pathways different from those observed for the Gilch synthesis of para-PPVs. The observations are in line with competing Diels-Alder-type reactions of ortho-quinodimethane species as proposed by theoretical modelling.[6]

Ultra-fast and quantitative $^{29}$Si NMR of zeolites and PSS materials:  
The perks of CPMG and MC-CP MAS  

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Thorough NMR characterization of Si-containing materials is hampered by the low abundance of the NMR active $^{29}$Si nuclei and their long spin-lattice relaxation times. This causes $^{29}$Si NMR measurements to be challenging and time-consuming. These $^{29}$Si sensitivity issues are commonly dealt with by cross-polarization experiments, which remain mere qualitative. In order to gain insight into the level of connectivity and micro-structure of PSS materials, a new interesting type of spherolite-based polymers, quantitative NMR experiments are however essential.

Multiple contact cross-polarization under MAS (MC-CP MAS) provides such sensitivity enhancement with full quantitation. Even in the presence of a variety of distinct spin baths, which is the case for PSS materials, MC-CP MAS still provides quantitative data (Figure 1).

Zeolites, a specialized class of microporous aluminosilicates, pose an additional problem. $^1$H densities in zeolite frameworks are usually very low and preclude an essential spin bath. Efficient magnetization transfer in CP MAS is thus rendered absent for zeolites.

The Carr-Purcell-Meiboom-Gill pulse sequence offers the opportunity to obtain reliable and quantitative $^{29}$Si NMR data in a matter of minutes. With CPMG MAS NMR, a train of many Hahn echoes is acquired in just one scan with a constant delay between each echo recording. Summation of the individual echoes considerably increases the signal intensity per scan, allowing for much higher signal-to-noise ratios in less time (Figure 2). Separate analysis of each echo, taking into account $T_2$ relaxation times and finite number of echoes effects, allows to retrieve full quantitative information.
Abstracts for posters in session "New approaches to MR measurements"
Optimal control to design pulses providing high SNR and sharp resolution in geophysical surface NMR

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Surface nuclear magnetic resonance is a geophysical technique providing non-invasive characterization of groundwater systems (Legchenko and Valla, 2002). This promising method is the only geophysical technique providing direct sensitivity to water content. However, the technique commonly suffers from low signal to noise ratios. We aim to design pulses specific for surface NMR conditions capable of improving SNR and ensuring high-quality images using optimal control theory. The surface NMR method involves laying a large coil of wire at the ground surface (~50–100 m in diameter) and using this coil to perturb and subsequently measure the return to equilibrium of a magnetization present at depth that originates from the immersion of water molecules in the Earth’s magnetic field. The technique is most commonly used to produce depth profiles of the water content and relaxation times as functions of depth (to depths of ~50–75 m). The low signal to noise ratio in surface NMR is a consequence of the measurement being conducted at Earth’s field (without the use of prepolarization) and that the signal exists in a frequency band (~2 kHz) where the noise level can be over an order of magnitude larger than the NMR signal. One recent approach to improve signal to noise in surface NMR is the use of an adiabatic excitation pulse in place of the traditional on-resonance pulse (Grunewald et al., 2015). The advantage of using an adiabatic pulse is that it offers a means to maximize the signal amplitude given the transmit conditions in surface NMR, where the use of a surface coil leads to an extremely heterogeneous applied magnetic field (B1). However, one concern with such an approach is whether the signal enhancement comes at the expense of spatial resolution in the resulting depth profiles.

To design pulses capable of both improving SNR and ensuring the production of high resolution images we employ an optimal control approach (Vinding et al., 2015) to generate a pulse waveform capable of reproducing a desired B1 sensitivity (i.e. a desired transverse magnetization as a function of B1 magnitude). We aim to produce a B1 sensitivity profile resembling a step function, where a fully transverse magnetization is produced for B1 larger than some threshold while no transverse magnetization is produced less than this threshold. This particular sensitivity profile was selected based on surface NMR forward modeling where it was observed to provide enhanced SNR and improved spatial resolution with respect to the standard on-resonance approach. The optimal control approach is given the freedom to sweep the transmit frequency and modulate the current amplitude during the pulse in order to produce the desired perturbation. We present a numerical analysis contrasting the performance of the optimal control pulse against the standard on-resonance approach; signal amplitudes, spatial resolution, and depth penetration of each method is contrasted.

Use of $^{13}$C-NMR diffusion experiments to characterize complex polymeric formulated system

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Fast and effective structural/compositional analysis on formulated systems represents one of the most challenging and complicated tasks performed by the analytical groups within the chemical industry. The deformatulation analysis includes identification and quantification of several chemical species present in the final sample, such as flame retardants, initiators, catalysts and surfactants to name a few. Given the complexity of these materials it is of key importance to obtain a detailed insight in the final composition.

$^1$H-DOSY experiments are regularly used to simplify the analysis of the formulated system by filtering out the fast diffusing species (additives, catalyst) from the main component (such as a polymer) from the NMR spectrum. However, the limited spectral resolution of the $^1$H experiment does not allow a complete characterization of the samples. To overcome this limitation, the use of $^{13}$C-diffusion experiments was explored.

In this work we show that $^{13}$C NMR diffusion is a practical and useful tool for separating mixtures and hence accelerating structure determination. In addition, we have proposed a novel convection compensated pulse sequence to effectively suppress the thermal convection caused by the $^1$H decoupling. The implementation of this pulse sequence increased the robustness of the $^{13}$C-DOSY approach and allowed the generic applications of $^{13}$C-detected diffusion to systems from mixtures of small molecules, polymer blends, and copolymers to actual complex formulated systems.

**Figure 1:** Double PGSTE-refocused INEPT sequence for $^{13}$C-detected diffusion. The narrow and wide rectangular boxes represent 90° and 180° RF pulses, respectively. The line-filled box is the gradient pulse, and the sinuosoidal box is the spoil gradient pulse. The frames in dotted lines define the function of each segment in the pulse sequence. $\tau_1$ and $\tau_2$ are set to 1/(4J) and 1/(6J), respectively, to generate the refocused and positive $^{13}$C peaks.

Hou et al., Magn. Reson. Chem. (2016), “Generic applications of $^{13}$C-detected NMR diffusion to formulated systems with suppression of thermal convection induced by proton decoupling.”
Encoding of chemical shifts using multiple indirect evolution in Homo- and Heteronuclear experiments

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We propose a new general approach for chemical shift encoding based on the introduction of an additional evolution time period in homonuclear (DQF-COSY) [1] and heteronuclear (HSQC) pulse sequences. The additional evolution time period (t1’) results in a signal splitting along F1 as a function of the difference between the corresponding signal’s resonance and the carrier’s resonance during that period. The “low-precision” chemical shift measured in the signal split combined with the ambiguous but “high-precision” chemical shift of aliased signals, [2,3] makes it possible to reconstruct full 2D spectra with high resolution in F1. [4]

Figure 1. DQF-COSY (A) and HSQC (B) pulse sequence including the additional t1’ evolution time to produce chemical shift dependent splitting of signals. C) DQF-COSY spectrum obtained using a 3-fold aliasing in F1 and a splitting of signals relative to a carrier set to 10 ppm during t1’:

Spatially resolved multidimensional NMR relaxometry and diffusometry for rock core analysis at high temperatures and pressures

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Fluids confined in reservoir rocks may differ significantly in their properties depending on temperature or pressure [1,2]. Here we report on a setup allowing to saturate and flood rock cores under reservoir conditions in the laboratory. To this end a commercial pressure cell (Daedalus Innovations LLC), compatible for the use with NMR, was assembled within a 2 MHz NMR Rock Core Analyzer (Magritek Ltd.). This setup allows for temperatures of up to 150°C and a pressure of up to 6000 psi.

We implemented relaxation measurements to obtain spatially resolved 1D $T_1$ and $T_2$ distributions [3,4]. Both methods enable one to obtain resolved pore scale information along the rock core axis. Moreover, when applying the two methods subsequently it allows one to investigate the heterogeneity of rock samples based on the differences of $T_1$ and $T_2$ distributions in each slice.

Different fluids saturating and flooding rock cores at the same time may be discriminated by their molecular mobility. Therefore, the diffusion coefficient ($D$) was added as an additional dimension for the case of the aforementioned $T_2$ imaging method as shown in Fig. 1. This allows to distinguish different fluids (flowing or resting) while changing experimental parameters such as temperature or pressure, ultimately leading to a better understanding of the flooding process.

Figure 1: Spatially resolved $D$-$T_2$ profile for a sample initially only saturated with hydrocarbons (left). Flooding progress is demonstrated for partial (middle) and full (right) flooding with water at 25°C. Two reference planes are indicating hydrocarbons (left grey plane) and water (right grey plane) in each figure.

We recently introduced tailored mesoporous solids functionalised with homogeneously distributed radicals as polarizing agents (PAs) for DNP.[1, 2] These materials, dubbed HYPSO, have several potential advantages over previous PAs which require the use of additional agents (glassing agents and free radicals) that need to be removed before the hyperpolarized liquid can be injected into cell culture or into patients. The PAs can sometimes be eliminated by precipitation/filtration, or by solvent extraction, but the methods are substrate specific, and the contact PAs/polarized sample prior to separation results in signal loss.

With HYPSO materials, production of pure hyperpolarized liquids free of additional agents was shown to be possible by a simple wetting-polarization-filtration sequence. In addition, any persistent radical (trityl, TEMPO…) can be incorporated into a HYPSO, allowing both direct $^{13}$C polarization or $^{1}$H-$^{13}$C Cross Polarization (CP)[3] approaches to generate $^{13}$C hyperpolarization. The first generation of HYPSO materials allowed production of $[1^{13}$C]-pyruvate having a $^{13}$C polarization of ca. 25% after dissolution using TEMPO radicals and cross-polarization.

Here we describe several new variants of HYPSO in which we tune the anchoring group used to incorporate the radicals onto the solid surface, or we tune the overall porous architecture of the material. By changing the manner to link the radicals to the solid (i.e. Azide-Alkyne Huisgen Cycloaddition instead of peptide coupling), we managed to improve the overall polarization of an impregnated solution. The use of a cubic structuration (SBA-16) having 3D interconnected pore network further improved the polarization, with a potential of $^{13}$C polarization after dissolution of ca. 55%.

Observations of the influence of Taylor-Couette geometry on the onset of shear-banding in surfactant wormlike micelles

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For over 30 years, nuclear magnetic resonance (NMR) techniques have been used to study materials under mechanical deformation [1]. Collectively these methods are referred to as Rheo-NMR [2].

A recently developed Rheo-NMR drive-shaft [3] has been used together with a series of Taylor-Couette (TC) geometries to study the influence of the inherent strain rate variation between the curved surfaces of the geometry on the onset of shear-banding in cetylpyridinium chloride/sodium salicylate (CPCl/NaSal) wormlike micelles (WLM) during steady strain rate controlled experiments.

Velocity profiles acquired with NMR were used to calculate local strain rates and to infer when the fluid began to shear-band. This revealed that the onset of shear-banding in the CPCl/NaSal/brine system was dependent on the TC geometry dimensions where greater differences in curvature between the two shear surfaces caused shear-banding to begin at a lower applied strain rate.

These findings were confirmed by a second Rheo-NMR [4] apparatus which allows for the acquisition of shear stress in the NMR magnet while recording local velocities simultaneously. Results from these measurements showed that the observed applied strain rate at which shear-banding began was the same for both the bulk rheometry (i.e. flow curves) and NMR velocimetry data [5].

Figure 1: (a) Local strain rates next to the stationary wall obtained from 1D NMR velocimetry in two TC geometries; $r_i/r_o = 6.00/9.00$ (△) and 8.00/9.00 (●). The shear-banding transition can be estimated by the onset of the plateau for the local strain rates. (b) Bulk flow curves measured simultaneously including a reference, collected with a cone and plate geometry on a commercial rheometer (△).

Simplification of NMR spectra by broadband homonuclear decoupling applied to diverse homonuclear 2D experiments

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Broadband homonuclear decoupling [1] in 1D and 2D $^1$H NMR experiments eliminates the effect of $J_{^1H^1H}$ scalar couplings, leading to a reduction of multiplets into singlets and thus greatly improving spectral resolution. A variety of methods had been proposed to achieve this goal.

We applied some of them (nemoZS [2], PSYCHE [3], BIRD [4]) to decouple the indirect F1 dimension of the recently introduced CLIP-COSY [5] and 2D DIAG [1] experiments. The former, based on in-phase magnetization transfer in the mixing period is an alternative to the standard DQF-COSY experiment, with the difference of generating only positive signals and thus overcoming the problem of signal cancellation. Further spectral simplification can be achieved with the 2D DIAG experiment where $^1$H-$^1$H correlations are eliminated, resulting in only F1-decoupled diagonal signals well separated along the first dimension.

The combination of the homonuclear decoupling and spectral aliasing leads to high-resolution spectra that can be acquired over a short experimental time (order of a few minutes).

The advantages, limitations and complementarity of these 2D homonuclear experiments using nemoZS, PSYCHE and BIRD decoupling blocs will be discussed and compared.

Figure 1. Simplification of the 2D spectra (left to right: DQF-COSY, CLIP-COSY, DIAG) of a sample of raffinose.

Double Resonance On A Chip

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The integration of high-resolution NMR spectroscopy with microfluidic devices offers unique possibilities for the characterisation of biological systems. We have recently demonstrated a highly optimised planar transmission line probe, which accommodates generic microfluidic devices, to obtain high-resolution proton NMR spectra from 2 µl samples at 1 mM concentrations on the chip.¹

In the present contribution, we show that this detector can also be used for ¹H–¹³C double resonance spectroscopy. This opens new possibilities for the characterisation of metabolic mixtures, but we also demonstrate that protein spectroscopy on a chip is made possible with this system. Indeed, we have shown that the HSQC spectrum from 50 µg of β-2-microglobulin obtained with this detector is of comparable quality to that from a state-of-the art cryoprobe system using 300× more sample.

The detector uses two parallel conductors with a constriction focusing sensitivity on the sample. The detector is attached vertically to the probe, parallel with the B₀ magnetic field, minimising susceptibility broadening effects. The detector was fabricated from copper-laminated PTFE; microfluidic chips are manufactured from PMMA by laser cutting and scoring. PMMA layers are thermally bonded with the help of a plasticiser. Tuning is achieved using a circuit design adapted from Mispelter et al.² with a high frequency band stop and a lower frequency band pass. This design is optimised to be more efficient at the higher frequency, made use of by detecting on the proton channel.

Heteronuclear Single Quantum Coherence (HSQC) experiments are useful for mixture analysis and structure determination. Fig. 1 shows the HSQC spectrum of a mixture of amino acids in solution at 1 mM concentration. More complex variants of the HSQC experiment are also possible, such as Total Correlation Spectroscopy (HSQC-TOCSY), which provides additional connectivity information.³ Using covariance processing a ¹³C-¹³C TOCSY spectrum can be taken showing connectivity of ¹³C atoms within a molecule such as the α and β form of glucose in solution (Fig. 2).

References:
New metal-air battery cell design for in-operando NMR measurements

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To develop and improve metal-air battery systems, nuclear magnetic resonance (NMR) with experimental setups for in-operando investigations presents a viable option to reveal transport properties and transformation reactions on microscopic as well as macroscopic length scales. This contribution reports on our development and evaluation of an in-operando NMR battery cell housing. The entire in-operando NMR setup was systematically optimized for the operation in strong and homogeneous magnetic fields (patent application submitted).

The in-operando NMR setup includes a customized high-power, broad-band, high-temperature probe. It can be used in a 400 MHz and a 600 MHz wide-bore magnet. The electrochemical cell is supported by a 3D printed mount with an integrated 15 mm NMR coil. Since conductive parts, such as wires, current collectors and electrodes lead to disturbances of the magnetic field and screening of the NMR pulses, metallic parts in the cells were minimized.

To test this setup, we performed in-operando measurements on lithium-ion batteries (LMO/Li, LMNO/Li and Li/graphite). The gas-tight Li-ion in-operando cell (iOC) shows a very good cycling behavior and can be charged and discharged inside the NMR spectrometer numerous times, hence it was ideally suited for a systematic improvement of the cell housing. No failure in operation due to leakage or contamination occurred thus far, and tests with over one hundred charge/discharge cycles were successful and highly reproducible.

For in-operando measurements of silicon-air batteries, the greatest challenge was to distinguish between the corrosion products (95% at.) and the products from electrochemical discharge reactions (5% at.). To solve this problem, silicon corrosion in different electrolytes was measured time-resolved, such that reaction constants and reaction pathways could be determined by solving kinetic equations. For the discharge operation, the iOC metal-air NMR setup enabled almost the complete consumption of the metal anode before the battery current flow ended. Corresponding experiments will be presented and possible mitigations to reduce corrosion reactions are discussed.

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Figure 1. In-operando NMR optimization measurement of a Li/graphite battery cell.
Kinetics of rapid exchange in the cucurbituril-xenon host-guest system

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Synopsis: The reversible binding of xenon to host structures is fundamental to the development of biosensing employing hyperpolarized xenon (hpXe) and chemical exchange saturation transfer. The rates of xenon entering and leaving the host directly affect the sensitivity or contrast of the approach. While the exchange mechanisms and respective rate coefficients for the widely used host cryptophane-A are known, such data are not available for the attractive cucurbituril molecules, and may be difficult to obtain because of high exchange rates. Here, exchange spectroscopy is modified for analysis of exchange kinetics at rates in the range of kilohertz. The underlying exchange mechanisms and rate coefficients are determined for the cucurbit[6]uril-xenon host-guest system.

Method: Consider a solution where the host molecule and hpXe combine and dissociate (Fig. 1a). A non-selective rf pulse (blue) inverting free xenon magnetization resonantly but rotating the magnetization of host-bound hpXe in a complete cycle around the respective effective field puts the magnetization of both sites in anti-phase along the static magnetic field. For a resonance offset Δ of the host-bound spins, the rf amplitude Δ/√3 suffices for a rectangular rf pulse shape. At high field or large Δ, the pulse may be of short length to enable clean spin preparation despite rapid exchange. In the subsequent delay τex, the free and host-bound spins freely exchange such that the oppositely oriented magnetization in both pools compensates each other. The extent of magnetization depletion in dependence of τex is monitored by a non-selective read pulse (red) and subsequent detection. The depletion curves (Fig. 1b) for both sites are mono-exponential with identical rate koff = (c + k[Xe]) / (1 + M_CXe / M_Xe) as shown recently; c and k are the rate coefficients for dissociative or degenerate exchange mechanisms, respectively. For the ratio M_CXe/M_Xe of the signal intensities of host-bound and free hpXe in dynamic equilibrium given, a linear dependence in the free hpXe concentration [Xe] results.

Results: For 4.5 mM CB[6] in PBS at 298 K and 7 T (Δ=6 kHz), depletion rates were determined at various [Xe] (partial pressure) with the sequence in Fig. 1a and mono-exponential fitting. Using weights (1 + M_CXe/M_Xe)1 from independently taken spectra, almost identical linear dependencies were obtained for free and CB[6]-bound hpXe, clearly indicating the presence of dissociative and degenerate exchange processes in the CB[6]-hpXe system (Fig. 2). The mean rate coefficients are c=1140 s⁻¹ and k=129300 M⁻¹s⁻¹.

Fig. 1. (a) Pulse sequence and (b) depletion for free (red) and CB[6]-bound xenon (black).

Fig. 2. Depletion rate for free (red) and CB[6]-bound hpXe (black) with weight (1 + M_CXe/M_Xe)1 in dependence of xenon partial pressure (tor Xe).

18 Abstracts for posters in session "NMR physics"
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The density matrix theory of triple-quantum filtered COSY NMR experiments applied to an AX system of spins S=1: an example of passive coupling in two-dimensional NMR spectroscopy.

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The 2D NMR Triple-Quantum Filtered (TQF) COSY spectrum of an AX spin system of spin-1 has been calculated by numerical density matrix calculations. The basic phase cycle to achieve triple quantum filtration and phase-modulation of the signal during \( t_1 \) consists of 12 steps. If nuclei A and X are not coupled, the calculations predict no peaks at all. If nuclei A and X are coupled and the multiplets are not resolved in the conventional 1D spectrum, the calculations again predict no peaks at all. It is therefore not possible to distinguish between the uncoupled and the coupled cases unless the multiplets are resolved in the conventional 1D spectrum. If nuclei A and X are coupled and the multiplets are resolved in the conventional 1D spectrum, only diagonal peaks are detected. This is due to the fact that the coupling is passive. It is believed that the calculations presented here will be needed to calculate the Exclusive COrelation SpectroscopY (E.COSY) of an AX system of spins S=1.
NMR is a powerful tool for obtaining detailed molecular structure, dynamics and interactions information of heterogeneous system when in solution, solid and gas phase. In the last decades Magic Angle Spinning (MAS) NMR has become an extremely valuable tool not just for solids but for semi-solid samples also. Despite its widespread applications MAS NMR instrumentation presents sealing limitations for the case of liquids or semi-solid samples. When at medium – to – high spinning speed the ceramic cell – rotor cap(s) sealing fail. The centrifugal force induced pressure.

Over the last two decades have been several attempts to successfully hold the fluids inside the active volume of the MAS rotors. From O-ring based to insert used design configurations various attempts were employed. Each of them presents its own limitations and drawbacks.

Here we propose a new sealing method and tools for both, single or double open-end design MAS rotors, which successfully seal the sample active volume [1]. The new rotor cap(s) are designed to work with commercial available ceramic sleeves, are reusable and don’t require special tools to work with (Figure 1). The cap(s) design’s efficiency was tested for various liquids (density, viscosity temperature, etc) and the results at 35 kHz speed were presented.

References:

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A faster, simper 2D correlation spectroscopy experiment based on a pulse-imposed Fourier encoding

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In a typical 2D correlation spectroscopy (COSY) spectrum the diagonal contains the most intense peaks, but the symmetrically placed cross peaks contain most of the relevant information. This paper proposes a method to suppress diagonal peaks and remove redundant information, by delivering only one of the two symmetrical cross peaks to be observed. The pulse sequence is shown in Figure 1. Polychromatic selective pulses PC1 excite the desired peaks [1], and the diagonal peaks are suppressed by two consecutive excitation sculpting blocks [2]. To exemplify the proposed scheme, Figure 2 illustrates the sequence’s operation on a butyl methacrylate (C8H14O2) sample. PC1 excites the resonances marked in I, II and III, and the blue traces in Figure 2c are decoded from only three 1D spectra acquired with the pulse sequence shown in Figure 1a. Shown as red traces are the cross sections from the conventional COSY shown in Figure 2a, with the peaks marked I, II and III corresponding to diagonal resonances. Notice that these are suppressed in the blue traces, which will show only off-diagonal resonances. Notice as well that these spectra, an their pseudo-2D composite in Figure 2b, are simpler than their conventional counterpart in Figure 2a, and are devoid from the strong, uninformative diagonal peaks.

Figure 1. Pulse sequence for a faster and simper COSY experiment is illustrated. The shaped pulse PC1 is a polychromatic (PC) selective 90° pulse imposing a phase-modulation on the peaks to be decoded by a discrete Fourier-matrix approach.[1] The shaped pulses PC2 are PC selective 180° pulses that address the same resonances as PC1, but without involving a phase-modulation.

Figure 2. COSY experiments on a butyl methacrylate(C8H14O2) sample, recorded on a 500 MHz Varian spectrometer and probe. (a) Conventional COSY spectrum acquired in 10 min using 256 t1. (b) Pseudo-2D diagonal-suppressed COSY spectrum reconstructed from 1D traces (blue) in Figure 2(c). (c) Traces (red) taken along the signals at I, II and III in the indirect dimension in Figure 2(a) and a diagonal suppressed version (blue) based on 1D acquisitions shown in Figure 1a. Peaks I, II and III are selected by PC1, and are flipped by PC2. The experimental time for (b) was 6s.

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Spin dynamics in Wigner space

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A general method to visualize operators of coupled spin systems in a Wigner representation was introduced in [1]. This technique provides a general approach to systematically analyze coupled spin systems and their time evolution by representing operators as linear combinations of spherical harmonics and depicting them as three-dimensional shapes. Examples are applicable to research and education and include density operators that describe the state of a quantum-mechanical system (e.g., spin systems or quantum bits from quantum information processing), Hamilton operators that specify energy terms, and unitary transformations that model the time evolution.

Here, we focus on determining how to directly compute the time evolution of an operator in its Wigner representation. Building on earlier results of [2], the Wigner transformation of multispin product operators is described by mapping single-spin operators onto separate spheres. This gives rise to a natural basis for the Wigner formalism of coupled spins as direct products of spherical functions. By obtaining Wigner representations of both the Hamiltonian \( H \) and the density operator \( \rho \), the time evolution is computed based on the spatial form of the corresponding shapes. An analogue of the Liouville-von-Neumann equation \( i\partial\rho/\partial t = [H, \rho] \) for the Wigner space is developed for systems consisting of an arbitrary number of spin \( 1/2 \). Denoting the Wigner transform of \( H \) and \( \rho \) as \( W_H \) and \( W_\rho \), the time evolution of the Wigner function of a single spin \( \partial W_\rho/\partial t \) is exactly given by the so-called Poisson bracket on the sphere \( \partial W_\rho/\partial t = \{W_\rho, W_H\} \), which consists of combinations of spatial derivatives along the curved coordinates \( \theta \) and \( \phi \) of \( W_\rho = W_\rho(\theta, \phi) \) and \( W_H = W_H(\theta, \phi) \). For an arbitrary number of spins, a generalization of the Poisson bracket for multiple spheres is used, and pictorial representations convey how our approach works in practical examples: Figure 1 discusses the case of two coupled spins.

Figure 1: Time evolution of the initial density operator \( I_{1z} \) under the bilinear coupling Hamiltonian \( H = \pi J I_{1z} I_{2z} \). Wigner representations are plotted at three different times \( 0, 1/(4J), \) and \( 1/(2J) \) for the Hamiltonian (upper row), the density operator (middle row) i.e. \( \rho(0) = I_{1z}, \rho(1/(4J)) = (I_{1x} + 2I_{1y} I_{2z})/\sqrt{2}, \) and \( \rho(1/(2J)) = 2I_{1y} I_{2z} \), as well as the time derivative of the density operator (lower row).

A fast approach to 3D HSQC-based spectroscopy based on Fourier encoding of pre-targeted resonances

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Traditionally, the duration of an NMR experiment increases with spectral dimensionality and with demands for indirect-domain resolution. This work presents a novel approach to acquire 3D spectra deriving from 2D HSQC experiments (e.g., HSQC-TOCSY, HSQC-NOESY), whose experimental time does not increase with these parameters by virtue of relying on a Fourier-based phase-encoding of pre-targeted peaks. A schematic diagram of a 3D HSQC-TOCSY NMR experiment based on these principles is illustrated in Fig. 1a. It relies on modulating the phases of two polychromatic pulses (PC5, blue and PC3, red) applied during the HSQC portion of the experiment, whereby peaks become Fourier encoded.[1] This involves imparting artificial phase factors $e^{i2\pi(m-1)n_1/N}$ and $e^{i2\pi(m-1)n_1/N}$ onto the known F1- and F2-dimensional resonances $S_n$ and $I_n$ of the $n^\text{th}$ cross peaks in the 2D HSQC spectrum (Fig. 1a), during the $m^\text{th}$ scan of the 3D acquisition. Here $N$ is the number of resonances, $n_5+n_1 = n - 1$, and $n = 1, 2, \ldots, N$. Assuming $S_{mn}$ is the signal from an $I_n$ spin which is bound to $S_m$ spin, $S_{mn'}$ is the signal from $I_n$ spin which is bound to $S_{m'}$ spin and is coupled with $I_n$ spin (where $n' = 1, 2, \ldots, N$), then the total signal $S$ acquired in the $m^\text{th}$ scan will be

$$S = \sum_{n=1}^{N} (S_{mn} + \sum_{n'\neq n} S_{mn'}) e^{i2\pi(m-1)(n-n')/N}. \quad (1)$$

As each resonance is phase encoded by the PC pulses, the resulting spectra will show phase-twists, of which shown on the left of Fig. 1b. Upon performing a Fourier transform of the resulting spectra along the $m$ index, the intensities of the resulting traces (right-hand panel in Fig. 1b) will be given by

$$S_{\text{decod}}(k) = N(S_{m\alpha} + \sum_{n'=1}^{N} S_{m'n'}) \delta(n - k). \quad (2)$$

Equation (2) indicates that every decoded spectrum $S_{\text{decod}}(k = n)$ includes the signals $S_{mn}$ and $S_{mn'}$. Accordingly, the positions of the various cross peaks $(I_n, S_m, I_n)$ and $(I_n, S_m, I_{n'})$ in the final 3D correlation spectrum, can be found by performing a number of scans given solely by the number of peaks in the original, previously known 2D HSQC spectrum.

Quantum Detection and Control of Individual Nuclear Spins

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The detection of individual nuclear spins is crucial issue towards single molecule NMR which can be applied in biology, material science, medicine, geology and physics. The sensitivity, spatial resolution and stability of magnetometry can be enhanced by special tragedy with NV center in diamond. A shallowed implanted NV center allows strong coupling between an atomic sensor and sample nuclei. Here we demonstrate the realization of nano-NMR with single nuclear spin sensitivity [1], and then we try to study molecule diffusion properties by correlation spectroscopy [2] which can enhance the linewidth resolution of nano-NMR. In last we show the demo to resolve interaction between nuclear spins [3].

Magnetic field-induced effects on the NMR properties of benzene

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An apparent magnetic field dependence of the one-bond carbon-proton and carbon-deuteron spin-spin couplings, $J_{\text{CH}}$ and $J_{\text{CD}}$, as well as the deuteron quadrupolar splitting, $\Delta \nu_D$, are revealed by the NMR spectra of 1,3,5-$d_3$-benzene measured at various magnetic fields. These effects arise from the partial orientation of the molecule in a strong magnetic field leading to incomplete averaging of the anisotropic (dipolar and quadrupolar) interactions. Current experiments make feasible the determination of the anisotropy of the diamagnetic susceptibility, the orientational order parameter, and the sign of the $^2\text{H}$ quadrupolar splitting. The field dependence of the NMR parameters is also studied with quantum chemical calculations. The orientational order parameter of the symmetry axis of 1,3,5-$d_3$-benzene appears to be negative leading to positive dipolar couplings and negative $^2\text{H}$ quadrupolar splittings. The diamagnetic anisotropy, determined from three independent NMR properties, is on average $-878 \times 10^{-30} \text{JT}^2$.

The study shows that when moving to higher magnetic fields in NMR spectrometers, the dipole-dipole couplings become significant in cases where the molecular diamagnetic anisotropy is large. Hence, spin-spin couplings, commonly used for molecular structure elucidation, cannot be determined with high accuracy on molecules with large diamagnetic anisotropy without taking into account the field dependence.
19 Abstracts for posters in session "Protein relaxation and dynamics"
The Role of Dimer Asymmetry and Subunit Dynamics in Enzyme Catalysis

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Enzymes greatly accelerate biochemical reactions by providing a scaffold to bind and recognize substrate, position catalytic units, and facilitate formation of stabilized transition states. Despite the long history of enzymology, the role of protein dynamics in catalysis is still a highly debated topic. Here, we use freeze-trapped X-ray crystallography (FTX), NMR and molecular dynamics (MD) simulations to examine functional states, dynamics, and quaternary structure using fluoroacetate dehalogenase (FAdC), a homodimeric enzyme from Rhodopseudomonas palustris as a model system. X-ray crystallography provides high-resolution structures of key states (apo, Michaelis complex, covalent intermediate, and product-bound states) associated with the reaction and showed binding and catalysis of substrate in only one of subunit in a dimer. 39 crystal structures solved by FTX and 15N,1H NMR establish a clear allosteric pathway between protomers in the heterogeneous substrate-bound dimer in the conformational ensemble as the reaction ensues. MD simulations and computational rigidity analyses further corroborate the allosteric pathway and correlated motions within the dimer. 19F NMR highlights functional dynamic processes and sampling of these states, in a manner consistent with conformational selection. Crystal structures at key catalytic steps show pronounced asymmetry in local dynamics, where the empty protomers exhibit pronounced disorder. These increases in configurational entropy associated with the empty protomers, corresponding increased protomer exchange and domain specific dynamics likely lower the activation free energy barrier and drive sampling of the transition state. This detailed mechanism provides insights into how substrate-coupled allosteric modulation of structure and dynamics facilitate catalysis by lowering the activation free energy barrier of catalysis in a homodimeric enzyme through half-sites reactivity.
Insight into the dynamic coupling networks of a PDZ domain using exact NOEs

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The 96 residue long second PDZ domain (PDZ2) of the human tyrosine phosphatase 1E (hPTP1E) is involved in the regulation of apoptosis [1, 2]. As there is no significant structural change between the free and the ligand bound form [2, 3] it is most likely that in PDZ2 allosteric regulation is based on dynamical changes, i.e. “stiffening” of side-chain motion, as suggested by Cooper and Dryden [4]. Several networks have been previously suggested, an evolutionary [5], a structural [6] and a dynamical network based on the side-chain order parameters [3], those networks are not identical but show an overlap. The employed methods only provide information as to whether two residues are coupled or not and do not allow insights into the interaction mechanism.

The aim of this work is to detect coupling networks that link distal molecular docking sites based on the use of eNOEs and compare them to previously published networks [3, 5, 6] to detect similarities and differences.

Ensemble–structures of the free and the ligand–bound form of PDZ2 domain are determined based on the use of eNOE data [7, 8, 9], Hα–Hα, Hα–Hβ and aromatic N–Cα and Cα–Cα scalar couplings, allowing for a detailed description and understanding of protein function from the 3D atomic-resolution structure and an accurate description of the dynamics and the conformational space of the protein[8]. Calculated ensemble–structures will also allow the identification of possible small conformational changes of the PDZ2 domain upon ligand binding and concerted domain motions, which have so far escaped detection by the classical structure determination procedure. This data will advance the understanding of allostericity on an atomic level and the understanding of the relationship between dynamics and function of a protein.

Characterization of Fibril Dynamics on Three Timescales by Solid-State NMR

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Amyloid fibrils have been of great interest in recent years due to their role in many diseases-in particular neurodegenerative diseases, and also because of their biological role as functional amyloids. Despite their importance, however, dynamical information on formed fibrils has been limited. In order to understand the complex dynamic behavior of fibrils, we have performed experiments to elucidate both H–N motion and Hα–Cα motion, and improved experimental and processing methods to allow deconvolution of multiple motions.

We have measured $R_1$, $R_1\rho$, and REDOR on the HET-s (218-289) prion protein for both $^{15}$N and $^{13}$Cα nuclei, in order to obtain a full dynamical characterization of the HET-s backbone motion. Data was fitted to motional models using a complement of Redfield theory for calculation of relaxation for fast motions and explicit simulations of relaxation for slow motion.

Our experiments and analysis allowed us to characterize site-specific motion, using a model with three timescales, where each motion was characterized by a correlation time and amplitude. $^{15}$N and $^{13}$Cα analysis gave complementary information, providing information predominantly on backbone rigidity and side-chain motion. Additionally, both $^{15}$N and $^{13}$Cα analysis revealed global motion of the fibrils.

We also performed molecular dynamics simulations, where we found good agreement between experiment and simulation. We used MD simulation to aid in interpretation of the solid-state NMR data, by determining timescale-specific correlation of the H–N or Hα–Cα bond motion with motion of surrounding bonds.

Figure 1. Site-specific H–N nanosecond motion from solid-state NMR and molecule dynamics simulation. A and B show amplitude ($\theta_{\text{ns}}$) and correlation time ($\tau_{\text{ns}}$) of nanosecond motion determined by experiment and simulation. C maps the experimental amplitude of motion onto the HET-s (218-289) structure. D shows correlation coefficients of the H–N nanosecond motion with neighboring bonds, as determined by simulation.
Proteins are inherently flexible, and their dynamics often play a central role in biological functions, influencing diverse processes, such as conformational selection in molecular recognition, catalysis, and allosteric regulation. However, over the biologically important \( 10^{-8}-10^{-4} \) s range, NMR has a “blind spot”, indicating that it is difficult to evaluate the nanosecond timescale range using NMR methods. In this study, we exploit paramagnetic relaxation enhancements to gain site-specific information about protein mobility on the nanosecond timescale. However, the present novel approach is relatively unconventional. Specifically, we propose the transient generation of radicals to address the problems originating from the presence of radicals in the sample.\(^1\) To obtain nuclear relaxation rates in radicals with lifetimes of only several microseconds, we harness a spin hyperpolarization technique termed Chemically Induced Dynamic Nuclear Polarization (CIDNP), which detects indirectly via spin polarization of their recombination products. The time-resolved version of CIDNP enables a highly precise characterization and evaluation of radicals, as well as their reactivity and spin relaxation properties. In addition, CIDNP increases the inherently low sensitivity of NMR by several orders of magnitude. The developed technique enables the determination of both motional parameters, the correlation time \( \tau_c \) and the order parameter \( S^2 \); furthermore, differences in these parameters for different protons of the same amino acid residue can be reliably determined.

The paramagnetic relaxation times of the aromatic and \( \beta \) protons of the native ubiquitin Tyr59 and His68 residues and of the non-native ubiquitin Tyr59 residue were determined from an analysis of CIDNP kinetics obtained during the photoreaction of the protein and 2,2'-dipyridyl excited in the triplet state. Using the paramagnetic relaxation times determined earlier for the radicals of free amino acids as an internal standard and assuming that the hyperfine interaction (HFI) anisotropy is very similar for the radicals of free amino acids and corresponding radicals of amino acid residues in the proteins, we determined parameters that characterize the intramolecular mobility of different protons in native and two non-native states of ubiquitin. The latter are denatured at pH 2 and 57 \(^\circ\)C, and the A-state at pH 2 in a 60%/40% methanol/water mixture. The determination of both parameters of intramolecular mobility \( \tau_c \), and \( S^2 \) was only possible by analyzing paramagnetic relaxation data obtained at two magnetic fields (4.7 T and 9.4 T) using nuclear magnetic resonance spectrometry. Intramolecular correlation times fall into the submicrosecond-microsecond timescale. Longer correlation times and higher order parameters were defined for the less accessible Tyr59 residue than for the His68 residue, as well as for the more buried \( \beta \) protons than for the aromatic protons for both of the protein residues in the native state. For Tyr59, intramolecular mobility increases following the loss of the tertiary structure of ubiquitin. These findings strongly support the reliability of the obtained data.

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Internal motions of autoregulating domain of transcription factor Sigma A

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Transcription is a key biomolecular process in the genetic information transfer. One RNA polymerase transcribes all genes in bacteria. However, numerous transcription factors diversify molecular machinery responsible for transcription and enable bacteria to adapt to different needs. The number of DNA recognition subunits, sigma factors, vary in different bacteria from just one sigma factor to several dozens. We focused on the sigma A factor (SigA) from \textit{Bacillus subtilis}. SigA is composed of four domains, \(\sigma_1, \sigma_2, \sigma_3,\) and \(\sigma_4\) that are connected by flexible linkers. \(\sigma_{1.1}\) binding to \(\sigma_2\) and \(\sigma_4\) prevents DNA binding prior to formation of RNA polymerase holoenzyme.

Molecular motions have a significant impact on the function of proteins. In order to describe internal motions of \(\sigma_{1.1}\), we analyzed relaxation of the amide \(^{15}\text{N}-^1\text{H}\) spin system. Auto-relaxation rates \(R_1\) and \(R_2\), longitudinal and transverse cross-correlated relaxation rates, and steady-state Nuclear Overhauser enhancement (Fig.1) were evaluated. Data were obtained on 600 MHz, 850 MHz, and 950 MHz spectrometers. In addition to molecular motions on the ns-ps timescale, the measured relaxation rates indicated the presence of a slow conformation exchange on the \(\mu\text{s}-\text{ms}\) timescale. The CPMG relaxation dispersion experiment was performed to further investigate the slow motions. The results confirmed the presence of a slow exchange for several non-glycine residues.

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Unambiguous determination of the ionization state of arginine side chains

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Arginine in proteins exists in its protonated, guanidinium form almost without exception, due to its strongly basic character. Because of this strong basicity, reports that demonstrate that a protein arginine residue is charge-neutral are rare and often controversial. Although the ionization state of arginine residues could preferably be determined by directly counting the number of protons that are bound to the head group, NMR experiments relying on $^1$H detection are compromised due to chemical and conformational exchange effects, leading to strong exchange broadening. Here, we present a novel two-dimensional NMR experiment for the detection of arginine charge states in proteins that are insensitive to the chemical and conformational exchange effects. We make use of $^{13}$C detection, and thus correlate the arginine head group nitrogen $^{15}$Nε and $^{15}$Nη chemical shifts with that of the directly attached carbon $^{13}$Cζ. The number of protons can be counted by encoding the $^{15}$N chemical shifts without $^1$H decoupling if exchange with the solvent is slow enough to observe $^{15}$N–$^1$H scalar couplings. In the presentation, we demonstrate how the experiment can be implemented and adapted for unambiguous determination of the charge state of arginine residues.
Study of intrinsically disordered proteins from Ebola virus

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Ebola virus causes severe hemorrhagic fever with high mortality rates. Its negative single strand RNA encodes at least for seven proteins, four of them are involved in the replication-transcription complex: the nucleoprotein (NP), VP35, VP30 and the viral polymerase (L). NP forms filamentous structures that encapsidate the viral genome and protects it from the degradation. The NP N-terminal part is required for NP-NP self-association and RNA interaction. The C-terminal part is essential for the binding with matrix proteins in order to form virus-like particles. The NP comprises an intrinsically disordered region (NP412-640) the molecular resolution and biological function of which remain unknown. The viral polymerase cofactor VP35 is responsible of the immune signaling inhibition and the RNA synthesis. Recently, its N-terminus was found to be disordered and to chaperone the N-terminal lobe of NP in the monomeric form prior to encapsidation of the genome. In this project, we used nuclear magnetic resonance, spin relaxation and other biophysical methods to identify the molecular dynamic properties and the role of NP and VP35 in the RNA replication process.
Gaining insight into collagen structure from $^{15}$N-labelled synthetic model peptides and mouse bone.

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Collagen is the most prevalent component of the extracellular matrix. Due to its diverse structure and composition it serves many functions, such as providing structural and mechanical support for the surrounding cells, and importantly, playing roles in cell-to-cell communication.1 Nonetheless, despite collagen being at first glance a simple protein formed by three similar polypeptide chains each consisting of repetitive three-amino-acid triads which trimerize into a triple helix, it is a highly versatile and complex system. There are over 3000 amino acids in each triple helix, and 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 of proteins such as collagen.

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 the sequence dependence and neighbor effects of $^{15}$N relaxation to be characterized. A first is assignment of the $^{15}$N spectrum using techniques such as 2D $^{13}$C-$^{15}$N DCP correlation spectroscopy (Fig. 1. (a)).

While $^{13}$C NMR of mouse bone shows only the predicted signals for collagen (and its glycosylations), in $^{15}$N spectra additional, unexpected resonances around 40 ppm are observed. We hypothesize these correspond to immature collagen cross-links that are developed during maturation of collagenous tissues. These are primarily derived from collagen telopeptide hydroxyl-aldehydes, the major cross-link being the hydroxylysino-5-ketonorleucine (HLKNL). In the case of lower levels of hydroxylation of the helical residues in bone collagen the cross link, hydroxylysino-norleucine (LKNL), is formed between an additional keto-imine following reaction with a helical lysine.3 To the best of our knowledge, this is the first observation of collagen cross links by NMR and is a potential route for further study of these components which are crucial to collagen ordering and mechanical properties.

References:
Fast Field Cycling NMR as a method to study protein dynamics and aggregation of therapeutic proteins

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Fast Field Cycling NMR relaxometry (FFCR) is a low-field technique which measures the longitudinal (spin-lattice) relaxation rate dependence of the magnetic field strength (NMRD) over a wide range of magnetic fields (from a few kHz upwards), giving important information on the range of molecular motions, such as rotation and diffusion, including slow motions, present in a substance or complex mixture. Herein we will show how FFCR can be used to obtain important information on proteins.

¹H NMRD profiles can detect motions occurring on time scales from $10^{-6}$ to $10^{-9}$ s, thus allowing the detection of reorientation times of proteins from a few kDa up to MDa. The accessibility of these correlation times can be very useful for monitoring protein aggregation, protein folding, and optimizing protein solution conditions affecting protein tumbling [1,2]. The ¹H NMRD profiles show stretched dispersions with respect to the Lorentzian function, possibly due to the combination of fast internal mobility, proton lifetimes shorter than the reorientation time, and aggregation effects. The profiles can be reproduced by a sum of a finite number of Lorentzians, as customarily done in model-free approaches [3]. The largest correlation time obtained in the analysis can provide information of the overall reorientation time of the system, and thus on the presence of aggregation.

The aggregation of therapeutic proteins is an important problem in the bio-pharmaceutical industry. Protein product aggregates are potent inducers of immune responses to therapeutic protein products, thus manufacturers of therapeutic protein products should ensure that their products contain minimal product aggregates. There is a real need for new and improved analytical methods for defining protein aggregates [4,5]. FFCR shows considerable promise for routine assessments of therapeutic protein aggregation. NMRD can be used to characterize very large aggregates because of the very low frequencies achieved and does not suffer from aggregate fractionation or separation as the system is measured [6].

Figure 1. ¹H NMRD profiles of a therapeutic protein in non-aggregated and artificially aggregated states

High-power $^1$H CPD provides artifact free Exchange-mediated Saturation Transfer (EST) experiments

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Abstract

Exchange-mediated saturation transfer (EST) provides critical information regarding dynamics of molecules. In typical applications EST is studied by either scanning a wide range of $^{15}$N chemical shift offsets where the applied $^{15}$N irradiation field strength is on the order of hundreds of Hertz or, scanning a narrow range of $^{15}$N chemical shift offsets where the applied $^{15}$N irradiation field-strength is on the order of tens of Hertz during the EST period. The $^1$H decoupling during the EST delay is critical as incomplete decoupling causes broadening of the EST profile, which can result in inaccuracies of the extracted kinetic parameters and transverse relaxation rates. Currently two different $^1$H decoupling schemes have been employed, intermittently applied 180° pulses and composite-pulse-decoupling (CPD), for situations where a wide range or narrow range of $^{15}$N chemical shift offsets are scanned, respectively. We show that high-power CPD provides artifact free EST experiments, which can be universally implemented regardless of the offset range and irradiation field-strengths.
Multi-scale dynamics of an intrinsically disordered protein from NMR relaxation and MD simulations

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Intrinsically Disordered Proteins (IDPs) lack a stable three-dimensional structure and their function is encoded both in their conformational heterogeneity and dynamics. Nuclear Magnetic Resonance (NMR) spectroscopy provides many probes to derive ensemble descriptions of IDPs. In particular, NMR relaxation rates report on the timescales and amplitudes of ps-ns motions. However, because NMR relaxation rates are ensemble-averaged values, traditional analytical tools (e.g. the conventional model-free analysis) are not sufficiently insightful.

We measure an extensive range of both auto- and cross-correlated $^{15}$N relaxation rates at different magnetic fields over a large range of temperatures (268-298K) on three constructs of different length of the Ntail domain of the nucleoprotein of Sendai virus. By introducing a robust procedure based on an Arrhenius-type relationship to analyse up to 61 relaxation rates per residue, we are able to assign the physical origin of different dynamic modes in this archetypal IDP.

Molecular Dynamics (MD) simulations have the potential to complement NMR relaxation data. However, in order to sample the vast conformational space of IDPs, either extremely long (up to hundreds of microseconds) trajectories have to be calculated, requiring dedicated hardware that is not generally available; or enhanced sampling techniques have to be used, modifying the features of the conformational landscape and therefore hampering a meaningful comparison of the calculated motional time scales with the experimental ones.

We propose a solution based on the calculation of several short (~150 ns) MD trajectories initiated from conformers that are distant in the free-energy landscape. We show that a combination of these trajectories provides an adequate sampling of the conformational space, as measured by comparison with an experiment-derived ensemble. We introduce a framework termed Averaged Block Selection Using Relaxation Data (ABSURD) that filters out those segments of the trajectories that are not compatible with the experimental NMR relaxation data. ABSURD eliminates most of the limitations that available force fields suffer when reproducing the dynamics of IDPs without affecting significantly the conformational properties of the parent trajectories and allows us to simultaneously reproduce with good accuracy a set of sixteen NMR relaxation rates measured at four different magnetic fields, from 600 to 950 MHz.

Increased Protein Conformational Dynamics Rescues Catalysis in the Proline Isomerase Cyclophilin A

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To understand the function of a biological macromolecule, it is of critical importance to complement a high-resolution three-dimensional structure with detailed insight in its molecular dynamics. For a growing number of systems, it has been found that slow collective motions can be rate limiting for biological function - it is, however, challenging to relate specific conformational changes directly to protein function.

In the human proline isomerase cyclophilin A (CypA) there is a network of residues undergoing exchange between major and minor conformations that have previously been associated with the catalytic conversion of cis- to trans-peptide substrates. This dynamic network extends from the active site into the protein core and a previously designed second-shell mutation (S99T), not directly contacting the substrate, strongly stabilized the minor conformation and severely impaired catalysis.

Here, using directed evolution we identified two mutations that partially restore the enzyme’s catalytic activity. The analysis of their conformational exchange by NMR spectroscopy (using CPMG relaxation dispersion and CEST experiments) reveals a concomitant increase in protein dynamics for residues in the dynamic network that connect to the active site for these mutants. Residues in loops adjacent to the active site undergo exchange on a faster time scale ($k_{ex} > 2000 \text{ s}^{-1}$) in both the mutant and wild-type proteins, and their exchange characteristics remain unaltered.

Room-temperature X-ray crystallography data was collected on these mutants to understand the structural basis of the increased dynamics. We show that the “evolved” mutations reduce steric clashes that destabilize the transition to the major conformation of the surrounding residues in the allosteric network.

Our experiments provide further support for a direct rate-limiting role of the major-minor conformational exchange in the catalytic cycle of CypA. The presented data provide a first glimpse of the evolutionary trajectory of an enzyme’s energy landscape. The mutations arising from directed evolution have reshaped the dynamic framework surrounding the fully conserved active site residues.
Structural Plasticity of MHC I: Integrating Cellular Modeling and Protein Function

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The control of the immune system plays a key role in host defense. The presentation of peptides at the surface of most nucleated cells by major histocompatibility complex class I molecules (MHC I) is crucial for eliciting or evading an immune response. Since the pool of surface presented peptides only presents a small sample of all possible peptides understanding their selection process is crucial for the development of treatments such as vaccines, which rely on the selection of specific peptides. The peptide selection process is mediated primarily by a weakly interacting multi-protein peptide loading complex (PLC) at the center of which is the modulation of MHC class I conformation.

To gain a better understanding of the mechanisms of peptide selection by MHC I, we developed computational systems models encoding distinct mechanistic hypotheses of PLC function. Using \textit{in vivo} biochemical data we were able to infer that the system is under kinetic control and that a conformational intermediate of MHC I is significant for peptide selection.

We investigate the molecular determinants of peptide selection using a combination of X-ray, NMR, and biophysical techniques together with molecular dynamics simulations. Using this approach we show that peptide selector function correlates with protein plasticity rather than structure. This in turn was tested experimentally \textit{in vivo} and extended to the PLC resident chaperone tapasin.

By combining computational systems models with in-cell biochemical data and structural methods we identify a previously undetected correlation between protein plasticity and \textit{in vivo} peptide selector function of MHC I, with implications for host defense and immunotherapy.


Protein dynamics from NMR relaxation (parameter correlations, frequency limitations and potential contribution of relaxometry): results from Markov Chain Monte Carlo simulations and fractional Brownian dynamics

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NMR instrumentation and methodology have reached a mature state in the study of protein dynamics. It provides extremely efficient tools to selectively measure various spin relaxation processes, and therefore to probe molecular motions with an atomic site specificity. The connection between molecular motions and spin relaxation is given by relaxation theory, and is embodied in the spectral density function $J(\omega)$ of the relevant magnetic interactions, which must in turn be related to molecular motions through a dynamical model. The practical problem at hand amounts to fitting model parameters from measured data. But, due to the relative scarcity of independent relaxation measurements, the model complexity is strongly limited. At this point, a microscopic approach to dynamics, such as provided by MD simulations can be used to test physical models of internal dynamics, including various kinds of stochasticity. A question of important practical relevance is to determine what dynamical information can reasonably be expected from relaxation measurements. Indeed, in addition to the limited number of parameters, several issues arise, such as the correlation of their values during fitting, which is in particular the case for overall and internal dynamical parameters. Moreover, the spectral density function $J(\omega)$ is sampled in restricted regions of the frequency spectrum, and the determination of short time scales is limited by the magnetic field strengths used.

We used a Markov Chain Monte Carlo approach.[1] This provides marginal probability density functions for each model parameter or set of parameters, whilst avoiding model selection. We used this strategy to investigate the possible contribution of relaxometry to improve the determination of model parameters by NMR. We used a spectral density function based on fractional Brownian dynamics, introduced recently in order to account for distributions of time scales in protein dynamics, with a fixed number of parameters. [2] Simulations showed that the use of relaxation measurements (from 23.4 to 0.4 T) may improve the determination of the overall correlation time $\tau_0$, but mainly for large values of the latter. It was found that for small proteins ($\tau_0 \approx 4$ ns), no such improvement was obtained. Interpretation of these findings will be given.

References


Conformation dynamics comparison of receiver domains of histidine kinase receptors CKI1 and ETR1 from Arabidopsis thaliana

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Cytokinins are essential plant hormones that control cell division, shoot meristem, leaf and root differentiation, senescence and other growth and plant developmental processes. In Arabidopsis thaliana, cytokinin signal transduction is mediated via a protein phosphorylation cascade known as the multistep phosphorylay pathway (MSP). Key components of the phosphorelay consist of the histidine kinase (HK)-type receptors, histidine phosphotransfer proteins (HP), and nuclear response regulators (RRs) that serve as transcriptional regulators. Sensor histidine kinase CKI1 (Cytokinin independent 1) is incapable of binding to cytokinins but is has been shown that it can phosphorylate and dephosphorylate HPs, what plays an important role in the cytokinin signaling. Moreover, CKI1 can initiate the cytokinin signaling phosphorelay by inducing responses independently of cytokinins.

Besides to responses caused by cytokinins, plans sensitively respond to ethylene molecule, which acts as a gaseous hormone but its perception differs from the cytokinin one. Ethylene, which activates the ethylene pathway cascade, can be either synthesised by plant or perceived from the environment and is in control of different responses. Ethylene binds to the ethylene receptors ETR1, ERS1, ETR2, ERS2 and EIN4 localised at the endoplasmic reticulum. ETR1 (Ethylene response 1) receptor possesses all sequence motifs of canonical HK domains including the HK activity, necessary for the signal transduction via parallel MSP, causing a wide spectrum of responses. Accumulating evidence suggests a role of ETR1 in integrating ethylene recognition with the MSP mediated signaling.

One of the major objectives of our project is the structural and functional comparison of two homologues receiver domains of histidine kinases, CKI1 and ETR1. Although both domains have the same tertiary structure, they significantly differ in the orientation of a so-called gamma-loop, which connects β-strand 3 and α-helix 3 of the receiver domains. The 15N-CPMG relaxation dispersion experiment revealed a striking difference in the conformational exchange in this region, well correlated with the distinct functions of studied receiver domains in the signal transduction.

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Abstracts for posters in session "Paramagnetic systems"
NMR shifts in paramagnetic metalloproteins from first-principles calculations

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Paramagnetic NMR (pNMR) shifts provide very useful restraints in biomolecular structure determination. The reliability of the structural interpretation may be substantially enhanced by the recently introduced first-principles pNMR calculations.

We present an approximation that enables quantum chemical calculation of long-range pseudo-contact shifts (PCSs) in an entire protein. We relate the paramagnetic molecular susceptibility tensor to the electron paramagnetic resonance (EPR) tensors, hence providing the unifying link between the traditional susceptibility-based PCS interpretation and modern pNMR theory employing magnetic property tensors.

The formalism is applied to two classes of metalloproteins, the CoII-substituted human matrix metalloproteinase 12 and the copper–zinc superoxide dismutase derivatives. The calculations are able to reproduce the experimental solid-state PCS data, thus enhancing the available structural information on the paramagnetic center. For the first time, the point-dipole approximation underlying the susceptibility-tensor-based formalism is validated by comparison to full quantum chemical pNMR calculations including the effects of zero-field splitting. The full pNMR calculations in the short-range regime provide the so far unavailable assignment of the experimental pNMR shifts.

References:
Solid State NMR investigations of Na-based cathode materials.

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Sodium ion batteries have regained the interest of the scientific community in the recent years due to the uniformly distributed and inexpensive ores of the sodium in the world (1). The search for commercially viable batteries based in sodium demands that new electrode materials and electrolytes are found and optimized, in order to obtain batteries that are more economic, safer, and with a longer life. One of the most promising family of cathode materials for Na-ion batteries are the NaMO2 layered oxides (M = Cr, Mn, Fe, Co, Ni, etc. and mixtures of 2-3 transition metals) because of their high capacity and structural simplicity (2,3). Solid-state NMR spectra of such materials are challenging due to the large paramagnetic interactions induced by the transition metal ions in the nuclei under observation (23Na in the present work). These compounds can induce huge shifts of the NMR resonances of up to several thousands of ppm, especially when strong paramagnetic ions are involved like Fe3+ or Mn3+ (4).

We will show in this presentation, some recent work carried out in our laboratory where fast MAS NMR (>50 kHz) in combination with low fields where successfully applied, in order to obtain very valuable information about the structure and the ionic mobility in this family of materials. Furthermore, we will show how, ex situ solid-state NMR has been used as a tool to understand the structural transitions that undergo during the sodiation/desodiation processes present during electrochemical cycling.

In particular, the impact of Fe2+, Mg2+, Zn2+, Ti4+, H+ and other dopants on the local structure and dynamics of Na+ in Na-based layered oxides was followed by 23Na solid state NMR. The information obtained is related to the performance of the materials providing a useful tool to rationalize the effects of different dopants on the electrochemistry.

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Figure 1. 23Na ex-situ solid state NMR spectra of Na3Ni2SbO6
New reduction stable lanthanide chelating tags for PCS NMR Spectroscopy

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Pseudocontact shift (PCS) NMR spectroscopy is a powerful tool to study the structures and interactions of proteins and other biomacromolecules in solution. High affinity chelating tags displaying a rigid conformation around the lanthanide metal centre are particularly well suited for creating large PCSs. We have used a seven-fold methylated DOTA scaffold and a pyridine-sulfone linker to create a lanthanide chelating tag (LCT) that fulfills the above criteria and that is, in addition, stable towards strongly reducing buffer conditions as they are found e.g. in living cells. Figure 1 depicts the reaction scheme for the loading of the tag with lanthanide cations and the conjugation to a surface exposed cysteine of a protein to form a thioether bond. Thioether bonds are very inert not only to strongly reducing potentials, but also towards nucleophilic substitution in aqueous solution as well as extreme pH conditions.

![Figure 1. Reaction scheme for the metallation and conjugation of the new tags.](image)

The new tags deliver large PCS > 6 ppm and cause strong alignment of the tagged protein with the magnetic field and give thus rise to ample residual dipolar couplings > 30 Hz. The new family of LCTs allows therefore to monitor long-range interactions > 60Å under conditions that were not accessible previously and thus bear a great potential to study proteins and their interactions in virtually any buffer composition.
Natural monoisotopic $^{19}$F has an NMR resonance frequency close to that of $^1$H and exhibits sensitivity comparable to $^1$H (83%). Fluorine concentration in organisms is virtually zero and, therefore, the lack of background in fluorine-based images enables "hot-spot" imaging. Moreover, only small hardware and software adjustments of standard $^1$H scanners are needed for $^{19}$F detection.

However, the $^{19}$F nucleus present in organic molecules has usually a very long $T_1$ relaxation time requiring a long delay between excitation pulses.\cite{1}

It has been shown that the introduction of highly paramagnetic lanthanide(III) ions to the close vicinity of the fluorine atom(s) leads to significant shortening of the relaxation times.

It is known that, despite its low overall electronic spin ($S = 1$) and magnetic momentum ($\mu_{\text{eff}} \sim 3$ B.M.), Ni(II) ion can induce a large paramagnetic chemical shift and relaxation enhancements comparable to that of lanthanide(III) ions with higher $S$ and $\mu_{\text{eff}}$.

Thus, we decided to study the $^{19}$F NMR relaxation properties of Ni(II)$^{[2]}$ and Co(II/III) complexes. These ions fit perfectly into the cavity of 1,4,8,11-tetraazacyclotetradecane (cyclam) and its derivatives are well-known to form complexes with high thermodynamic stability. The 2,2,2-trifluoroethyl side arm was chosen as a group containing a high number of equivalent fluorine atoms (Figure 1).

Effects of ligand structure, central metal ion electron configuration, external field $B_0$ and temperature were studied. The most promising systems were tested by MRI imaging of phantom samples and in a mouse (Figure 2). Significant advantage of these complexes is their presentation in $^1$H $T_2$-weighted images which can be utilized for localization of the $^{19}$F-image in the tissue.

References:


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$^{19}$F-MRI using fast relaxing d-element based complexes
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Figure 1: Ligand structures in the Ni(II) or Co(II/III) complexes

Figure 2: In vivo image of a mouse injected with Ni-based contrast agent; $^{19}$F MRI image – red; $T_2$-weighted $^1$H MRI image – gray scale; pure contrast agent as a reference is shown in the left corner.
A High Performance Control and Read-out Device for the Optically Detected Magnetic Resonance

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This paper presents a fully-fledged, high performance, compact and easy re-configurable device, which is designed for the control and the measurement for the nitrogen-vacancy center based optically detected magnetic resonance. The device includes fast arbitrary waveform generator (AWG), high resolution pulse generator, and high precision read-out circuit including time-to-digital convertors and fluorescence photon counters. All these units are FPGA-based. The AWG has 2 channels with a maximum sampling rate up-to 1.2 Gsps and a DC-400MHz bandwidth. The pulse generator includes 18 channels, 10 of them are designed using the 3.3V Transistor-Transistor logic (TTL), and the other 8 using the 1.8V Low-Voltage-Complementary-Metal-Oxide-Semiconductor (LVCMOS). All of the pulse channels can work under a maximum resolution of 50 ps in both duration and delay. As an option, the pulse resolution is configurable and an additional 625 ps resolution is available. The read-out circuit provides a 30 ps time measurement resolution and a maximum counting rate of 100 Mcps, and a configurable accumulation function is available. The device is suitable to be applied in the magnetic resonance based solid-state quantum physical systems, such as the quantum precision measurement and the quantum information processing.

Figure 1. Block diagram of the fully fledged control and read-out device for the N-V center based ODMR.
High Resolution solid-state NMR of paramagnetic systems using 111 kHz magic angle spinning

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We investigate here the advantages for the study of paramagnetic materials offered by the recently developed 0.7 mm probe with MAS rates higher than 100 kHz. Solid-state NMR studies of paramagnetic materials using moderate MAS rates have often suffered from poor resolution due primarily to the large anisotropic interactions between the nuclei and the unpaired electrons, resulting in large shifts and shift anisotropies, and short relaxation times. When spinning at 111 kHz MAS, the improved averaging of dipolar couplings, the efficient separation of broad spinning sideband manifolds, and the shorter rotor periods produce a spectacular increase of resolution, sensitivity and coherence lifetimes compared to previously available MAS rates.

For example, a virtually sideband-free $^{31}\text{P}$ MAS spectrum can be achieved in 1D on the paramagnetic cathode material LiFe$_{0.25}$Mn$_{0.75}$PO$_4$, where large paramagnetic shifts and anisotropies span more than 4000 ppm (Figure 1).1

We also perform a complete examination of broadband RF irradiation schemes,2 by using experimental and numerical simulation results in this spinning regime, and we notably demonstrate the high efficiency of low-power broadband RF elements for >100 kHz MAS rates.

Finally, we expand the repertoire accessible to the NMR spectroscopist by developing multiple-band frequency-swept pulses, which are conceptually similar to single-sideband-selective adiabatic pulses (S$^3$APs),3 but multiple sidebands are irradiated simultaneously. These pulses allow complete population inversion and coherence refocusing with only moderate power requirements, and simultaneously compensate for biasing introduced by schemes sweeping over only one sideband.

\textbf{1H and 13C Chemical Shifts in Paramagnetic Rare Earth Metal Complexes: A First-Principles Study}

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The interest in calculations of NMR chemical shifts for paramagnetic open-shell species (pNMR) has been growing fast in recent years. \(^1\)-\(^4\) Although NMR techniques are extensively used to characterize paramagnetic metal complexes in great detail, it can often be very difficult to interpret the NMR spectra without theoretical support. It is known that density functional theory (DFT) has problems in accurate description of zero-field splitting (ZFS), EPR \(g\)-tensors, especially for systems with orbital degeneracies, low-energy excited states, or strong spin-orbit (SO) couplings. In such difficult cases, accurate pNMR shifts can only be obtained by high-level calculations based on \textit{ab initio} wave function theory (WFT).

Herein, we present a theoretical \textit{ab initio} approach to investigate the paramagnetic effects on NMR chemical shifts for three paramagnetic rare earth metal (REM) complexes viz. Nd(III), Eu(III), and Yb(III). The computed values are compared with the experimental high-field NMR results. To calculate the hyperfine coupling tensor (HFC), \(A\), the four-component relativistic all-electron matrix Dirac-Kohn-Sham (mDKS) approach \(^5\) with a hybrid PBE0 functional was used. The \(g\) and ZFS-tensors were computed using \textit{ab initio} complete active space self-consistent field (CASSCF) methods. These results were combined to obtain the total paramagnetic chemical shifts. \(^6\) The experimental pNMR shifts as well as the sizable difference of the \(^{13}\)C NMR shift for these iso-electronic species are well reproduced by the calculations.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{a) Molecular structure of Europium Complex. Experimental and calculated \(^1\)H (b) and \(^{13}\)C (c) chemical shifts for the Europium Complex. \(H_{\text{opt}}\): DFT/BP86/Def2-TZVP, \(\text{Full}_{\text{opt}}\): DFT/BP86/Def2_TZVP/COSMO (Turbomole).}
\end{figure}

Abstracts for posters in session "Relaxation and transport phenomena"
The analysis of complex mixtures is a problem that chemists face almost daily. Unfortunately, the utility of NMR in this area is often hampered by an excess of information stemming from the combination of spectra that already bear an inherent complexity.

One frequently used NMR technique for the screening and characterization of a mixture of analytes is diffusion ordered spectroscopy (DOSY), which seeks to differentiate the NMR signals of different molecules based on their different translational diffusion rates. Although the setup of DOSY experiments is rather straightforward, several complications can severely limit the effectiveness of this technique. In particular, data processing can be difficult in the case of strongly overlapping signals, and even more so when the separation in the indirect dimension is based on small differences of molecular diffusion coefficients.

The use of stationary phases (e.g. chromatographic silica) has proven to be effective in perturbing the analytes diffusivities, thus potentially improving the quality of DOSY experiments. Unfortunately, this approach introduces a considerable signal broadening due to local-magnetic field inhomogeneities, which are generally tackled by spinning the sample at the magic angle or by matching the magnetic susceptibilities of the solid and the liquid phase [1]. In this context, we describe two different strategies to alleviate (or circumvent) the problem of local-field inhomogeneities [2]. The first one is the use of micrometric hollow silica spheres as a stationary phase, which allows the use of standard solution-state probes and opens the possibility of choosing among different (deuterated) solvents for an optimal “pseudo-chromatographic” separation. The second one is the use of quasi-stationary phases such as monolayer-protected nanoparticles, that can be easily tailored to target specific classes of molecules. The potentials of the two approaches in different situations will be discussed.

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Long-Lived Nuclear Spin States in Monodeuterated Methyl Groups


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Nuclear magnetic resonance (NMR) experiments are time-limited by relaxation dynamics. Observing non-equilibrium magnetization is restricted to timescales governed by the longitudinal relaxation time $T_1$. The use of long-lived states (LLS) offers a promising means to transcend this limitation [1,2]. LLS are configurations of nuclear spins that are protected against relaxation. In systems of spin-$1/2$ pairs, the LLS is called singlet order and the decay time constant is denoted $T_S$. LLS lifetimes exceeding $T_1$ by a factor of 50 have been observed, with a lifetime exceeding 1 hour in room-temperature solution in one case [3]. LLS have also been observed in the 3-spin-$1/2$ systems of rapidly rotating methyl groups in solution [4]. Unfortunately, the LLS only gives rise to observable NMR signals through an incoherent cross-relaxation mechanism [5].

Since methyl groups are ubiquitous in nature, the exploitation of methyl LLS is still potentially attractive. We show that in certain cases, it is possible to achieve coherent access to a methyl LLS with high conversion efficiency into observable NMR signals. The conditions are: (1) the methyl group is monodeuterated, leaving a proton pair; (2) the local environment is chiral, so that a small chemical shift difference is induced between the CH$_2$D protons [6]. We show that the small chemical shift difference allows coherent access to the long-lived singlet order of the proton spin pair, using known radiofrequency pulse techniques [7]. We report an observation of a proton LLS in the N-CH$_2$D group of (N-CH$_2$D)$_2$-methylpiperidine. We observe singlet lifetimes with values between 20 and 60 seconds (dependent on temperature), exceeding $T_1$ by a factor of ~3. This result shows that is feasible to exploit methyl LLS with high signal amplitude, in suitable cases.

References
The high-frequency asymptotes of the spin correlation function for a dilute Heisenberg paramagnet

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The line shape of correlation function can yield information about, for example, rate of equilibration in heterogeneous spin systems that is relevant to the problems of ergodicity, thermalization, spin transport, and many-body localization.\(^1\)\(^\) Its wings decrease exponentially with frequency when the spin autocorrelation function (ACF) has singular points on the imaginary time axis. The coordinates of the singular points that determine exponential decay were calculated for regular spin lattices.\(^2\) The dependence of coordinate on the magnetic concentration does not seem to have been considered. It should be noted that the disordered system can be replaced by the regular lattice of spins at average distance to obtain the coordinate of singular point.\(^3\) However, the legitimacy of such a substitution is questionable, since the calculations of the central part of the ACF line shape indicate the importance of taking into account a non-uniformity in spatial distribution of spins.\(^4\)

Our analysis leads to conclusion that the spins of magnetically dilute system that play an important role in establishing equilibrium between different parts of system and shape the center of the ACF spectrum are located at average distance. However, the wings are determined by clusters which have small probabilities and give large contribution to the modulation frequency.

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**Figure 1.** Coordinate of the nearest singular point as a function of the magnetic concentration for contributions from close (Z=3—solid; Z=5—dash) and residual spins \(\delta^2b^2\) (from top to bottom): 0; 0.03; 3; 30, where \(c\delta^2 = M_2 - cZb^2\), \(M_2\)=second moment, \(b\)=coupling constant between close spins.
Probing the translational diffusion of antioxidants in different membrane mimetic environments by NMR pulsed field gradient experiments and molecular dynamics simulations

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Antioxidants are molecules which absorb free radicals produced as by-products during normal metabolism and cause oxidative damage to human body cells. We study the translational diffusion [1] of two antioxidants: curcumin and rosmarinic acid in Dipalmitoylphosphatidylcholine(DPPC) liposome and Sodium dodecyl sulfate as membrane mimetic by using NMR Pulse Field Gradient (PFG) [2] and Molecular Dynamics Simulation [3] techniques. The diffusion time scale for both these techniques is in the range of milliseconds and nanoseconds respectively. From NMR PFG we find that antioxidant molecules follow anomalous diffusion equation

\[ I = I_0 \exp \left( -D \gamma^2 g^2 \delta^2 \left( \Delta - \frac{\delta}{2} \right)^n \right), \alpha \neq 1 \] [4], and diffusion coefficients [5] are extracted by fitting intensity with gradient. The above systems are simulated in GROMACS software using gromos based force field and mean square displacement is studied as a function of time. For anomalous diffusion \( \langle z^2 \rangle \propto t^n \), \( n \neq 1 \). From the simulation results it is found that the systems follow anomalous diffusion.

From our results we can conclude that antioxidants in membrane mimetic follow anomalous diffusion for both the time scales.

References


Permutation symmetry theory of LLS

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Long-lived nuclear spin states (LLS) have been first demonstrated for ¹H nuclei in 2,3-¹³C-dibromothiophene [Phys. Rev. Lett. 92, 153003 (2004)]. After that in the last decade about 150 publications have been presented on this topic. Many studies have shown several LLS applications: for exploring weak ligand-protein interactions, investigating glycine protons in Ala-Gly, in para-ethanol, and in the investigation of slow-diffusion processes. In addition they can be found as a "byproduct" in dissolution DNP experiments.

Recently a LLS of more than one hour in solution and at room temperature has been presented [Angew. Chemie Int. Ed. 54, 3740 (2015)].

Here we propose a theoretical approach that considers LLS as naturally arising from the nuclear exchange symmetry properties of the complete Hamiltonian operator describing the system under investigation. Figure 1 reports the characteristic block diagonal structure for the internal dipolar matrix representation of a CH₃ group, that is typically the fingerprint of a possible LLS.

The nuclear wavefunctions, as well as coherent and fluctuating terms of the complete Hamiltonian can be classified according to their permutation symmetry properties. The proposed toolbox is useful in the nuclear wavefunctions classification, in labelling the spectral transitions of the 1D NMR spectrum, and in the prediction and analytical expression of long-lived orders as population imbalances.

The simplicity and compactness of the formalism grounded on well-known group theory theorems makes permutation symmetry theory a handy and easy way to analyse and predict long-lived orders on different molecular environments. Its validity, under a set of ad hoc introduced approximation, is demonstrated in rigid and non-rigid molecular systems and may offer a way for the experimentalist to visualize the degree of magnetic asymmetry a spin system can display.

As opposite to the traditional quantum mechanical approach that employs the Liouvillian space for the quantum mechanical operator representation, permutation symmetry theory can be formalized in the much smaller Hilbert space thus reducing the computational effort and improving the time performance of the calculations.

Figure 1: Matrix representation of the internal dipolar Hamiltonian for a CH₃ group with \( \tau_R = 10^{-17}, 10^{-18}, 0 \) in a), b), c) respectively. The symmetry of the dipolar changes with \( \tau_R \), until a block diagonal structure is reached for \( \tau_R = 0 \).
Pulsed-field gradient diffusometry of binary water/ethylene glycol oligomer mixtures

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Pulsed field gradient NMR is an established method for measuring self diffusion coefficients in solutions. NMR spectrometers with high spectral resolution, where the Fourier transform of the detected echo signal yields spectra with resolved peaks from different components in the solution, are nowadays routinely used for diffusion measurements. Here we report on measurements on binary mixtures performed on a low-field (20 MHz) NMR instrument. For lack of spectral resolution time-domain signals were analyzed. Pulsed field gradient experiments with echo-train detection for improved signal intensity were carried out. The diffusion coefficients of the two components could be reliably distinguished if they differ by a factor of two and even less when the molar fractions of the two components are similar.

Binary mixtures with water were investigated for mono-, di-, tri-, and tetraethylene glycol over the full range of concentrations. Biexponential Stejskal-Tanner fits yielded two distinct diffusion coefficients, one for the CH\textsubscript{2} groups of the glycol and one for the OH groups of glycol and water, which undergo rapid exchange. Hence only the self-diffusion coefficient of the glycol component is obtained directly, while the one for water must be calculated from the measured weighted average value of water OH and glycol OH groups. Good agreement with previous work [1] and measurements on our samples with pulsed field gradient FT NMR was found. Using the Stokes-Einstein equation, \( D = kT/(6\pi\eta R_h) \), and viscosities \( \eta \) measured with a capillary viscometer, the self diffusion coefficients were converted to apparent hydrodynamic radii (\( R_h \)). The concentration dependence of \( R_h \) observed for the different ethylene glycol oligomers will be discussed in terms of the possible structure of the solution.

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**Sparse (or not) regularization of Inverse Laplace transform in protein studies and reaction monitoring**

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Inverse Laplace transform (ILT) is employed to process data from diffusion and relaxation studies by NMR. As the transform is mathematically unstable and extremely prone to noise many different methods of regularization of ILT were proposed. One of the recent regularization methods takes the form of:

$$\min_{A \geq 0} ||\Phi A - \Psi||_2^2 + \tau ||A||_{\ell_p}.$$  \hspace{1cm} (1)

Where $\Phi$ is ILT matrix, $\Psi$ processed signal, $A$ reconstructed diffusion/relaxation profile. In case of $\ell_p = \ell_1$ the regularization method is referred as ITAMeD[1] and is in fact the sparse regularization. For the samples with unknown polydispersity, and thus $A$ deviating from strict sparsity, the method called tailored norm regularization[2] was proposed. This method is based on automatic tuning of the $\ell_p$ parameter in the range from $\ell_1$ to $\ell_2$ to guarantee the most proper regularization for each signal.

Such methods can be also utilized for joint Fourier Inverse Laplace transform together with non-uniform sampling in Fourier and Laplace dimensions[3].

In this poster we present the implementation of aforementioned methods in:

- Insulin aggregation studies
- Protein relaxation measurements
- Reaction monitoring

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**References**


Weak anion binding to poly (N-isopropylacrylamide) studied by electrophoretic NMR

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The Hofmeister effect is ubiquitous and plays an important role in a wide range of fields. It was proposed that different ion binding ability to proteins is one of the reasons responsible for specific ion effects and numerous of research has been conducted to quantify specific ion binding. Yet the mechanism behind it is largely unknown. In order to elucidate the nature of ion binding, quantitative data on ion binding to non-ionic molecules at very low salt concentration is necessary to avoid interference of any strong electrostatic interactions. There have been such studies at the air/protein/water interface. However, there are only scarce observations in bulk solution. One significant problem might be the lack of technique which could detect such weak binding in bulk solution. As we are going to show, electrophoretic NMR (eNMR) is a suitable method for this purpose and provides insight on specific ion effects.

We investigated a carefully characterized polymer, poly (N-isopropylacrylamide). eNMR was used to measure the average effective charge of polymer molecule which is gained as a result of ion partition between the surface of polymer and the bulk. We judiciously extended the instrumental limits of eNMR in order to quantify the very weak binding. Hence, we are able to quantify ion binding of 1 elementary charge per 100 kDa. The results show that the binding can be described by simple Langmuir-type isotherms in the 10-400 mM salt concentration range with binding strength following the Hofmeister series SCN⁻>ClO₄⁻>I⁻>Cl⁻>F⁻≈SO₄²⁻≈0 where all the “chaotropes” having weak binding while “kosmotropes” showing no binding. The partition free energy is calculated and is found to correlate well with the partition free energy from water into organic solvents which indicates the solvation of ion as the main factor responsible for the observed behavior.

Abstracts for posters in session "Sensitivity enhancement"
Planar probe for 400 MHz DNP – NMR

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DNP requires microwaves of sufficiently high power to saturate electron spins. On the other hand, dielectric heating of samples due to microwaves causes a decrease in the enhancement of nuclear polarization. High-field (~ 9 T) DNP is generally performed without a microwave resonator and may require a high-power microwave irradiation. Here, we report 1H DNP experiment at 9.3 T using a novel DNP-NMR planar probe[1,2,3]. The top part of the planar coil (Figure 1) is a wire grid, which allows good microwave transmission (~ 260 GHz), and the bottom part is a copper block with a high thermal conductivity acting as a ground plane (low E field) and as a heat sink. We performed 1H DNP using the planar probe with increasing microwave powers far beyond the level needed to reach saturation. The DNP signal intensity obtained by the planar probe reaches a maximum at about 100 mW and remains constant up to 3 W at 20 K. Instead, by using a solenoid coil, the signal reaches the maximum at a similar power, but decreases rapidly with increasing a microwave power. This power dependence of the planar coil implies much lower dielectric heating generated by microwaves in comparison to a solenoid coil.

References


Figure 1: Planar probe (top view)
Towards solid-state photo-CIDNP MAS NMR $^{13}$C-$^1$H correlation experiments

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Since the discovery of solid-state photo-CIDNP (photochemically induced dynamic nuclear polarization) effect by Zysmilich and McDermott in 1994 [1], it has been developed towards an analytical tool for studying the electronic structures of small photosynthetic cofactors embedded in huge protein-membrane complexes, since it provides dramatic nuclear spin order that can be observed as strong signal enhancement (by the factor of 10000-80000 for $^{13}$C) by magic-angle spinning (MAS) NMR experiments [2, 3]. Successful combination of signal enhancement from selectively and strongly hyperpolarized $^{13}$C nuclei with $^{13}$C-$^1$C 2D homonuclear correlation experiments such as RFDR and DARR allowed for unambiguous signal assignment of electron donor and acceptor in bacterial reaction center of Rhodobacter (R.) sphaeroides [4, 5]. On that basis, the molecular electronic structures of the cofactors forming the primary radical pair in the electronic ground state [4], the charge separated state [6], as well as the molecular triplet state [3] have been obtained. It has been shown that the primary acceptor, a bacterioheme cofactor is electronically not affected by matrix interaction [7], while the donor dimer is tuned by side-chain effects [5]. In addition, kinetic studies provided direct access to the spin-dynamics [6].

Up to date, the solid-state photo-CIDNP effect is reported for $^{13}$C and $^{15}$N nuclei, and $^1$H MAS NMR experiments on photosynthetic RCs failed to show the effects. Therefore, the information on the role of protons in electron transfer process occurring during photosynthesis is limited. Preliminary studies [8] showed the possibility to transfer the photo-CIDNP enhanced polarization from $^{13}$C nuclei to nearby protons, providing the in principle the possibility to investigate them indirectly via heteronuclear correlation experiments. In the present study, we attempt to combine strong enhancement of nuclear polarization from labeled carbon atoms with dipolar- and scalar-based heteronuclear $^{13}$C-$^1$H correlation experiments. These types of experiments can open the access for studying the properties of protons of photosynthetic cofactors in their native protein environment and might allow for better understanding of the role of the proton pool around the electron donor in the electron transfer processes.

SABRE polarization of dipyridyl stabilized Ir-complex at high, low and ultralow magnetic fields

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The limitation of NMR is its inherent low sensitivity that can be overcome by using an appropriate hyperpolarization technique. Presently, dynamic nuclear polarization and spin-exchange optical pumping are the only hyperpolarization techniques that are used in applied medicine. However, they are relatively complex in use and expensive. Here we present a new modification of SABRE (Signal Amplification By Reversible Exchange) – SABRE in stabilized Ir-complex. Originally SABRE was found to be a promising method for hyperpolarizing different substrate molecules like pyridine [1]. However Mewis at al. have shown that dipyridyl and phenanthroline can be used for deactivation of SABRE to gain longer lifetime for hyperpolarized free substrate [2]. Here we present another approach. We found that the deactivated Ir-complex (here we term is “stabilized complex”) is still involved in hydrogen exchange, therefore it can be hyperpolarized by para-hydrogen. We have used different hyperpolarization transfer techniques and demonstrated that 1H and 15N nuclei of stabilized Ir-complex can be hyperpolarized over a wide range of magnetic fields from few µT up 10 T with 15N polarization ~3% (see Figure 1). Thereby our study opens a perspective to incorporate investigated complex or its modifications into biomolecules of choice; the para-hydrogen exchange will allow one to continuously generate hyperpolarization of an investigated compound using an appropriate polarization transfer scheme.


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Advanced instrumentation for DNP-enhanced MAS NMR for higher magnetic fields and lower temperatures

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Sensitivity enhancement of magic-angle spinning NMR (MAS NMR) using dynamic nuclear polarization (DNP) is becoming increasingly popular at moderate external field conditions \( B_0 < 9.4 \) T and temperatures \( T > 90 \) K, but less accepted at higher fields. High-field condition is crucial for gaining spectral resolution and basic NMR sensitivity, but decreases the efficiency of the cross effect-based DNP, progressively diminishing the merit of high-field DNP.

In coping with this issue, advanced hardware development should play a key role. Here, we describe two innovations we have recently made in instrumentation for DNP-MAS-NMR at \( B_0 = 16.4 \) T (700MHz for \(^1\)H, 460 GHz for electron):

1. **Closed-cycle helium-cooling MAS probe system**. The low sample temperature (~30K) improves both the DNP efficiency and the Boltzmann nuclear polarization, recovering the sensitivity gain at high fields. This system cools the compressed spinner gases with Gifford-McMahon cryo-coolers, spins the rotor while cooling, and re-compresses the return gas, for the first time, in a completely closed gas circulation path. Thus, the system does not consume any helium to sustain stable MAS (4-12 kHz) at cryogenic temperatures (35-120 K) for an extended period of time (e.g. >2 weeks) with low running cost (~$3/hr for mostly electricity expense). The long-term stability has enabled routine use of high-dimensional spectroscopy at cryogenic temperatures, strongly promoting application of high-field DNP to very complex and/or high-molecular weight chemical/biological systems. With the present system, we have so far obtained the sensitivity gain of over 300 from DNP and the temperature effect combined at 35 K.

2. **Double-frequency sub-millimeter wave (SMMW) irradiation system**. Many exciting possibilities will open up for high-field DNP NMR if the spectrometer is able to handle two SMMWs in different frequencies. The system involves two 460 GHz frequency-tunable and frequency-agile gyrotrons, quasi-optical transmission system, universal polarizer, and a custom designed beam combiner. Recently, we have confirmed in a simple experiment the benefit of using double-gyrotron irradiation. In the presentation, the latest experimental DNP data will be presented.

2. Y. Matsuki et al., *JMR* 264, 107- (2016)
Dynamic nuclear polarization of silicon-29 micro- and nanoparticles

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The intrinsic difficulty for a wide application of hyperpolarized probes obtained with dynamic nuclear polarization (DNP) is their relatively short lifetime given by the T1 relaxation of the target nuclei. Most of the up-to-date reported probes are based on small, selectively labelled carbon-13 molecules. In such a case, the hyperpolarized signal can be observed for up to few tens of seconds allowing to track only relatively fast biochemical processes.

More recently, an attention was brought to the polarization of elemental silicon-29 in the form of micro- and nanoparticles [1]. The naturally occurring oxidation defects on the surface of the particles serve as a source of electron polarization, excluding the need to dope the sample with an exogenous radical. Similar to schemes applied for other nuclei, the electron polarization is transferred to the surrounding nuclei by microwave irradiation and further spread to the interior of a particle by spin diffusion. However, the lack of interactions with other hetero nuclei and the weak homonuclear interactions in pure polycrystalline silicon, result in T1 times on the order of tens of minutes, even after dispersion of the particles in a liquid. The large nuclear polarization generated by DNP is hence effectively “locked” inside the particle and can be observed on a time scale much longer than the ones reported for any other molecule [2-3].

Nonetheless, little is known what properties of the silicon particles make them a good candidate for obtaining large nuclear polarization with DNP. In this report, several micro- and nano-powders of silicon obtained with different methods are studied in terms of their ability to be effectively polarized. In particular, the maximum achievable polarization is correlated with the results obtained from XRD, EPR and MAS NMR spectroscopy. In addition, the effect of microwave field modulation and the use of oversized quasi microwave cavity on the obtained silicon nuclear polarization level are discussed.

[1] Silicon Nanoparticles as Hyperpolarized Magnetic Resonance Imaging Agents, Aptaker et al. ACS Nano, 3 (12), 2009

Figure 1. Decay of Si-29 nuclear polarization at the field B₀=9.4T and at room temperature. The sample was composed of ~100mg of ball milled polycrystalline silicon powder with APS=1.5 um
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 about 50'000 [2].

However, $^1$H→$^{13}$C CP has so far been suboptimal because of the rapid proton relaxation arising from the presence of PAs. We show in this work that $T_{1p}(^1H)$ can be significantly extended, and therefore CP greatly improved, by briefly switching off the irradiation prior to CP. During this interruption of microwave irradiation, 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, $T_{1p}(^1H)$ is extended by several orders of magnitudes and CP contact times can be lengthened to achieve optimum transfer.

The use of microwave gating in this context has two favourable effects; (i) preventing excessive 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 $P(^{13}$C) as high as 65% was achieved in acetate with an overall polarization build-up time constant as short as 3 min. A record $^{13}$C polarization of 78% was even achieved in $^{13}$C labelled urea.

Figure 1. Build-ups of the carbon-13 polarization during multiple CP with continuous microwave irradiation (red) and with microwave gating (blue).

Coherent spin order transfer in coupled electron-nuclear spin systems

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A common strategy of many methods for generating nuclear spin hyperpolarization is the transfer of electronic spin polarization to the nuclear spin reservoir of choice. Two factors determine the resulting hyperpolarization, namely the usable level of electronic spin order and the efficiency of the polarization transfer process. In standard DNP the electrons are thermally polarized, while in alternative techniques, such as CIDNP or optical nuclear polarization (ONP), the electron spins get their polarization via an optical excitation cycle. For the transfer Overhauser DNP relies on relaxation, i.e., on stochastic processes, whereas in solids also coherent transfer pathways are supposed to be operative. To analyze in detail such coherent transfer, which is usually much more efficient than its stochastic counterparts, time-resolved measurements of electron-proton polarization transfer have been performed on small spin ensembles in single-crystalline matrices with a restricted number of interacting spins. These systems allow the investigation of the fundamental electron-nuclear spin coupling without additional complexities arising from interactions among like spins and from molecular dynamics within the systems. The experiments have been performed under various experimental conditions with laser flash excitation followed by various rf-pulse sequences using different doped molecular systems (Acridine/Fluorene and Pentacene/Naphthalene). The experimental results show well pronounced oscillations (see Figure 1) with characteristic frequencies, which are independent of the molecular systems and the applied pulse sequences, and constitute hence a generalized feature of the polarization transfer dynamics. These oscillations allow therefore the direct experimental investigation of the predominant transfer mechanisms constituting the fundamental steps in the polarization transfer process in coupled electron-nuclear spin systems.

In particular, the coherent nature of electron-nuclear polarization transfer is clearly demonstrated and its pathways are analysed. As a result, new directions for efficient manipulation and optimization of the nuclear hyperpolarization process are shown, which will be discussed in detail.

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Figure 1. Optically-induced nuclear polarization (ONP) of protons in molecular crystal Pe-d14/Na-h8 as a function of time, $t_y$, for the pulse sequence $\pi_x - t_y$. Here $\omega_{rf} = 230$ MHz $= \omega_0$, $\omega_n = 1.2$ MHz, $T = 250$ K.
Investigations of beer carbohydrates with increased 13C sensitivity

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Complex carbohydrates are an integral part of foods and the structural assignment of these complex oligo- or polysaccharides is a necessary step for understanding their biological functions and biosynthetic pathways. NMR is a powerful and often used technique in this structure determination and it often relies on natural 13C abundance, which comes with severe limitations – low sensitivity and highly overlapping spectra. Insight into the multifaceted mixture of different carbohydrates at natural 13C isotopic abundance was achieved through 13C projections from two-dimensional 1H-13C HSQC experiments using high-resolution in the second dimension, thereby providing a highly detailed mapping of a central compound class that is not easily amenable to other detection modalities. Carbohydrate structures were investigated by use of the anomeric reporter signals (Figure 1A). Multivariate data analysis techniques were used to examine the carbohydrate profile of ale, lager and Lambic beer types to identify differences (Figure 1B), which could stem from differences in the fermentation processes. Moreover, the carbohydrate profiles are correlated to the beer alcohol content, thereby providing information about biological and biotechnological processes in beer production.

Figure 1. A) 13C spectra created from positive projection of F1 in 1H-13C HSQC spectra of 42 beers. b) S-line plot from an OPLS-DA model on 13C projected spectra of beers (ale and lager).
Temperature-jump DNP with Frequency Modulation at 9.3 T

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Modulation of the microwave frequency in DNP-NMR experiments has been shown to be effective in increasing the enhancement obtained [1][2]. With the help of a home built frequency tuneable and rapidly switchable gyrotron [3], we have been able to achieve sweep rates of several kHz and amplitudes of up to 100 MHz, and have shown improvements in static $^1$H DNP enhancement of up to 70% in frozen glassy solutions of TEMPOL from 20 K to 100 K (Figure 1).

A temperature jump is an obvious means of achieving hyperpolarisation. We tried a version of this technique for $^{13}$C DNP where a sample containing TEMPOL is hyperpolarised in its frozen state and then melted with a brief exposure to high power microwaves. The switchability of our gyrotron allows for a transition time shorter than the nuclear relaxation time, leading to enhancements of about 500 for $^{13}$C liquid state NMR relative to thermal equilibrium (Figure 2). As both the hyperpolarisation and melting are performed by the same gyrotron, no modification is necessary to the standard DNP-NMR probe.

References:


Selective protein hyperpolarization in cell lysates using targeted DNP

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Nuclear magnetic resonance (NMR) spectroscopy has the intrinsic capabilities to investigate proteins in native environments1-3. In general, however, NMR relies on non-natural protein purity and concentration to increase the desired signal over the background. One way of improving NMR sensitivity is to use Dynamic Nuclear Polarization (DNP) enhancement. Conventional DNP uses a statistical distribution of radicals in the sample and will provide similar enhancement for target protein and background. Notably tough, a couple of studies have described the placement of the radical in a more defined position, providing the opportunity to obtain localized information4-10.

We here report on the usage of a covalently biradical-labeled ligand (Bak-peptide) that, after binding to the targeted protein (Bcl-xL), can selectively direct hyperpolarization to the desired protein instead of the whole sample. Radical labelling is realized by a maleimide modification of the TOTAPOL biradical and ligation to a cysteine residue of the ligand. Using this targeted-DNP setup we were able to restrict the hyperpolarization to the protein of interest while maintaining comparable signal enhancement with about 400-fold less radicals than conventionally used. We could selectively filter out our target protein directly from crude cell lysate obtained from only 8 ml of fully isotope enriched cell culture. Our approach is simple and widely applicable, and contributes effective means to study proteins with atomic resolution in increasingly native concentrations and environments.

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Welcoming natural isotopic abundance in solid-state NMR: A new strategy for structure determination of organic nanoassemblies using DNP

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Organic nanoassemblies find applications in fields ranging from medicine to molecular electronics. Their rational improvement requires precise structural knowledge, which includes their supramolecular assembly. Solid-state NMR (ssNMR) spectroscopy is potentially a powerful technique for providing atomic-scale information on such assemblies. However, it still faces strong limitations regarding samples that contain 13C and 15N at their natural isotopic abundance (NA).

In this contribution, we illustrate the immense possibilities lying in the use of magic-angle spinning dynamic nuclear polarization (MAS-DNP) for 3D structure determination of such samples. In particular, we demonstrate that low NA is not necessarily an annoying obstacle. On the contrary, when combined with DNP, NA is the key for recording unique and very informative spectra that are impossible to obtain with fully labeled material. For example, the observation of long distance 13C-13C couplings becomes much easier, owing to the spin dilution and thus greatly reduced dipolar truncation at NA. In addition, the theoretical description of the occurring spin dynamics is straightforward, since it can be reduced to isolated two-spin systems.

More specifically, it will be shown that the so-called NMR crystallography approach can be extended by NA DNP-enhanced 13C-13C and 15N-13C correlation spectra, allowing complete resonance assignment in otherwise ambiguous cases.[1] Secondly, we will show the importance of measuring 13C-13C interatomic distances at NA and their subsequent impact on structure determination. The detailed analysis of polarization buildup curves from 1D and 2D dipolar recoupling experiments combined with numerical simulations clearly produces structural information about both intra- and inter-molecular distances up to 7 Å. Furthermore, we succeed in efficiently recoupling aromatic carbons with high CSA, making it possible to observe π-π interactions through 2D 13C-13C correlation spectra. Finally, the particular challenges for future de novo structure determination at NA based on NMR distance measurements will be discussed.

Interpolating and extrapolating NUS reconstruction: seeking a $t_{\text{max}}$ for optimal sensitivity, resolution and frequency accuracy.

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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 how far one should sample in the indirect dimensions to obtain optimal sensitivity, resolution and peak accuracy. Rovnyak et al. (1) have previously emphasized that the optimal sampling evolution time is $\pi T_2$ when sensitivity is no issue and $0.4\pi T_2 (= 1.256 T_2)$ when sensitivity is an issue. This, based on uniform sampling analogs, Gaussian distributed white noise, and no time-equivalence.

To explore these questions about sensitivity, resolution and peak accuracy, we create a test situation to evaluate how to best cover the maximum evolution times. We also evaluate the simulation against the situation in a HSQC spectrum. In both cases all reconstructions are done to $2T_2$. The first data point in the graphs indicate the result when hmsIST (2) reconstructing $1/8^{\text{th}}$ of the data uniformly obtained plus $7/8^{\text{th}}$ non-obtained values for extrapolation; the last value is a reconstruction of $12.5\%$ sparsity NUS over the entire acquisition range of $2T_2$. The intermediate points are acquired with a mixture of interpolation and extrapolation.

Figure 1. Graph of observed sensitivity measured from simulation. The synthetic data contains one single Lorentzian signal centered on a Nyquist frequency, and noise added. The value for the noise is derived from a portion of the spectrum away from the signal. By reducing the noise by half the curve with open triangles, the shape alters and yields a maximum around $T_2/2$.


Figure 2. Graph of observed sensitivity measured from the HSQC spectrum of GB1. The measures are derived from the average peak height over the maximum value pixel in the first row in the spectrum, where only noise is presented. The value hence represents $\text{SN}(\ell_{\infty})R$. The figure indicates a maximum around $t_{\text{max}} = 60$ msec, which is somewhat less than $T_2/2$ since GB1’s $T_2$ of Nitrogen is in the range 140 to 150 msec.
Apparatus for gas dissolution at elevated pressure in situ NMR: Application to PHIP and SABRE

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During the past few decades hyperpolarization methods in NMR and MRI are rapidly developing. Parahydrogen Induced Polarization (PHIP) and Signal Amplification By Reversible Exchange (SABRE) are two techniques where the parahydrogen spin isomer is used as a source of non-thermal nuclear polarization. The initial step in homogeneous reactions is the dissolving of parahydrogen gas in liquids. Due to the low solubility of hydrogen at ambient pressure in liquids, especially in water, H₂ dissolution becomes the limiting step for the hydrogenation reaction.

A simple and low-cost apparatus for the hydrogen dissolution directly in the NMR sample tube is presented (figure 1). Hydrogen gas is dissolved in the liquid by bubbling through a thin plastic capillary inserted in a standard screw cap NMR sample tube. The pressure in the capillary is set higher than the pressure above the liquid surface, so that there is a constant flow of gas through the liquid. The NMR sample tube is tightly screwed to the Teflon adapter that has an inlet and an outlet. The system is closed and operates at the pressure up to 8 bar. The setup allows bubbling by two gases (e.g., hydrogen and nitrogen) and the application of vacuum for cleaning the connecting pipes. The control of gases is done by magnetic solenoidal valves that are operated by fast TTL-pulses synchronized with the RF pulses in the NMR pulse program.

The device allows to measure NMR spectra immediately after starting of hydrogen dissolution with a delay less than 1 s. Due to the computer controlled bubbling the highly reproducible PHIP spectra with a high spectral resolution are obtainable. This technique allows to measure the kinetics of PHIP polarization on the second time scale at different concentrations of dissolved parahydrogen. It is shown (figure 2) that an increase in pressure from atmospheric to 6 bar results in an increase of PHIP signal up to 50 times in the hydrogenation reaction of Fmoc-tyrosine-(propargyl)-OH.

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Figure 1. Principal scheme for in situ high pressure bubbling set-up with the solenoidal valves controlled by TTL-pulses from NMR console.

Figure 2. PHIP spectra of polarized Fmoc-tyrosine-(allyl)-OH taken at different bubbling pressure of para-hydrogen.
Continuous-flow DNP polarizer for small animal in-vivo MRI applications

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The Overhauser DNP technique allows achieving significant enhancements of signals for liquid-state NMR spectroscopy at 1.5 T [1]. This phenomenon was exploited also in MRI for small animals to improve image contrast by using a continuous flow out-of-bore polarizer [2]. Here we report on new results from a modified in-bore 1.5 Tesla DNP MRI polarizer equipped with a new multimode resonator and a more powerful 42 GHz source resulting in a flow rate up to 1 mL/min of aqueous TEMPOL solution which is a 3-fold increase in comparison to the previously reported in-bore polarizer [3,4]. Besides, 20-fold image contrast improvement has been measured with the new setup. The new polarizer is equipped also with a fluid flow diverter that can help to choose necessary timing of perfusion into the animal. Besides, temperature monitor and a chiller for polarized substrate has been implemented to keep temperature of the hyperpolarized substrate compatible with in-vivo physiological conditions. DNP test results on different 2-D and 3-D phantoms will be presented.


Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI) are essential methods widely applied in medicine and drug research. Currently, functional imaging or molecular imaging are improved to get deeper insight into processes on a molecular scale.

Nonetheless, magnetic resonance applications are limited by the inherent low sensitivity which is mainly caused by the low population difference of the nuclear spin levels. During the last decades efforts were made to increase this population difference, among them Para-Hydrogen-Induced Polarization (PHIP). This method is based on polarization transfer via a hydrogenation reaction with para-enriched hydrogen. In fact an unsaturated moiety must be present in the molecule of interest, which can be efficiently hydrogenated with the para-H₂ in a pairwise manner within the lifetime of the hyperpolarized species. Due to this restriction to the unsaturated moiety a surprisingly small number of simple biorelated molecules have been studied to date using this hyperpolarization method.

The poster present a short summary of my recent research based on the general challenge to find structurally low invasive building blocks which contain an unsaturated moiety to generate biomolecules for applications of PHIP. To test the scope of PHIP towards possible applications, e.g. in MRI, several unsaturated peptides have been studied. After getting first insights towards the PHIP-susceptibility of exemplary unsaturated oligopeptides, an unsaturated protease inhibitor is investigated as a first biologically active target for PHIP. The PHIP activity is achieved, in a series of novel bioactive derivatives of the sunflower trypsin inhibitor-1 (SFTI-1), by labeling with L-propargylglycine, O-propargyl-L-tyrosine, or 4-pentynoic acid. ¹H NMR signal enhancements of up to a factor of 70 is achieved in aqueous solution.

This systematic study of the PHIP phenomenon of a diversely functionalized bioactive peptide leads to the assumption that the propargyltyrosine residue may find general application as an efficient building block to selectively access PHIP in bioactive peptides. This enables new approaches to the investigation of biomolecules and may be of crucial importance in the development of new signal-enhanced MRI methods.

Figure 1. Structure and overlays of energy-minimized 3D models of the several investigated SFTI-1 variants (acetylene groups are depicted in magenta, trypsin catalytic triad highlighted in green) as co complex with trypsin to visualize the spatial position and orientation of the PHIP labels.
DNP Enhanced Solid-State NMR Spectroscopy at High Magnetic Field and Fast Magic Angle Spinning

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Solid-state NMR spectroscopy has become one of the most important analytical techniques for structure elucidation and dynamic studies for solid biological samples as well as for a wide range of functionalized materials. However, its applications are often hampered by its intrinsically low sensitivity. Dynamic Nuclear Polarization (DNP) is currently emerging as a powerful new approach which can overcome this sensitivity issue by transferring the large polarization of unpaired electrons to neighboring nuclei. Continuous wave Magic Angle Spinning (MAS) DNP can proceed via several mechanisms including Solid-Effect (SE), Cross-Effect (CE), and Overhause Effect (OE). Many aspects of these mechanisms have recently received attention in order to optimize DNP performance, like the design of polarizing sources optimized for one or the other effect, and spectacular progress has been achieved along these lines. Although sample spinning is an intrinsic part of these experiments, the sample spinning rate itself has so far received little attention. For biradicals featuring the Cross Effect, simulations predict a decrease of the enhancements with increasing MAS rate.[1-4] Using a prototype 1.3 mm MAS probe operating at 18.8 T (800 MHz) and ~ 100 K we have recently shown that signal amplification factors could be increased by up to a factor two when using smaller volume rotors as compared to 3.2 mm rotors, and reported enhancements of around 60 over a range of sample spinning rates from 10 to 40 kHz.[5]

Here, we will present new investigations on the MAS frequency dependence of the other DNP mechanisms. In particular we will show that using BDPA in OTP, enhancement factors of more than 100 can be reached at 40 kHz MAS and 18.8 T. The overall sensitivity enhancement factor in this spinning regime will be discussed and new applications will be presented.

Production of hyperpolarized contrast agents by heterogeneous parahydrogen-induced polarization

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Hyperpolarization of nuclear spins is a highly efficient way to overcome the issue of inherently low sensitivity of NMR and MRI. Parahydrogen-induced polarization (PHIP) is one of these hyperpolarization techniques, which is based on pairwise addition of parahydrogen (p-H2) to some unsaturated asymmetric substrate. Pairwise hydrogen addition can be mediated by either homogeneous or heterogeneous catalysis. Homogeneous catalysts (transition metal complexes) provide high NMR signal enhancement, but their applications are significantly limited by difficulties of catalyst separation. This problem can be solved by utilization of heterogeneous catalysts (e.g. supported metal nanoparticles), which also enable continuous production of hyperpolarized liquids and gases, though the level of polarization still needs to be improved.

Hyperpolarized (HP) propane is a promising inexpensive alternative for hyperpolarized noble gases as a contrast agent for MRI of lungs. It was demonstrated that propane gas produced by heterogeneous hydrogenation of propene with p-H2 over Rh/TiO2 catalyst can be utilized for fast high-resolution 3D 1H MRI of various phantoms[1]. The drawback of HP propane is its short (~ 0.6 s) relaxation time, which can be improved up to 6.0 ± 0.3 s by the use of low magnetic field, e.g. 47.5 mT, and deuterated precursor (propene-d6)[2].

Also we present an approach for production of catalyst-free aqueous solution of hyperpolarized ethanol[3]. Heterogeneous gas phase hydrogenation of vinyl acetate with parahydrogen over Rh/TiO2 catalyst led to formation of hyperpolarized ethyl acetate with high (more than 95 %) yield. Subsequent dissolution of gaseous ethyl acetate in aqueous solution of NaOD allowed to obtain catalyst- and organic solvent-free hyperpolarized ethanol and sodium acetate. Another promising approach is heterogeneous liquid phase hydrogenation of vinyl acetate over Rh/TiO2 catalyst. The use of elevated pressures allowed to obtain ethyl acetate with pronounced PHIP hyperpolarization in high yield. Combination of this approach with magnetic field cycling enabled polarization transfer to 13C nuclei of ethyl acetate carboxylic groups. Level of polarization is sufficient to observe hyperpolarized 13C resonances not only in case of isotopically labeled substrate (98 % 13C enrichment), but also when vinyl acetate with 13C at natural abundance (1.1 %) was utilized.

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Hyperpolarization of Amino Acids with Para-Hydrogen

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Para-Hydrogen Induced Polarization (PHIP) is a technique of hyperpolarization that utilizes the singlet-triplet conversion of para-hydrogen upon its addition to an unsaturated bond to create high levels of polarization.1 Signal enhancements of over 10 000-fold can be achieved with PHIP and offer possibilities for the improved design of molecular imaging contrast agents for disease targeting.2 In particular, the direct observation of metabolic events is one of the main strengths of hyperpolarized contrast agents.3,4 PHIP has found limited applications for metabolic targeting in the past due to the requirement of an unsaturated bond in metabolite precursors.5 Recently, the PHIP-SAH technique (PHIP by means of sidearm hydrogenation) was introduced in which an unsaturated sidearm is attached to the carboxylic acid moiety of pyruvate that can be hyperpolarized followed by polarization transfer to a 13C species in the target molecule.6 Subsequently the sidearm is cleaved and hyperpolarized pyruvate yielded which may then be used as a metabolic tracer. Amino acids are another group of metabolites that are of particular interest to hyperpolarize: Firstly, they can be used as molecular imaging agents.7 Secondly, amino acids are building blocks for peptides and proteins and their hyperpolarization with PHIP may serve as a rapid technique for signal enhancement in liquid state structural biology.8,9

In order to work towards this goal, we have synthesized amino acid precursors that contain a side-arm with an unsaturated bond and can be hyperpolarized with para-hydrogen. The precursor is dissolved in acetone together with a suitable hydrogenation catalyst and the hydrogenation is initiated by supplying 5 bar of para-hydrogen. After the reaction, the polarization is transferred from the protons to a 13C species in the amino acid by rapidly dropping the sample into a magnetic shielding and slowly bringing it back into the earth’s magnetic field. If the sample is transferred at this stage into a NMR spectrometer the 13C signal enhancement of the amino acid species can be observed. In order to cleave the sidearm from the amino acid we have added a concentrated aqueous sodium hydroxide solution to the reaction mixture in acetone after the field-cycling step. Two phases separate and the pure amino acid is yielded in the aqueous phase. We believe that our approach can be utilized in the future to broaden PHIP-applications in detecting metabolic events that involve amino acids. Additionally, this approach may be extendable to hyperpolarize peptide and protein structure and may serve as a useful tool for structural biology in the liquid state.

References

A high saturation factor in Overhauser DNP with nitroxide derivatives: the role of $^{14}$N nuclear spin relaxation

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The performance of Overhauser DNP experiments strongly depends on the capability of saturating the EPR line. To investigate and optimize the saturation behavior of the nitroxide polarizers, Overhauser DNP enhancements of toluene were measured at a magnetic field of 0.35 Tesla in a series of chemically functionalized nitroxide radicals. We observe that the enhancements increase systematically with polarizer size and rotational correlation time [1]. Examination of the saturation factor of $^{14}$N nitroxides by pulsed ELDOR spectroscopy led to a quantitative interpretation of the enhancements, for which the saturation factor increases up to almost unity due to enhanced nuclear ($^{14}$N) relaxation ($w_n$, as internal relaxation between different hf EPR transitions) in the nitroxide radical. The $w_n$ increases with the correlation time and crosses over the Heisenberg exchange rate (for 1.5 mM polarizer) at a correlation time of ~0.15 ns. Such an increase in the nuclear relaxation rate finds some precedents in ELDOR studies of the $^{15}$N-TEMPO radical in solvents of different viscosities [2] as well as studies of spin labeled lipids [3]. Overall, our results demonstrate that saturation factors close to unity can be achieved in Overhauser DNP with nitroxide radicals.

Spin Hyperpolarization in Dynamic Nanostructures

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The efficiency with which both electron and nuclear spin hyperpolarization can be generated in solution is often strongly dependent upon molecular motions and intermolecular interactions. By using self-assembling nanostructures such as reverse micelles to encapsulate molecules in a small water pool it is possible to modify these key factors. Here we present preliminary results from our investigation into hyperpolarization efficiency as a function of water pool size.

There are a number of chemically induced dynamic electron polarization (CIDEP) mechanisms that lead to spin-polarized radicals; these can be observed through transient EPR spectroscopy \cite{Forbes2013}. Many CIDEP mechanisms polarize only transient radicals whose concentration decays rapidly due to radical recombination or chemical reaction. In the radical triplet pair mechanism (RTPM) \cite{Blättler1990}, a transiently generated triplet state can produce a large net polarization of a stable radical. As this hyperpolarized radical persists in solution there is a possibility that polarization transfer to nuclei could be achieved via the Overhauser mechanism. This offers a new route to Dynamic Nuclear Polarization (DNP) utilizing hyperpolarized electrons, removing the Boltzmann limit on maximum NMR sensitivity enhancement arising from the $\gamma_e/\gamma_n$ ratio. To maximise nuclear polarization efficiency a good understanding of the initial electron hyperpolarization step is required. As the RTPM depends upon intermolecular quenching encounters between triplet state dye molecules and the radical, the relative motion of these species is crucial to determining the magnitude of the electronic magnetization generated (Figure 1). Encapsulation of dye and radical molecules within a reverse micelle should increase the probability of the desired quenching encounter, whilst minimizing unwanted triplet-triplet annihilations. Here we present our first study of RTPM generated electronic hyperpolarization as a function of reverse micelle size.

For our initial attempts to detect optically generated nuclear hyperpolarization we are performing ~15 MHz $^1\text{H}$ NMR measurements at ~0.34 T in an X-band ENDOR resonator. To test this system we have undertaken microwave-pumped Overhauser DNP studies. As Valentine \textit{et al.} recently highlighted reverse micelles offer not only increased dipolar contact with the polarizing radical (which may increase the eventual DNP enhancements on substrates) but also small water pools counter the problems of limited skin-depth and heating associated with microwave irradiation of an aqueous sample \cite{Valentine2014}. We have therefore investigated microwave-pumped DNP enhancements as a function of water pool size in the same reverse micelle system used for our RTPM study.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Figure1.png}
\caption{Electronic hyperpolarization of Rose Bengal / TEMPOL solution after a 532nm laser pulse varies with viscosity.}
\end{figure}

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Parahydrogen-induced polarization using group IV and group V heterogeneous catalysts

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Parahydrogen-induced polarization (PHIP) is a relatively simple hyperpolarization technique, which does not require very sophisticated equipment. The efficiency of the method, however, crucially depends on the catalyst mediating parahydrogen interaction with substrates. The most efficient catalysts are dissolved precious metal complexes, which are difficult to isolate fast from the hyperpolarized substances. As solid heterogeneous catalysts are easy to separate from reaction mixtures, heterogeneous hydrogenations using parahydrogen provide an efficient approach for producing hyperpolarized gases and liquids. For the last decade several heterogeneous catalytic systems were found to produce PHIP in numerous reactions with parahydrogen.\[1,2\] In most of the cases the catalysts were based on group VIII metal nanoparticles or complexes supported on oxides.

Herein, we show that supported complexes of group IV and group V elements can provide hyperpolarized compounds in hydrogenations with parahydrogen. Several catalysts with V, Ta, Zr and Hf complexes as active components were used. It was shown that in spite of a relatively low activity, these catalysts demonstrate high stability over long reaction runs. For example, Figure 1 presents the results obtained with mesityl V complex in propene hydrogenation. It is demonstrated that the production of hyperpolarized propane takes place over a wide range of temperatures (250-500 °C). For comparison, immobilized Rh complexes tested in the same reaction are active only for a very limited reaction time, and easily reduce with temperatures exceeding 80 °C.\[3\] It was demonstrated that catalytic activity of V catalysts is the highest among the examined catalysts. In contrast to V and Ta catalysts, Zr and Hf catalysts were rather more active at low temperatures (below 0 °C), but their activity was low.

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Trityl- and TEMPO-Based DNP Samples at 7 T Field

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The development of dissolution DNP into a standard technique requires further progress in methodology and hardware design. Specifically, the need for very low temperatures in the 1 K range and the resulting requirements for hardware pose an obstacle for ease of use and make polarizers complex. Recent years have seen a development for dissolution DNP polarizers towards higher static magnetic fields. This might offer a route to work at higher temperatures since the electron polarization reaches unity already at higher temperatures. In addition, recent experimental data suggest that higher absolute polarization levels can be reached at higher magnetic fields at the cost of slower polarization build-up times [1].

We have designed and built a dissolution DNP polarizer at a static magnetic field of 7 T following the same design principles as realized in our 3.5 T [2] systems. Here, we present the design of the polarizer and data recorded on the 7 T system. In particular, we analyze the polarization enhancements and build-up rates obtained with the two most popular radicals, TEMPO and trityl, and compare the data to data obtained at 3.5 T magnetic field.

Trityl-based samples are used typically for direct $^{13}$C polarization for in-vivo MRI, which requires as much polarization as possible for optimum resolution and signal-to-noise. We have analyzed different factors affecting the maximum achievable polarization level in neat $[1-^{13}]$C-pyruvic acid doped with trityl radicals. Additionally, we discuss the problem of reproducibility of obtained polarization levels with the purpose of comparison with the values reported in the literature [1,3,4].

TEMPO-based samples are often used for more methodological or spectroscopic work. We present results on work, which looks into the effect of deuteration of the solvent for TEMPO-based samples and how the time dependence of the polarization can be described in the context of a spin-thermodynamic model that has been previously used with data obtained at lower field [2].

Parahydrogen-based hypersensitive NMR/MRI tool for catalysis and beyond

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To hyperpolarize nuclear spins, parahydrogen is still predominantly used in combination with homogeneous processes in solution catalyzed by dissolved transition metal complexes. Since this is not suitable for a number of applications, the development of the strategies for producing hyperpolarized fluids that are free from organic solvents, metal-containing catalysts and other impurities is highly important. We have successfully demonstrated PHIP effects in heterogeneous hydrogenations [1] using as catalysts various metal complexes immobilized on solid supports, metal nanoparticles and clusters supported on oxide materials, and unsupported metals and metal oxides. PHIP effects are thus quite common in heterogeneous (HET) catalysis. At the same time, the results to be presented show that they depend critically on the catalysts composition and pretreatment.

One of the objectives of HET-PHIP research is to provide signal enhancement for various practical applications of NMR and MRI. Contrary to their homogeneous counterparts, heterogeneous catalysts are easy to separate from the reaction products; furthermore, their use allows one to produce catalyst-free hyperpolarized fluids continuously. Combination of HET-PHIP with 1H MRI is promising for MRI of porous media, including functional lung imaging. Recent results are discussed, including PHIP-enhanced high- and low-field MRI of gases in model objects. Major efforts are directed toward the development of approaches for in vivo studies. In particular, several examples of liquid- and vapor-phase hydrogenation of unsaturated compounds with parahydrogen over heterogeneous catalysts followed by 1H to 13C polarization transfer illustrate a promising way to produce catalyst-free 13C-hyperpolarized substances for potential in vivo use.

Another remarkable possibility provided by HET-PHIP is to develop a hypersensitive NMR-based tool for catalytic research. The unique chemical specificity of NMR spectroscopy and the non-invasive nature of MRI, combined with the major signal enhancement provided by PHIP, can provide new knowledge about the mechanisms of important chemical reactions and the dynamic processes in operating reactors. Examples include the study of the mechanism of heterogeneous catalytic hydrogenation of unsaturated cyclic C6-hydrocarbons, and MRI of operating gas-solid catalytic reactors. Yet another promising direction of research is the extension of HET-PHIP to reactions other than hydrogenation of simple olefins and alkynes, as exemplified by the HET-PHIP study of the catalytic oligomerization of unsaturated hydrocarbons.

Applications of PHIP to homogeneous catalytic processes are far from being exhausted either. Metal-free catalysts such as frustrated Lewis pairs (FLP) are considered as alternative catalysts for cheaper and greener industrial catalytic hydrogenations. Observation of PHIP effects upon parahydrogen activation by FLP provides a novel powerful instrument for the detailed mechanistic studies of their functioning. SABRE technique is rapidly becoming a powerful and popular approach for hyperpolarizing suitable substances in solution, with recent examples extending its use to high magnetic fields, and to polarization of other (e.g., 31P) nuclei.

References.

Micro Imaging Probe-Head Design for Dissolution Dynamic Nuclear Polarization

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Dissolution Dynamic Nuclear Polarization is a technique used to generate large signal enhancements in liquid state NMR. A unique dual iso-center magnet system at the University of Nottingham allows for dissolution to be carried out in less than one second [1]. Due to its speed, this technique opens up the possibility of high resolution micro-imaging which will take advantage of the high signal to noise available from the hyperpolarized signal [2]. However, an appropriate probe head must be built to optimally utilize the large signal enhancement.

A multi-channel probe-head has been designed and constructed so that the rapid data acquisition technique, Sensitivity Encoding (SENSE) [3], can be used to generate micro-images from hyperpolarized samples on a timescale less than the $T_1$ relaxation time.

SENSE is a commonly used technique for clinical imaging, however it has seldom been used on the micro-scale due to the difficulty in constructing the necessary hardware. SENSE allows undersampling of the acquired MR data and also yields a fully reconstructed and artifact free image. The advantage of doing this is that the acquisition time is drastically reduced and so the data can be collected before the signal relaxes. Artefact free reconstruction is achieved through multiple receivers providing a further level of spatial encoding to that of the imaging gradients. In order to get a good quality image reconstruction, the performance of the probe-head is paramount.

We will present design aspects for the construction of a multi-channel, micro-imaging, probe-head, and demonstrate that the performance of the probe is at the necessary level to allow artefact free reconstruction of under-sampled, micro scale, imaging data. The design and concept presented will allow rapid acquisition of the hyperpolarized signal when combined with Dissolution DNP, in order to facilitate the imaging of biological processes within cells.

Figure 1. Left: Under-sampled images from two orthogonal coils. Middle: reconstructed Image. Right: g-factor map, showing the goodness of the reconstruction, where $g = 1$ is a perfect reconstruction.

References:
HP Xenon by d-DNP using the clinical GE SPINlab polarizer system

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Purpose: Hyperpolarized (HP) $^{129}$Xe for magnetic resonance (MR) has wide use in material sciences, structural chemistry and medicine. In the medical field $^{129}$Xe has been demonstrated as a useful probe for MR lung imaging and proposed as a blood tracer for in vivo perfusion imaging. The Xenon probe is therefore promising for clinical assessment of brain function, and may aid in the diagnosis and prognosis of brain diseases. Standard production of HP $^{129}$Xe is via spin exchange optical pumping (SEOP). However, reports of large polarization enhancements for $^{129}$Xe via dynamic nuclear polarisation (DNP) have raised expectations that DNP can be an alternative method for producing HP $^{129}$Xe for patients. We therefore investigated the possibility for production of HP $^{129}$Xe using the clinical GE SPINlab polarizer, extending the practical use of the system.

Materials and methods: Solid state samples of 0.80±0.01 mL 1-Propanol / 12 mM AH111501 radical, infused with natural abundance (n.a.) or enriched (70% $^{129}$Xe) Xenon gas at 5.0±0.2 bar partial pressure for 60±1 minutes at room temperature, were prepared and inserted into the 5 T SPINlab polarizer. Microwaves at 140.01 GHz were used to irradiate the samples for 150±2 minutes at 0.90±0.04 K followed by dissolution, transport and MR measurement. HP / thermal MR experiments were performed (n = 3 / 2) with 9°±1° / 90°±1° flip angles and TR = 1 s / 300 s on a 9.4 T small bore rodent MR scanner using a volume radio frequency (RF) coil with 1 / 200 averages. Transport times for the n.a. / enriched gas experiments were 32±1 s / 27±1 s at earth field.

Results: The experiments resulted in enhancements / polarizations of 214±22 / 0.18±0.02 % and 431±30 / 0.37±0.03 % with $T_1 = 29±2$ s and $26±2$ s for n.a. and enriched gas, respectively.

Conclusion: The experiments have shown that it is possible to hyperpolarize $^{129}$Xe using the SPINlab system. However, substantial work is required to achieve similar enhancements to SEOP. Strides should also be taken to prolong the $T_1$ lifetime of the resulting HP gas. These issues can be addressed on multiple fronts, including optimization of the radical and Xenon concentrations, using different solvents and transporting the HP gas in a dedicated electromagnetic carrier.

We report a general strategy that enables for the very first time the hyperpolarization of various gases and we demonstrate it on butane and ethylene. Gases are adsorbed on mesoporous silica matrices called HYPSO (HYbrid Polarizing SOLids) that contain paramagnetic polarizing agents covalently attached to the surface of the pores, and DNP is performed at low temperatures and moderate magnetic fields ($T = 1.2$ K and $B_0 = 6.7$ T). The high electron spin polarization is transferred from the unpaired electrons to the protons of the molecular gases by frequency-modulated microwave irradiation. The resulting $^1$H polarization is subsequently optionally transferred to $^{13}$C or possibly to other nuclear spins by cross polarization. In our proof of concept example, we show how $^{13}$C polarization of $P(^{13}$C) = 9 % for butane and $P(^{13}$C) = 26.9 % for ethylene are obtained in a few tens of minutes. Strategies for preserving this hyperpolarization upon dissolution or sublimation are currently under development.

**Figure 1.** Hiperpolarization of ethylene by DNP using HYPSO materials. Relatively high polarizations were obtained for ethylene at low temperature $P(^1$H) = 70.0 % and $P(^{13}$C) = 26.9 % in relatively short times (20 minutes).
A low-cost dissolution-DNP system at 6.7 Tesla

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Sensitivity is central to NMR. Among the methods to enhance sensitivity, dissolution dynamic nuclear polarization, first described in 2003 [1], is extremely versatile: It allows one to i) almost freely choose a target molecule, ii) achieve spin polarization from several percent to near unity on that molecule through microwave irradiation at a temperature of approximately 1 Kelvin, iii) acquire highly enhanced spectra on the dissolved sample using high-resolution NMR or MRI. In particular, dissolution-DNP allows one to study the metabolism of small molecules in vivo, as was demonstrated on human cancer in 2013 [2], only 10 years after the initial experiment.

Basic research is likely to foster new applications of dissolution DNP, but commercially available polarizers are often cost-intensive, with GE Healthcare’s SPINlab priced at 2.1M Euro and Oxford Instruments’ HyperSense exceeding 0.5 M. In addition, commercially available systems may not be suited for modifications to implement new techniques such as cross polarization [3]. At the same time, many NMR labs are in possession of a suitable magnet and the main instrumentation required are a microwave source, a cryostat and a roots pump, which in total cost an order of magnitude less than a HyperSense.

Here we describe the dissolution-DNP setup in Southampton, which uses a conventional Oxford Instruments NMR flow cryostat in a 6.7 Tesla magnet. The setup has become operational only very recently and thus far yielded estimated $^{13}$C polarization levels of 18% in the solid state and 4% in the liquid state. We expect these figures to increase substantially in the near future.

In addition to the above mentioned equipment, various engineering tasks need to be solved. The helium level needs to be controlled, sufficient microwave power needs to be transported to the sample and the NMR circuit needs to be tuned/matched at its operating temperature. Furthermore, a dissolution setup has to be built for dissolving the sample; a magnetic tunnel and an injection device may be important to preserve magnetization. Taken individually, each of these tasks is perhaps not too challenging, but, with the devil residing in the detail, significant resources have to be budgeted if each one is to be solved from scratch. We will publish our design online in great detail, including suppliers and part numbers, circuit board layouts, mechanical drawings and detailed procedures. We will argue that this resource will greatly simplify the construction of dissolution-DNP equipment.


Maternal-fetal exchange and metabolism followed in real-time by dynamic hyperpolarized $^{13}$C imaging on pregnant rats

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The mammalian fetus relies on the placenta to mediate exchanges between maternal and fetal gases and metabolites, and to excrete fetal metabolic wastes. Two thirds of all cases of stillbirth are connected to placental dysfunction. Therefore the ability to non-invasively characterize specific maternal-fetal exchanges by MRI may offer valuable insight to better understand fetal metabolism, developmental physiology and a variety of neo-natal diseases and malformations such as preeclampsia, hyperoxia and hypoxia [4]. This study presents the combination of MRI with dissolution DNP [1-3] to monitor the transport of pyruvate across the placental barrier and its subsequent conversion to lactate in a non-invasive way.

Wistar pregnant rats at late pregnancy stage (embryonic days 17 to 21) were anesthetized with 3% of isoflurane in 1L/min of O$_2$ and their tail vein was canulated for the injection of hyperpolarized pyruvate. Dynamic $^{13}$C chemical shift imaging (CSI) centric-sampling experiments were performed on a Bruker Biospec 4.7T system using a cross-coil configuration (volume coil transmit / 20mm surface coil receive). A FOV of 5cm and a TR of 68ms was used with a matrix resolution of 12x12 leading to an acquisition time of 8.3s. In addition, T1 and T2 weighted $^1$H anatomical images were obtained using gradient- (6.3ms TE, 615ms TR) and spin-echo (29ms TE, 5s TR) sequences with gating. $^{13}$C CSI images were reconstructed with Matlab and each was normalized to the highest intensity.

$^{13}$C-pyruvate was mixed with Ox63 (15mM) and hyperpolarized in an Oxford Instruments Hypersense operating at 94GHz and 1.4K. A 3ml bolus of the resulting 80mM hyperpolarized $^{13}$C-pyruvate solution was injected into the tail vein of the rat. $^{13}$C CSI were recorded at the end of the injection of hyperpolarized pyruvate or once half of the bolus was injected.

$^1$H anatomical imaging enabled the identification of all key maternal/fetal compartments including the maternal uterine artery, vena cava and the kidneys; the placenta; the fetus and its liver and heart. Series of $^{13}$C-pyruvate images recorded with sufficient signal-to-noise ratio (SNR) for over 30s following injection revealed a rapid build-up and decay in the maternal compartments (uterine artery, kidney, vena cava) and the placenta. The conversion of pyruvate to lactate was also monitored by a series of $^{13}$C-lactate images. For the maternal kidney a rapid decay of lactate signal is observed. In contrast slow buildup (starting at 16s) and eventual decay (to 56s) is observed in the placenta. This is in accordance with previous data of hyperpolarized urea, which showed slower signal decay after crossing of the placental barrier. In addition, weak, short-lived $^{13}$C-alanine signals were also observed in the fetal livers.

This study proves the applicability of hyperpolarized MRI for studying fetal metabolism in real-time. It may serve as a basis for the potential detection of fetal metabolic conditions such as preeclampsia, hyperoxia and hypoxia.
Long-lived Singlet States to Sustain SABRE Hyperpolarised Magnetisation

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The applicability of the magnetic resonance (MR) technique in the liquid phase is limited by poor sensitivity in general and fast nuclear spin relaxation in some cases. Here we illustrate a fast and simple method to address both of these issues simultaneously by harnessing long-lived hyperpolarized spin states that are formed by the Signal Amplification by Reversible Exchange (SABRE) technique. The whole process is simple and low cost compared to other hyperpolarisation techniques. It is also reversible in nature, thus allow to re-use the sample multiple times with very little going into waste. We achieve more than 4 % net 1H-polarisation in a long-lived form (SABRE-LLS) that remains detectable for several minutes by reference to proton pairs in the biologically important molecules whose in vivo imaging will offer a new route to probe disease in the future.

Figure 1. Graphical representation of the SABRE-LLS process.

We have studied a range of related molecules of nicotinamide, pyrazine and aminothiazole. Our studies include dependency of singlet lifetime and the enhancement factor over a range of parameters e.g. catalyst, concentration, solvent, magnetic field, temperature, RF power etc. A theoretical model has been developed to understand the relaxation dynamics of our study and our simulations show good match with the experimental results.
Lighting-up benchtop NMR spectroscopy using para-hydrogen hyperpolarisation

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The development of robust, high-resolution, benchtop NMR spectrometers based on permanent magnets creates the potential for new applications of NMR spectroscopy outside of the typical laboratory setting.1 Of particular interest is the use of benchtop NMR in industry for on-site process monitoring and control. One of the significant barriers to progress in this area is the limited sensitivity that arises from the relatively low magnetic fields (1-2 T) employed in these devices. In this work we aim to overcome the sensitivity issue through the use of hyperpolarisation. We are focused in particular on para-hydrogen (p-H2) induced polarisation (PHIP) because p-H2 is relatively cheap and easy to produce and so does not significantly compromise the overall size and affordability of benchtop NMR devices.

Para-hydrogen, the nuclear spin isomer of H2 in which the protons form a nuclear singlet state, can be used to generate NMR signal enhancements by means of a chemical reaction that results in the former p-H2 protons being chemically and/or magnetically inequivalent in a product molecule.2 In the signal amplification by reversible exchange (SABRE) approach,3 a reversible reaction is used such that the target molecule is hyperpolarised without the need for it to be hydrogenated (see Figure 1a).

In this work, we demonstrate that high levels of hyperpolarisation can be observed on a benchtop NMR spectrometer (Figure 1b) using both hydrogenative PHIP and SABRE (Figure 1c). We investigate how factors such as substrate concentration and T1 relaxation affect the observed enhancement levels and explore how this approach could be used for reaction monitoring on a benchtop NMR spectrometer, particularly for detecting species present at low concentration.

References


Figure 1. (a) SABRE hyperpolarisation scheme. (b) 1 T Magritek spinsolve benchtop NMR spectrometer. (c) Comparison of 1 T 1H NMR spectra of pyridine (with SABRE catalyst in d4-methanol) acquired with thermal polarisation (top) and SABRE hyperpolarisation (bottom).
Abstracts for posters in session "Small molecules and pharmaceuticals"
PSYCHEDELIC: A general method for extracting individual $^1$H-$^1$H couplings from crowded spectra

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Homonuclear couplings are a double-edged sword. They deliver a wealth of structural information, but equally they are detrimental to spectral resolution, impeding their accurate measurement. One way to disentangle individual couplings from complex spectra is the SERF experiment, which delivers a 2D J-resolved spectrum containing only selected couplings [1]. A variant of this experiment, G-SERF, uses the Zangger-Sterk pulse sequence element to deliver simultaneously all the individual couplings to one selected resonance [2]. Other recent variants [3,4] incorporate band-selective and Zangger-Sterk real-time pure shift acquisition [5]. However, all these methods can break down in crowded spectra, either because of signal overlap or because chemical shift differences between coupled spins are too small.

Here, we present the PSYCHEDELIC (Pure Shift Yielded by CHirp Excitation to DELiver Individual Couplings) experiment [6], derived from the PSYCHE pure shift method [7]. It delivers simultaneously all individual couplings to a selected proton, with minimal restraints imposed by spectral overlap and chemical shift differences (Figure 1). It provides the usual pure shift resolution, high sensitivity, spectral purity, tolerance to strong coupling and ease of experimental set-up of PSYCHE, making it of great value for measuring homonuclear scalar couplings or RDCs in crowded spectra.

Further variations on the experiment, allowing for instance measurement of couplings between protons with nearly the same chemical shift, and measurement of heteronuclear couplings, will also be discussed.

Elucidation of conformational preferences of L-histidine derivatives in solution by $^1$H NMR and DFT calculations

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Extensive structural research has been conducted on conformational equilibrium of amino acids and small peptides, in an attempt to elucidate their dynamic role in proteins formation.\(^1\) As a good approximation to the electronic environment of an amino acid residue in a protein chain we have proposed the study of amino acid methyl esters (R-OMe) and their N-acetylated derivatives (Ac-R-OMe), where R = amino acid,\(^2\) since these compounds do not form zwitterions and are soluble in organic solvents and, thus, they can be studied in solution. The present study provides an insight about the conformational preferences of two L-histidine derivatives (HisOMe and AcHisOMe) in solution using the NMR method of variation of the $^3J_{HH}$ with the solvent, supported by theoretical calculations of electronic structure and solvation theory (IEF-PCM).

Conformers were initially searched through potential energy surfaces using the GAUSSIAN 09 program, and they were named according the three arrangements showed in Fig. 1. For HisOMe, the calculations revealed the Ia and Ila conformers (Fig. 2a) as the most stable of the eight geometries obtained in isolated phase (populations of about 40% each), whereas for AcHisOMe the trans-Ia (Fig. 2b) is the predominant one (~100%). Experimental $^3J_{HH}$ obtained in different solvents showed changes with the increase in solvent polarity indicating, therefore, that both conformational equilibria are sensitive to the media. These coupling constants $^3J_{HH}$ were faced with the theoretically predicted ones for each conformer at oB97X-D/aug-cc-pVTZ level and with inclusion of solvent effect via IEF-PCM model. A joint analysis of these data allowed elucidate the behavior of the most stable conformers in solution, e.g. that IIc for HisOMe (Fig. 2a) and trans-IIb and trans-IIIb for AcHisOMe (Fig. 2b) are the most stabilized in polar solvents, whose stabilities were attributed to an interplay between steric effects and hyperconjugation.

![Figure 1](image)

**Figure 1.** Newman projections showing the dispositions a, b and c of the side chain, resulting from rotation around the $\alpha$-$\beta$ bond.

![Figure 2](image)

**Figure 2.** Most stable conformers of (a) HisOMe and (b) AcHisOMe, obtained at the oB97X-D/aug-cc-pVTZ level. They are ordered according to their relative energies in isolated phase.


FAPESP.
Spectroscopic and theoretical studies of conformational behavior of N-acetyl-L-cysteine methyl ester

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Amino acids and their derivatives have been subject of many investigations, since protein folding pathways, which determine the protein tridimensional geometries, are primarily restricted by the conformational space of each individual amino acid residue. Although there is great interest in recognize the conformational preferences of these compounds, studies related to the responsible forces for their behavior deserve greater attention, because they have been solely attributed to presence of intramolecular hydrogen bonds (IHB), while the important role of steric effects and hyperconjugations are neglected. Therefore, this study is aimed to investigate the conformational equilibrium of N-acetyl-L-cysteine methyl ester (Ac-Cys-OMe) in isolated phase and solution, as well as to describe the intramolecular interactions responsible for the obtained preferences. To this end, theoretical calculations combined with NMR and IR spectroscopies were employed.

Initially, the geometries and energies for the most stable conformers of Ac-Cys-OMe were calculated at the \( \omega B97X-D/\text{aug-cc-pVTZ} \) level, which indicated the predominance of only one conformer, \( \text{trans-I} \), in isolated phase (Fig. 1a). In solution, however, \( \text{trans-I} \) is destabilized with the increase in solvent polarity and had its population decreased to about 70% in DMSO, the most polar medium analyzed. At the same time, occurs the stabilization of the conformer \( \text{trans-II} \) (Fig. 1b), which represents ~30% of the conformational equilibrium of this compound in DMSO.

![Figure 1. Spatial representations, dihedral angles and dipole moments of the conformers (a) trans-I and (b) trans-II of Ac-Cys-OMe in isolated phase, obtained at the \( \omega B97X-D/\text{aug-cc-pVTZ} \) level of theory.](image)

Experimental \( J_{\text{HII}} \) spin-spin coupling constants were obtained, in several solvents (ranging from \( \text{C}_6\text{D}_6 \) to DMSO-\( d_6 \)), and compared with the corresponding theoretical values for each conformer (at BH\( \text{andH}/\text{aug-cc-pVTZ} \) level and with inclusion of solvent effect via IEF-PCM model). Also, IR data could estimate the populations of the two conformers in solvents of different dielectric constants. Further theoretical calculations, in the framework of the quantum theory of atoms in molecules (QTAIM), non-covalent interactions (NCI) and natural bond orbitals (NBO), at the \( \omega B97X-D/\text{aug-cc-pVTZ} \) level, were also performed. Both experimental and theoretical outcomes are in excellent agreement and suggest that an interplay between steric and hyperconjugative interactions rules the conformational behavior of Ac-Cys-OMe.


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Distinguishing small structural differences with selective pure shift TOCSY

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TOCSY\(^1\) gives crucial \(^1\)H-\(^1\)H connectivity information for organic compounds, but signal overlap can greatly reduce its usefulness. Recently, 2D NMR pure shift experiments have been shown to be very useful in TOCSY analysis,\(^2\) improving spectral resolution and reducing signal overlap, but at the cost of long experiment times. Moreover, in complex molecules and in mixtures, the information provided by these experiments can be very difficult to extract. This limitation can be overcome by using selective pulses,\(^3\) observing only the connectivities of the signals of interest. Recently a band-selective 1D pure shift frequency selective TOCSY experiment has been proposed,\(^4\) but this restricts the correlations observed to a narrow region of the spectrum. Here a new broadband 1D selective TOCSY-PSYCHE method is presented that does not suffer from this restriction, and can aid synthetic chemists to distinguish small changes in chemical environment. The new experiment has been used to analyze a mixture of provitamin D\(_3\) and vitamin D\(_3\) (Figure 1). Due to the similarity of these molecules, the aliphatic region of the conventional \(^1\)H spectrum (Fig. 1a) is very crowded and impossible to interpret. In the PSYCHE spectrum (Fig. 1b), even after collapsing the multiplet structure into singlets information is still obscured because of the density of signals in this region. However, with the new experiment, the spectrum is simplified to the point that all the relevant resonances can be distinguished, even where (as in the case of protons \(\beta\)) the chemical shifts are degenerate.

Figure 1. 500 MHz a) conventional, b) PSYCHE, and c,d) 1D selective TOCSY-PSYCHE \(^1\)H spectra for a mixture of provitamin D\(_3\) (top left) and vitamin D\(_3\) (top right) in CDCl\(_3\). \(\H{1}\) of provitamin D\(_3\) (c) and \(\H{1}\) of vitamin D\(_3\) (d) were selected using 180° rsnob pulses.

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Investigation of spiropyrans and -oxazines and their norbornene polymers by
in situ irradiation NMR

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Photochromic compounds like spiropyran and -oxazines are widely used for the synthesis of stimuli responsive materials which are capable of changing their physical properties reversibly upon irradiation with light. Typically UV/Vis measurements are used to examine absorption wavelengths of photo isomers in photochromic systems. NMR spectroscopy as a complementary method can give information on the structure and the distribution of different species of the photochromic system. Here we present the capabilities of online NMR with in situ irradiation for the investigation of the photochromic spiropyrans [1] and -oxazines [2] (Fig. 1) and their norbornene homopolymers. Irradiation of the sample was applied in situ by using high power LEDs and an optical waveguide [3]. By this approach molar ratios of photo isomers can be determined during irradiation with UV light as well as in photo stationary states without knowing the molar extinction coefficients (Fig. 2 a). Thermal and induced relaxation can be characterized. Continuous irradiation enables application of multidimensional NMR experiments on samples containing metastable photo intermediates to obtain C-H and N-H correlations of those species (Fig. 2 b).

Figure 2. a) Molar ratios of ME upon irradiation with UV light and thermal relaxation of ME at different temperatures. b) HSQC spectrum of a mixture of SPO and ME (blue).

Chirp Excitation:
from Ultra-High-Resolution NMR to Ultra-Broadband Spectroscopy

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Swept-frequency pulses have a long history in NMR and MRI, but most applications have been limited to inversion and refocusing, because of the special advantages such pulses have when the RF level used satisfies the adiabatic condition. These advantages make them the method of choice when broadband spin inversion or refocusing, or insensitivity to $B_1$ field inhomogeneity, is desired. Swept-frequency pulses have not found wide application for excitation, as opposed to inversion or refocusing, for two main reasons: (i) below the adiabatic threshold, they are very sensitive to $B_1$ field strength, and (ii) since they irradiate different offsets at different times, they generate severe offset-dependent phase errors when used for excitation. These problems make them less attractive than hard pulses for most purposes. Here we show how to circumvent the problems and take advantage of the broadband nature of chirp pulses in two very different applications.

The first experiment, PSYCHE (Pure Shift Yielded by CHirp Excitation) [1-4], uses low power (low flip angle) chirp pulses in a stimulated echo sequence element to produce broadband homonuclear decoupled (pure shift) NMR spectra. It achieves this by combining frequency sweep with spatial encoding, suppressing unwanted signals by spatio-temporal averaging.

The second experiment, CHORUS (C Hirped, O Rdered pulses for Ultra-broadband Spectroscopy) [5], takes the broadband excitation introduced by Bodenhausen [6] and refined by Shaka [7] a step further, combining phase-corrected 90° and 180° chirp pulses to give broadband, $B_1$-insensitive, quantitative excitation over a frequency range of ~300 kHz. Although demonstrated in $^{19}$F NMR, CHORUS has the potential to be useful for a wide range of NMR and EPR experiments needing wideband excitation.

Investigation of Motional Dynamics of Cyclodextrin
Inclusion Complexes by NMR Spin Relaxation

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Abstract: Innovations in material chemistry have fueled the development of drug delivery systems (DDS), creating carriers that are biocompatible, targeting and stimulus-responsive. As a consequence it becomes interesting to analyze drug carriers in terms of their interaction with drug molecules. In the present study our main objective is to analyze motional dynamics of cyclodextrin encapsulated model drug molecules where cyclodextrin is typically used as a drug delivery system to enhance solubility and bioavailability of the drug. Figure 1(a) represents a cartoon of the encapsulation process while figure 1(b) depicts how ratio of non-selective to selective spin-lattice relaxation rate depends on the motional correlation time of a molecule.

![Figure 1.](attachment:image)

**Figure 1.** (a) Cartoonic representation of host-guest complex of cyclodextrin; (b) semilogarithmic plot of $R_{1ns}/R_{1se}$ of proton against $\omega_c \tau_c$ considering intermolecular dipolar interaction as the sole relaxation mechanism to be operative in solution; (c) stack plot of bi-selective inversion recovery with 0-15s recovery period for paracetamol-cyclodextrin complex.

Formation of intermolecular adducts alters selective ($R_{1se}$) and non-selective ($R_{1ns}$) proton spin-lattice relaxation rates at different extent, depending on molecular rotational correlation time ($\tau_c$). We report $\tau_c$ of the free drugs by using selective relaxation rate in the fast molecular motion limit ($\omega_H \tau_c<1$ and $R_{1ns}/R_{1se} \approx 1.500$), whereas that of the bound drugs were found from the ratio of $R_{1ns}/R_{1se}$ in the intermediate motion time regime ($\omega_H \tau_c \sim 1$ and $R_{1ns}/R_{1se} \approx 1.054$). The correlation time of the free drug was about $6.11 \times 10^{-11}$s in case of model drug paracetamol that increased almost 30 times in presence of cyclodextrin confirming formation of inclusion complex. We further report inter-proton spatial distance between drug and the drug carrier by monitoring 1D bi-selective spin-lattice relaxation time by simultaneous inversion of a pair of protons from the drug and cyclodextrin as given in figure 1(c). An excellent correlation between inter-proton distance and change in chemical shift of drug protons on encapsulation is observed in case of paracetamol.
NMR characterization of the Interaction between Mcl-1 and Pyridoclax

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Mcl-1 and Bcl-xL are anti-apoptotic proteins implied in ovarian carcinoma. Both proteins cooperate to protect cancer cells against apoptosis and their concomitant inhibition leads to massive apoptosis even in absence of chemotherapy 1. Whereas Bcl-xL inhibitors are now available (ABT-737 2) Mcl-1 inhibition, remains problematic. Pyridoclax is an abiotic foldamer 3 designed to target Mcl-1 hydrophobic pocket. Surface plasmon resonance assay demonstrated that Pyridoclax directly binds to Mcl-1, and Pyridoclax, without cytotoxic activity, induced apoptosis in combination with Bcl-xL-targeting siRNA or with ABT-737 in cancer cells 1.

The work presented here consists of a NMR study of the interaction between Mcl-1 and Pyridoclax. 1D and 2D NMR experiments, were used to evidence this interaction and to identify the "hot-spots" within the Mcl-1 protein. Collected experimental data will be then used to improve docking results.

3. Foldamers are non-natural oligomers with well-defined conformations inspired by biopolymers. They can be used as platforms to project recognition motifs in the space that mimic the functionality side chains of proteins, e.g. side chains on one face of the α-helix.

This work has been carried out with financial aid of the CRUNCH institution.
In the pharmaceutical industry, it is vital to be able to identify and quantify impurities down to 0.1% levels. $^{19}$F NMR is potentially very useful here: a high proportion of active pharmaceutical ingredients contain one or more fluorine atoms, and the very wide chemical shift range makes signal overlap much less likely than in $^1$H NMR. Couplings to $^1$H complicate spectra, but can be suppressed by broadband $^1$H decoupling; couplings to $^{13}$C are more problematic. Broadband $^{13}$C decoupling is possible, but can cause severe sample heating if good resolution is needed, requiring long acquisition times. A further, less obvious, problem is that even if all $^{13}$C couplings are suppressed, the relatively large secondary isotope effect on the $^{19}$F chemical shift can mean that distinct singlet $^{19}$F signals are seen for different $^{13}$C isotopomers.

Here we present a novel approach to measuring high dynamic range $^{19}$F spectra that suppresses interfering signals from $^{13}$C isotopomers and does away with the need for broadband $^{13}$C decoupling. The pulse sequence shown is compatible with several different hardware configurations; experimental results will be presented for a system with a single high band RF power amplifier and a ($^1$H/$^{19}$F),$^{13}$C two-channel probe with a double-tuned high band coil. Couplings to $^1$H are suppressed using broadband $^1$H decoupling, time-shared during acquisition if necessary. Two different strategies are used for suppressing signals from $^{13}$C isotopomers. First, signals from species with one-bond couplings $^1J_{CF}$ are edited out by generating a $^{13}$C-modulated $^{19}$F echo and applying a 90° $^{13}$C pulse to convert the antiphase $^{19}$F signal into unobservable heteronuclear quantum coherence. Signals with long-range couplings $^nJ_{CF}$ are then edited out by averaging the signals from $^{13}$C-modulated $^{19}$F echoes with a range of evolution times. This is equivalent to measuring a heteronuclear 2DJ spectrum and taking the cross-section at $F_1 = 0$. The result is a clean $^{19}$F spectrum with no resolvable $^{13}$C couplings, obtained at only very modest cost in sensitivity.

**Figure 1.** a) Pulse sequence, b) 470 MHz 1D $^{19}$F spectrum of $C_6F_3H_3$ and c) cross-section at $F_1 = 0$. 

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**Uncovering weak impurity signals in $^{19}$F NMR**

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In the pharmaceutical industry, it is vital to be able to identify and quantify impurities down to 0.1% levels. $^{19}$F NMR is potentially very useful here: a high proportion of active pharmaceutical ingredients contain one or more fluorine atoms, and the very wide chemical shift range makes signal overlap much less likely than in $^1$H NMR. Couplings to $^1$H complicate spectra, but can be suppressed by broadband $^1$H decoupling; couplings to $^{13}$C are more problematic. Broadband $^{13}$C decoupling is possible, but can cause severe sample heating if good resolution is needed, requiring long acquisition times. A further, less obvious, problem is that even if all $^{13}$C couplings are suppressed, the relatively large secondary isotope effect on the $^{19}$F chemical shift can mean that distinct singlet $^{19}$F signals are seen for different $^{13}$C isotopomers.

Here we present a novel approach to measuring high dynamic range $^{19}$F spectra that suppresses interfering signals from $^{13}$C isotopomers and does away with the need for broadband $^{13}$C decoupling. The pulse sequence shown is compatible with several different hardware configurations; experimental results will be presented for a system with a single high band RF power amplifier and a ($^1$H/$^{19}$F),$^{13}$C two-channel probe with a double-tuned high band coil. Couplings to $^1$H are suppressed using broadband $^1$H decoupling, time-shared during acquisition if necessary. Two different strategies are used for suppressing signals from $^{13}$C isotopomers. First, signals from species with one-bond couplings $^1J_{CF}$ are edited out by generating a $^{13}$C-modulated $^{19}$F echo and applying a 90° $^{13}$C pulse to convert the antiphase $^{19}$F signal into unobservable heteronuclear quantum coherence. Signals with long-range couplings $^nJ_{CF}$ are then edited out by averaging the signals from $^{13}$C-modulated $^{19}$F echoes with a range of evolution times. This is equivalent to measuring a heteronuclear 2DJ spectrum and taking the cross-section at $F_1 = 0$. The result is a clean $^{19}$F spectrum with no resolvable $^{13}$C couplings, obtained at only very modest cost in sensitivity.
Menthol and amino derivatives: configurational and conformational analysis

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The complete series of menthol (Figure 1) isomers and their corresponding amino derivatives (base and protonated/HCl forms), were investigated using experimental and theoretical data. Our study focused on conformational and configurational analysis, and revealed that experimental data should be used in combination with calculated data. Furthermore, even in the case of the highly studied member, menthol, large discrepancies were found among published data based on calculations. We show that the correct determination of the population mix is a must for the correct prediction of the absolute configuration (AC) of neoisomenthol. The neoiso forms are of special interest since a number of structural inconsistencies can be found in the literature. We present a stringent proof of the AC of neoisomenthol based on literature information. To the best of our knowledge, the AC of neoisomenthylamine is for the first time shown using experimental and calculated optical rotation data. A correction of a series of publications containing an important error in the assignment of (+)-menthylamine (correct: (+)-neomenthylamine) is presented. With 26 data pairs (experimental versus calculated) of optical rotation values a regression is performed (Figure 2). The AC of all 12 compounds, even the most difficult neoiso forms, could be predicted correctly using experimental low-temperature NMR data. Furthermore, if only experimental data with an optical rotation outside the range from $[\alpha] = -10$ to $[\alpha] = +10$ are used, all 12 compounds would have been correctly assigned without low-temperature NMR data as restraints.

Figure 1. Calculated conformer of (+)-(1S,3S,4R)-menthol (1eq3eq4eq) obtained by DFT (density functional theory) optimization at the mPW1PW91/cc-pvdz level of theory with a continuum solvent model using acetonitrile as solvent

Figure 2. Experimental and calculated optical rotation values (in total: 26) of the (+)-menthol and (+)-menthylamine (base and HCl/protonated) isomers; linear regression lines (in red) and prediction bands at a 99 % level of confidence (in green)
How Acidic Decomposition Products of CDCl$_3$ Influence the Orientational Properties of Alignment Media

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To observe anisotropic NMR parameters like residual dipolar couplings (RDCs), alignment media are used to partially align the analyte of interest. The molecule interacts directly with the alignment medium and is forced to a certain favored orientation with respect to the magnetic field. Using chiral media the interaction of each analyte enantiomer differs and they may become distinguishable. It is essential to understand the alignment process in detail to at least aim for the ability to obtain the absolute configuration via this approach in the future.

It seems that acidic decomposition products have an impact on the alignment process. They are supposed to interfere with hydrogen bonding. First research results show an inconclusive picture of the contribution of hydrogen bonding to the interaction between solute and alignment medium.[1, 2] Therefore we decided to focus on the influence of hydrogen ions on the alignment process.

As a model system we use isopinocampheol (IPC) as analyte molecule dissolved and oriented in poly-$\gamma$-benzyl-D-glutamate (PBDG) and deuterated chloroform. RDCs were used to determine order parameters. We are able to evaluate the discrimination of the enantiomers by comparison of the orientation tensor, that describes the orientation of the molecule with respect to the magnetic field.[3, 4] To investigate the influence of acidic environment we utilize purified CDCl$_3$ and CDCl$_3$, that contains acidic decomposition products. The hydrogen ions might affect the ability of the OH-group of IPC to form H-bonds, which is expected to be involved in the process of orientation.


Figure 1. Does hydrogen ion concentration influence the enantiodiscrimination of IPC in alignment media?
Macrocyclic molecules have gained attention in drug design for their ability to present diverse functionality and stereochemical complexity in a conformationally defined structure. Recently, we have reported utilizing macrocyclization to restrict FVIIa inhibitors into their bioactive conformation identified from the enzyme-bound X-ray crystal structure of an uncyclized inhibitor. Incorporation of a methyl group at the 3-position of the core phenyl ring (Figure 1 left) improved potency 10 due to additional interactions with FVIIa in the S2-pocket. This, and related substitutions in the macrocycle produced extensive heterogeneities in the respective NMR spectra. NOE-difference spectra at elevated temperatures confirmed the presence of slow conformational exchange as the source of the spectral heterogeneity. Solution NMR was employed to first determine the 3d-structure in solution of the unsubstituted macrocyclic precursor and to determine the rate of rotation of the aromatic ring in the phenyl glycine moiety. A modified noesy-difference experiment was employed to determine temperature-dependence of the rate of interconversion of the substituted compound which is depicted on the left in Figure 1. At 20°C the rate of rotamer exchange was determined to be 1/ 1800 sec⁻¹ suggesting the presence of true atropisomerism. Various substitutients were probed to break the observed atropisomerism. Quantum chemical calculations suggested a stabilization of the bio-active conformer by the proper placement of a methyl group at the benzylic carbon – see center in Figure 1. This compound and analogs exhibited FVIIa-activities in the low Nano Molar range. NMR structural calculations confirmed that the dimethyl substituted compound adopt its bio-active, protein bound structure in solution – see right side in Figure 1.

Figure 1. Left: macrocyclic FVIIa-inhibitor scaffold with methyl substitution & the C3-position, center: QM-calculations in a dimethyl substituted analogue, right: super-position of 3d-structures of dimethylated macrocyclic FVIIa-inhibitor.

References
Bridged Epipolythiodiketopiperazines from *Penicillium raciborskii*, an Endophytic Fungus of *Rhododendron tomentosum* Harmaja


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Three new epithiodiketopiperazine natural products [outovirin A (1), outovirin B (2), and outovirin C (3)] resembling the antifungal natural product gliovirin have been identified in extracts of *Penicillium raciborskii*, an endophytic fungus isolated from *Rhododendron tomentosum*. The compounds are unusual for their class in that they possess sulfide bridges between α- and β-carbons rather than the typical α−α bridging. To our knowledge, outovirin A represents the first reported naturally produced epimonothiodiketopiperazine, and antifungal outovirin C is the first reported trisulfide gliovirin-like compound.

We previously screened endophytic fungi isolated from the leaf tissues of this plant, assaying extracts for antimicrobial and antioxidant activity.[2] Three fractions exhibiting antioxidant activity and sufficient yield based on initial LC/MS screening of *Penicillium raciborskii* strain TRT59 extracts were selected for structural elucidation. The principal molecules from each of the three fractions differed from each other only by the number of sulfur atoms. The structures of the three compounds (Figure 1) were determined using multidimensional NMR and MS/MS methods.

STD NMR and Molecular Dynamics Unravel Differences in Recognition of Hyaluronan by CD44 and LYVE-1

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The lymphatic system serves as a major channel for the trafficking of leucocytes during immune surveillance and the dissemination of metastatic tumors. To gain access to the lymphatics, cells must first navigate through the surrounding tissue in a process that involves specific interactions between hyaluronan [HA, (GlcNAcβ1-4GlcAβ1-3)n] in the interstitial matrix and its cell-surface receptor CD44 expressed on the surface of migrating cells. Furthermore, entry to lymphatic capillaries appears to involve a related HA receptor LYVE-1 that is expressed abundantly in lymphatic endothelium. Currently, the details of the LYVE-1/HA binding interaction remain elusive due to the lack of a reliable structural model. Clearly, characterisation of this key interaction would present therapeutic opportunities for a range of disease states, particularly inflammation and cancer.

Here we use a combination of STD NMR spectroscopy and molecular dynamics (MD) to elucidate the structural features of the LYVE-1/HA complex and to account for its distinctive properties that segregate it from CD44. Based on NMR data at 800 MHz we have obtained the binding epitopes for a synthetic HA tetrasaccharide (HA4S) bound to both CD44 and LYVE-1. In each case the HA4S binds in a longitudinal fashion, sharing a key interaction with the N-acetyl group of the central GlcNAc. This is consistent with the published structure of the CD44/HA8 complex, which shows binding of the N-acetyl group to a hydrophobic pocket within a shallow binding groove. Furthermore, STD intensities predicted for the CD44 complex (CORCEMA-ST) show good agreement with the experimental data.

In the case of LYVE-1, homology modelling reveals a binding groove that agrees qualitatively with the ligand binding epitope. However, experimental STD intensities suggest that the ligand protons distal to the binding groove (Fig. 1) make additional contact with the protein in a manner not predicted by the current model. Fascinatingly, molecular dynamics simulations of the LYVE-1 homology model show spontaneous rearrangement of the non-conserved C-terminal region such that protein sidechains contact the distal side of the binding groove, in agreement with the experimental NMR data. This conformation is supported by the fact that this region is required for effective HA-binding, a feature that could not be accounted for in the previous model. Our results underscore the advantage of using MD simulations to improve the interpretation of STD NMR data when the 3D molecular models of the protein-ligand complex are based on homology build proteins.
Inhibitor Studies of ε-Trimethyllysine Hydroxylase by Means of NMR and Computer Modelling

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ε-Trimethyllysine hydroxylase (TMLH) is a homodimer enzyme located in the submitochondrial matrix. TMLH is a ferrous iron and 2-ketoglutarate dependent oxygenase that catalyze the stereospecific oxidation of ε-trimethyllysine (TML) to β-hydroxy-TML (HTML) that is a first step in the L-carnitine biosynthesis pathway. The inhibition of the TMLH is proposed to have the similar and more potent effect on the energy metabolism processes than meldonium (clinically used anti-ischemia drug). Thus, it will become possible to reduce significantly the concentrations of the L-carnitine and to shift the energy metabolism from the β-oxidation of long-chain fatty acids to glycolysis.

The potential TMLH inhibitors (originally synthesized in the Latvian Institute of Organic Synthesis) were tested in the enzymatic assay that was monitored by means of NMR. The results reveal that the majority of newly synthesized compounds (both active and inactive ones) behave like competitive substrates and undergo enzymatic reaction. Afterwards the same compounds were verified in the three NMR binding experiments: T1ρ, waterLOGSY and ST1D. Spectral data revealed that the binders and non-binders are the same as it was in the enzymatic assay. Overall, the binding effects observed were moderate but still sufficient to identify the pharmacophores responsible for binding.

The same compounds were used in the receptor-based ligand docking for prediction of the possible ligand binding modes. As the TMLH experimental structure is not available, the homology model was build based on the closest homolog – γ-butyrobetaine hydroxylase (GBBH). Due to low sequence similarity (28%), the modeled enzyme structure was relaxed by means of molecular dynamic simulations. As the sequence similarity for the active sites of TMLH and GBBH is in a better agreement than overall structure (similarity 75%), the resulting homology model still allowed to model reliable active site that afterwards was used as a receptor for several molecular docking series.

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Exploring the use of Generalized Indirect Covariance to reconstruct pure shift NMR spectra

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The development of novel experimental strategies to significantly enhance signal resolution by broadband homodecoupling is a current topic of high interest in 1H NMR spectroscopy1,2,3. The original Zangger-Sterk experiment has been modified and improved in several ways. All of these novel building blocks have been implemented in a number of 1D and 2D homo- and heteronuclear pulse schemes to provide resolution-enhanced pure chemical shift 1H NMR spectra, where signals appear collapsed to singlet. On the other hand, covariance processing methods have been used to generate challenging NMR spectral representations4. We present here the first attempts towards a general solution to generate Pure Shift NMR spectra by using Generalized Indirect Covariance (psGIC). The current strategy is based on the calculation of a new 2D psGIC spectrum from the combination of a parent homo- or heteronuclear spectrum and a reference 2D F1-homodecoupled 1H-1H correlation spectrum only showing diagonal cross-peaks (DIAG), which share a common 1H frequency dimension. Using psGIC, the F1 dimension in the DIAG spectrum can be transferred to the F2 dimension of the parent spectrum, thus generating a new pure shift 2D spectrum. Examples are provided for a set of 2D NMR spectra of the alkaloid strychnine.

Figure 1. Generation of Pure Shift NMR spectra by using Generalized Indirect Covariance (psGIC).

Hydration/dehydration processes in molecular crystal of (+)-catechin

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(+)-Catechin, a flavan-3-ols exerting strong antioxidant, as well as antiviral and antimutagenic properties, for years has been elusive for crystallographic studies. Only recently a synchrotron XRD and solid-state NMR combining studies have revealed the crystallographic structure of 4.5-hydrate (Figure 1). The hydrate possesses continuous water channels and, as a consequence, is very prone to dehydration resulting in the less hydrated forms of (+)-catechin. Here, we employ solid-state NMR to describe the dehydration and rehydration process of (+)-catechin. The process is completely reversible and easy to manipulate by varying relative humidity. Our studies reveal that the dehydration of 4.5-hydrate (form I) leaves the crystal structure of catechin intact, until water loss exceeds 1.5 molecules. After this partial dehydration form II, displaying different from form I $^{13}$C solid-state NMR spectrum, starts to emerge (Figure 2). The experimental results together with computations, employing Crystal Structure Prediction (CSP) and gauge-including projector-augmented wave density functional theory (GIPAW-DFT), led us to a conclusion that this partially dehydrated form of (+)-catechin contains 2 or 2.5 water molecules, but, similarly like in form I, crystal structure of form II is preserved until there is less than one water per catechin. Then, after further dehydration, the amorphous phase (form III) occurs.

According to the $^1$H-$^{13}$C FSLG-HETCOR and 2D-PASS experiments the dehydration affects the most hydrogen-bonding network around C-5 and the $^2$H NMR spectra and their simulations revealed different nature of water remaining after dehydration in the amorphous form III. Finally, the computational prediction of partially dehydrated (+)-catechin, verified by the experimental $^1$H chemical shifts and the components of $^{13}$C chemical shielding tensors resulted in the appointment of two similar probable structures of form II containing 2 and 2.5 water molecules per catechin.

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Screening and hit validation with 1D NMR

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The use of NMR for screening and hit evaluation in the lead discovery program at AstraZeneca in Gothenburg is presented through a set of examples, viz. (1) Fragment screening of mixtures using relaxation filtered experiments and dual solvent suppression; (2) Ligand affinity determination using a reporter ligand; (3) ATP binding competence of a kinase protein preparation; (4) The determination of affinity of two weak ligands via mutual competition experiments; (5) The identification of redox active compounds, eliminating them as false positive hits in the HTS hitlist; (6) The determination of solubility of compounds under protein target crystallization conditions. Also an overview of the role of NMR and other biophysical techniques in the hit finding process is given.
Thermal Solvent-Free Method of Loading of Active Pharmaceutical Ingredients into the Pores of Mesoporous Silica Particles – Solid State NMR Studies

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Developing the innovative methods for drug transportation in the body and increasing the therapeutic efficiency of new and old generation drugs with different physicochemical properties is one of the biggest challenges for pharmaceutical sciences. Both subjects (transportation and efficiency) can be greatly improved by introducing of strategy based on application of modern Drug Delivery Systems (DDSs). Today, the field of DDSs is growing very fast and became one of the most profitable approaches in pharmaceutical industry. The majority of DDSs are based on biological or inorganic/organic components.[1] To the latter group belong Mesoporous Silica Particles (MSPs) which were recently approved as drug carriers by Food and Drug Administration (FDA). The attractiveness and usefulness of MSPs as DDSs is due to their unique geometrical features, high surface area, large pore size and large pore volume that can be adapted to the specific needs.

In this talk we present our recent achievements related with study of ibuprofen embedded into MCM-41 (Mobil Crystalline Material 41). [2] We compared two methods (incipient wetness and thermal solvent free (TSF)) for the encapsulation of ibuprofen in the pores of MCM-41 through NMR (nuclear magnetic resonance) spectroscopy. We employed advanced NMR techniques for evaluation of the encapsulation methods included an analysis of the filling factor of the drug into the pores. The stability of Ibu/MCM in an environment of ethanol or water vapor was tested. Our study showed that TSF method for mixture of Ibu and MCM is a much more efficient approach of confining the drug in the pores compared to incipient wetness. The optimal experiments for the former method achieved a filling factor of approximately 60%.

In the second part of the talk we present the applicability of TSF method for confining of more complex systems into MSPs. As a model guest sample we employed cocrystal formed by benzoic acid (BA) and is penta-fluorinated analog (FBA). BA/FBA cocrystal and BA/FBA:MSN assemblies are fully characterized by \(^1\)H, \(^{19}\)F and \(^{13}\)C NMR experiments. [3] Finally, we show how to trap naproxen into MSN employing TSF approach [4].

References


Hydrosilation induced by N→Si intramolecular coordination.
NMR controlling reaction products.

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Our attempts to synthesize N→Si intramolecularly coordinated organosilanes 1a - 4a yielded aza-2-silaindols 1 - 4, respectively. Isolated organosilicon compounds 1 - 4 are an outcome of the spontaneous hydrosilation of the CH=N imine moiety induced by N→Si intramolecular coordination.

Compounds 1 - 4 were characterized by their 1H, 13C, 15N and 29Si NMR spectra and X-ray diffraction analysis [1, 2].

Mosher acid was added to a mixture of two enantiomers. After several hours, 1:1 signals of appropriate diastereoisomers appeared. This approach is planned to be used as an analytical tool in case of asymmetric synthesis of aza-2-silaindols.

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Q.E.COSY: Sign and Size of Small Deuterium Residual Quadrupolar Couplings based on extended E.COSY principle

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Residual quadrupolar couplings contain important structural information comparable to residual dipolar couplings. However, the measurement of sign and size of especially small residual quadrupolar couplings is difficult. Here, we present an extension of the E.COSY principle to spin systems consisting of a spin 1 coupled to a spin ½ nucleus, that allows the determination of sign of the quadrupolar coupling of the spin 1 nucleus relative to the heteronuclear coupling between the spins. The so-called Q.E.COSY approach [1] is demonstrated with its sign-sensitivity using variable angle NMR, stretched gels, and liquid crystalline phases applied to various CD and CD₃ groups. The sign-sensitive measurement of residual quadrupolar couplings that remain unresolved in conventional deuterium 1D spectra is particularly beneficial with the approach (Figure 1).


Figure 1. Q.E.COSY spectrum of Alanine-d₃.
Self-assembling hydrogels. Balancing on the edge of solid and liquid state
NMR spectroscopies

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Supramolecular hydrogels based on low molecular weight gelators (LMWG) behave as natural hydrogels made of microtubulins, actins and intermediate filaments. They mimic dynamics and structure of natural systems and are working models for investigating unique macroscopic properties of the cell. The in-depth understanding of the arrangement and mobility of the hydrogel molecules provides an opportunity to utilize their peculiarities in development of new generation of synthetic materials – self-healing and adaptive materials, drug-delivery machines, etc. Thus, LMWG-gels are of considerable interest for modern pharmaceutics, chemistry, and industry; and comprehensive analysis and description of these systems is in high demand.

However, LMWG hydrogels are not a trivial system to investigate by NMR; the interplay between intense dynamics and extremely low concentrations poses several problems – liquid-state NMR senses free molecules, while the molecules constituting a network stay NMR-silent; solid-state ¹H-NMR provide spectra with very broad and featureless lines and ¹³C spectroscopy suffers from a lack of sensitivity (see fig. 1).

In the current work we combine diverse NMR approaches to overcome these challenges and provide an insight in the gelation mechanism of the self-assembling system – OG2, which is targeted as a stimuli-sensitive drug-delivery machine. We use 1D and 2D liquid state NMR spectroscopy to characterize structure of molecules in the pre-gelation state. Double-quantum based solid-state spectroscopy is applied to reveal and estimate dipolar couplings retaining LMWG within the network. Self-diffusion coefficient measurements of the probe molecule doped into the gel-matrix supply information about pore size, shape and features. Relaxation time dispersion allows obtaining data on liquid-state invisible states.

Combining these different techniques we hope to fill the gaps in the understanding of the gelation processes and promote the most efficient design of the novel “smart” materials.

Figure 1. OG2 ¹³C CPMAS spectrum (Acquisition time is 3 days)  Figure 2. Schematic representation of the functional mechanism
In the last years, a new generation of pulse sequences based on the concept of a spatial frequency encoding (SFE) of the sample has emerged in the field of ultra-high resolution NMR. On the one hand, a considerable effort has been devoted to the implementation of pure shift sequences to acquire spectra along which \(^1\)H signals appear as singlets.\(^{[1]}\) On the other hand, it has been shown that it is also possible to generate along the sample a series of selective spin echoes allowing for the complete and fully resolved edition of the scalar couplings involving a selected proton site.\(^{[2,3]}\)

We will present here an original theoretical formalism to describe the key features of SFE NMR spectroscopy. We will show that it becomes possible to optimize both the sensitivity and the spatial resolution of pure shift and J-edited experiments to a point where their performance is at least comparable to that of competing methods.\(^{[4]}\)

An application of this analytical tool to the accurate determination of enantiomeric excesses in mixtures of enantiomers dissolved in a chiral liquid crystalline solvent will finally be presented.\(^{[5,6]}\)

Slice-selective one- and two-dimensional experiments to study concentration gradients in agar gels

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Agar gels are quite attractive media to study aqueous solution in 5-mm NMR tubes. They are easy and quick to obtain, show very low residual signals, avoid problems due to solvent convection and measurements can be made up to -10 C.

When adding a titrant solution above an agar gel containing a solution to titrate, a gradient of concentration can be generated and studied using slice-selective experiments. This makes it possible to determine weak-molecular interactions such as the affinity of β-cyclodextrin for a guest compounds.[1]

![Figure 1. The variation of chemical shifts combined with signal integration makes it possible to determine the binding constant of β-cyclodextrin for paracetamol (from ref. [1]).](image)

We present a general method to transform one- and two-dimensional NMR experiments into slice-selective experiments. It includes a protocol to solve the problem of the phase difference between hard pulses and soft-imaging pulses making it possible to fully automatize the acquisition of series of 1D and 2D spectra.

Intramolecular and Intermolecular Chemical Exchange Processes in Small Molecules studied by STD NMR and the novel “spin-STD” (SSTD) NMR

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Chemical exchange underlies very relevant bio- and physicochemical processes in which small molecules participate (e.g. drug-receptor interactions, conformational rearrangements, etc.). In the simplest case, a small molecule is in equilibrium between two different states (two-sites chemical exchange). In NMR we can monitor the motion of the nuclei of the small molecule from one environment to another, making accessible the chemical kinetics of the system (e.g. on and off rate constants). Fast chemical exchange (at least in the NMR relaxation time scale) is particularly interesting for NMR spectroscopy as it allows the observation of properties “transferred” from one molecular state to another (so-called NMR transfer techniques). In the particular case of saturation transfer experiments, we can detect the transfer of polarization from one, selectively, perturbed state to the other, unperturbed, one.

Saturation transfer difference spectroscopy (STD NMR) is a powerful technique for the structural and kinetics studies of intermolecular protein-small molecule interactions, and we have used to study relevant protein-ligand systems. What is more, we have developed a novel approach (spin saturation transfer difference NMR (SSTD) NMR, Figure 1) to obtain the kinetics parameters of uni-/intra-molecular chemical exchange processes in challenging small organic and organometallic molecules [1].

In particular, here we show first our results from STD NMR to characterize the complexes of novel inhibitors of Cholera toxin B (CTB), and provide evidences on how STD NMR is a straightforward tool to verify the proposed “three finger pharmacophore” requirement for the inhibition of the cancer related protein-protein interaction between p53 and MDM2 [2]. On the other hand, we demonstrate for the first time how a novel STD NMR based approach (SSTD NMR), is a powerful methodology to obtain kinetics parameters in intramolecular processes, exemplified on some organic and organometallic small molecules.

References
Structural Revision of Pyrone-related Natural Products by Using CAST/CNMR System

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The CAST/CNMR system is composed of two functions for prediction of 13C NMR chemical shift values from a query chemical structure (CAST/CNMR Shift Predictor) and elucidation of chemical structures from query 13C NMR chemical shift values (CAST/CNMR Structure Elucidator). These functions work using a database consisting of reported/observed 13C NMR chemical shift values associated with their chemical structures in 3D. These structures are described using the CAST (CAnonical-representation of STreochemistry) coding method. Those two functions are complementarily used in the study of chemical structure determination and chemical shift assignments. In the development of the database, we occasionally add our originally assigned data but mostly register reported data from peer-reviewed journals. However, even in such authorized journals, wrong signal assignments or mistake in structure characterization are sometimes reported. Wrong structures are often found particularly for recently reported new compounds.

We have applied the CAST/CNMR system to find and correct such wrong assignments or structures. One can locate wrong assignments of 13C signals by comparing their reported data with chemical shift values predicted using CAST/CNMR Shift Predictor. CAST/CNMR Structure Elucidator suggests other possible structures, which can help get ideas to revise structures. Those error data have been carefully corrected or removed from the database so that the accuracy of the CAST/CNMR system has been improves. We previously reported structural revision of several natural products such as brominated sesquiterpenoids aldingenins C and D.3 Here, we will present structural revision for a pyrone-related class of natural products. For example, we have revised the structure of 5-hydroxymethyl-furan-3-carboxylic acid, named flufuran4,5 to a well-known kojic acid, i.e. 5-hydroxy-2-hydroxymethyl-4H-pyran-4-one, based on the identical reported NMR data.6 We have found that reported NMR data for 5-hydroxy-4-hydroxymethyl-2H-pyran-2-one7 were also identical to those of kojic acid. We will demonstrate the structural revision of these compounds as well as other pyrone-related compounds and also other classes of natural products.

References
The polycyclic aromatic compounds attract scientific attention namely due to their conjugated π-π aromatic systems which promise many interesting and unusual optical and electronic properties. Some of these compounds were shown to be effective in the development of materials useful e.g. in molecular-based electronics.[1]

The electronic structure of these compounds is studied mainly by theoretical approaches.[2] The theoretical results can be correlated to some experimentally accessible parameters. The $^1J(^{13}C-^{13}C)$ values can provide an interesting insight into electron distribution in the target molecule. $^1J(^{13}C-^{13}C)$ values and $^2J(^{13}C-^{13}C)$ values and signs are sensitive to the nature of the substituent in the vicinity. The $^3J(^{13}C-^{13}C)$ values correspond well with the values of aromaticity. These values are accessible via number of experiments derived from original INADEQUATE experiment. The measurement was done on two series of substituted phenanthrenes. Furthermore, we would like to present new 2D $J$-resolved refocused INADEQUATE experiment which can observe also $^4J(^{13}C-^{13}C)$ couplings between two equivalent nuclei. The technique will be demonstrated on few basic molecules (e.g. benzene).

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References

INVESTIGATION OF HEPARIN-ANALOGUE PENTASACCHARIDES-ANTITHROMBIN-III INTERACTION WITH NMR AND MOLECULAR DYNAMIC METHODS

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Heparin, isolated from natural sources, has been used as an antithrombotic agent in clinical practice since 1937. Since its administration is associated with rare serious side effects, several research groups are making efforts to synthesize heparin-analogue oligosaccharides displaying better pharmacological properties than heparin.

During our research we studied the structure and dynamics of novel heparin-analogue pentasaccharides containing two or three sulfonatomethyl groups at specific positions and their interaction with antithrombin III (AT-III) using a combined NMR experimental–MD computational approach.

Although the chemical composition of these analogues is almost the same, the in vitro anticoagulation studies revealed significant differences in their biological activities. Specifically, the trisulfonate analogue exhibited markedly lower activity than the two other ones.

In order to identify the structural and/or dynamic factors behind the biological profile, the conformation along with the conformational flexibility have been investigated in both the free and the antithrombin-bound forms of the three pentasaccharides. Pleasingly, the conformationally relevant proton-proton distances and torsion angles assessed by the NMR and MD approaches were generally in good agreement. Noticeably, the three analogues in complex with AT-III displayed conformations alike, on the contrary, the free forms exhibited significant differences in their conformations and conformational flexibility, which might explain their different biological activities. To characterize the stoichiometry of pentasaccharide-AT-III complexes and the thermodynamics of their interactions, ITC measurements have been also performed.

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Cross-linked Poly(ethylene glycol) Diacrylate Gel – A Medium for Aligning Solutes in a Broad Range of Solvents

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Residual dipolar couplings (RDCs) have proven to provide valuable structural information for biomacromolecules [1] as well as for small organic molecules [2]. For their measurement appropriate weak partial alignment in so-called alignment media is necessary to induce the suitable anisotropy. Two types of alignment media, liquid crystalline phases and mechanically strained polymer gels, are commonly used to partially align solutes. However, both are typically restricted to certain solvents.

Earlier investigations with swollen cross-linked poly(ethylene glycol) hydrogel (PEG) have shown that it supports a wide range of solvents [3]. Bisacrylated derivatives of PEG, poly(ethylene glycol) diacrylate (PEG-DA), can easily be cross-linked by radical polymerization to yield homogeneous gels. They show considerable swelling in a various solvent range from polar to apolar solvents. Here, we present the applicability of chemically cross-linked PEG-DA to align solutes in water, chloroform and dimethylsulfoxide as the three most widely used NMR solvents.

Figure 1. Gel sticks in equilibrium in various solvents and comparison of the swollen volume relative to their initial dry state

Figure 2. CLIP-HSQC[4] of sucrose with 1DCH RDCs in stretched PEG-DA hydrogel in D2O ($\Delta v_Q$: 6Hz) and 2H-Image[5].

As far as enantiomeric discrimination of 2-alkyl-5-methyl-2-pyrroline goes

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From the analytical point of view, 1H NMR spectra have all required data for fast identification and quantitation, however enantiomeric discrimination is limited to the use of chiral solvating agents (CSA). The enantiomeric excesses have to deal with the superposition of multiplets due to scalar spin-spin couplings, which can be solved by transforming them into singlets by homodecoupling during the acquisition time (pure shift methodologies). Therefore, this work aims at the enantiomeric discrimination and enantiomeric excess determination of 2-alkyl-5-methyl-2-pyrrolines with scarce literature data on their enantiomeric separation. In this work, we evaluated the enantiomeric separation of 2-hexyl-5-methyl-2-pyrroline (1, Figure 1), which was synthesized by enzymatic transamination/cyclization of 2,5-undecadione. Three 1H NMR techniques were applied to study the enantiomeric discrimination of this pyrroline promoted by (S)-binol as chiral solvating agent (CSA): regular 1H experiment, PSYCHE1 and HOBS2. Figure 1 shows the results focusing on the signals of the H-5 in structure 1. Spectrum (a) was recorded in a 600 MHz NMR equipment. However, spectra (b) and (c) were recorded in a 400 MHz equipment, where the multiplets resolution were not sufficient to integrate the signals of interest. In this case, we applied PSYCHE (b) and HOBS (c) pure shifts methodologies, which showed consistent results (enantiomeric excess about 40-42%) when compared to 1H experiment at 600 MHz (42%). These methodologies were validated by capillary electrophoresis using β-cyclodextrin sulfate as CSA, the enantiomeric excess of 42% was consistent with the NMR. Therefore, these results attested that 2-alkyl-5-methyl-2-pyrrolines could be enantiomerically discriminated by using CSA, such as (S)-binol and β-cyclodextrin sulfate, and that this discrimination was successfully evaluated by 1H NMR techniques and capillary electrophoresis.

References
Studying the conformational ensemble of b^3/b^2-peptides using ROEs, J-couplings and RDCs

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NMR spectroscopy is the method of choice for determination of the three-dimensional structure of molecules in solution. Commonly, it is assumed that a single dominant molecular conformation in solution can represent all the experimental NMR data. However, molecules are constantly subjected to conformational changes in solution and representing the conformational ensemble as single structure can lead to over-restraining and thus to misinterpretation of the available data. Efforts to overcome this problem have mainly been focused on large biomolecules. For small and medium-sized molecules the small density of available restraints still renders a full description of the conformational ensemble difficult.

We have studied the solution-structure of the mixed b^3/b^2-peptides 1a and 1b in detail. It is known that b^3/b^2-peptides can exhibit antimicrobial activity, and only recently they were found to penetrate the lipid bilayer of eukaryotic cells [1]. Earlier studies suggested that a 12/10 helix is the dominant conformation of the terminally protected b^3/b^2-nonapeptide 1a in methanol. Deprotection (1b) is believed to lead to an equilibrium between a 10/12 and a 3_14 helix [2].

To investigate this hypothesis in more detail we have used an extended set of experimentally derived restraints, including RDCs, and a multi-copy simulated annealing procedure. The RDCs were measured using a stretched polyvinyl acetate gel in methanol. For comparison the structures were also calculated with the common single conformation procedure. The structures resulting from the two different methods are discussed.

Computer-Assisted Structure Elucidation of Two Isomeric, Highly Symmetrical Helical Molecules

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Two isomeric, highly-symmetrical helical molecules were synthesized by Rh(I)-catalysed cycloisomerisation of a suitable triyne substrate. The isomers were separated by preparative scale liquid chromatography before 10 mg of each isomer was dissolved with deuterated chloroform and placed in an NMR tube in order to confirm their structures. Here we describe the advanced computation method used for confirming the structures by NMR experiments only.

Due to the presence of molecular symmetry the NMR spectra of the resulting compounds exhibited very few peaks relative to the size of the molecule. That is, half the number of expected signals are observed in the 13C NMR spectrum and, in addition, several of the signals are extremely closely spaced (see Figure 1). Moreover the deficient hydrogen content of the compounds resulted in a low number of correlations in the 1H-13C HMBC spectra.

Computer Assisted Structure Elucidation (CASE) together with band-selective versions of the HSQC and HMBC experiments were employed to determine the structures and assign unequivocally the NMR data and thus confirm the new molecules. The final assignment of the two isomeric structures was accomplished by using 1H-1H 2D NOESY experiments.

Figure 1. Expanded region of 13C NMR (left), conventional (top right) and band-selective (bottom right) 1H-13C HMBC spectra.
An experiment for the measurement of $^{19}$F-$^{15}$N coupling constants at natural abundance and in the presence of $^1$H couplings

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Of the ca. 100 $^{19}$F-$^{15}$N coupling constants measured so far, only one was measured in an indirect detection experiment (HMBC) and that was for a compound void of protons. All of the other measurements were made in $^{15}$N spectra, at natural abundance or on $^{15}$N labeled compounds, using $^1$H broadband decoupling. In a couple of cases, the sensitivity was increased 10 fold using polarization transfer from protons.

We propose the experiment in figure 1 for the measurement of $^{19}$F-$^{15}$N coupling constants at natural abundance. This is an indirect detection experiment with polarization transfer from $^{19}$F, which in principle can provide an increase in the signal to noise ratio of up to 263 times compared to $^{15}$N spectra. The coupling constants are measured in $\delta$, as the difference between the $^{19}$F spectra obtained for the alpha and beta states of the $^{15}$N nuclei, by inverting the phase of the last 90° pulse on the decoupler. For the nucleus caring the magnetization of interest, the sequence is a succession of spin-echoes, which refocuses the $^1$H couplings, as seen in figure 2.

![Figure 1. Pulse sequence for the spin-state selective HSQC with refocusing of the $^1$H couplings. The filled pulses are 90°, the white ones are 180°. The phase of the pulses is x, unless specified otherwise. A two-step phase cycle was used, in which the first 90° decoupler pulse and the receiver are inverted on alternate scans.](image1)

![Figure 2. Spin-state selective HSQC spectra of 3-fluoropyridine taken with the pulse sequence in Figure 1. The $^3$JFN is measured as the difference between the two spectra, 4.5 Hz.](image2)
Rational Design of α-helical Antimicrobial Peptides Aided by Paramagnetic Relaxation Enhancement

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Antimicrobial Peptides (AMPs) hold great promise as future weapons against multi-drug-resistant pathogenic bacteria. The structure-activity relationships of these peptides are still not understood. Structural data on peptides are scarce, and the data usually do not take the orientation of the peptide in the cell membrane into account. We have developed a method for the simultaneous determination of membrane insertion and structure of antimicrobial peptides embedded in lipids.

Using micelles as model systems for cell membranes, and using paramagnetic environment relaxation enhancement, we investigated a series of derivatives of anoplin, a small, α-helical AMP. By comparing antimicrobial and hemolytic activity with PRE-derived data on the membrane insertion of the peptide variants, we suggest a simple set of rules for the improvement and design of α-helical AMPs.

Figure 1. Structure of anoplin embedded in a DPC micelle (transparent, grey sphere, phosphate head groups in yellow and red).
There is still a huge interest in chemical compounds found in plants that have a biological and/or pharmacological activity. A lot of medically important plants come from the South America. These from the Amazon Rainforest are used everywhere from medicine to food industry. More studies on plants used by native people are needed. The example of such a plant is *Geissospermum reticulatum* (Apocynaceae) which is believed to have anti-malarial, anti-cancer and nociceptive properties. It is widely regarded that these effects in most plants are exhibited due to occurrence of alkaloids.

In this work we report the HPLC, MS and NMR studies on the alkaloid fractions of *Geissospermum reticulatum* bark.

Seven samples of barks we collected in distinct localities of Peruvian Amazonia. The alkaloid fractions were prepared using a 3-step extraction with methanol and ethyl acetate. The HPLC (High Performance Liquid Chromatography) experiments were performed to elucidate the purity of alkaloids isolation from the crude extracts. These purified alkaloid fractions were then subject to MS (Mass Spectrometry) and NMR (Nuclear Magnetic Resonance) studies. The MS and NMR (1H, 13C, DEPT, HMBC, HSQC using Bruker Avance III instrument, 500 MHz) spectra were analysed to identify alkaloids.

The aforementioned methods were successfully applied in the present work to report two previously not described alkaloids from *Geissospermum reticulatum* bark. Further studies on characterization of these compounds are undergoing.
Crystal structure and tautomerism of Pigment Yellow 138 determined by X-ray powder diffraction and solid-state NMR

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Pigment Yellow 138 (P.Y. 138) is a commercial greenish-yellow pigment based on quinophthalone. It exhibits three possible tautomeric forms, which denoted as CH-form (1a), OH-form (1b) and NH-form (1c), Figure 1.

Due to the lack of good crystals for the single crystal X-ray diffraction analysis, the crystal structure of P.Y. 138 was determined by combining X-ray powder diffraction data (using real-space methods with subsequent Rietveld refinements) solid-state NMR and computational data. The tautomeric state was investigated by solid-state 1D and 2D multinuclear NMR experiments.

In the crystals, the compound exhibits the NH-tautomer with a hydrogen atom situated at the nitrogen of the quinoline moiety. Direct evidence of the presence of the NH-tautomer is provided by 1H-14N HMQC solid-state NMR at very fast MAS (70 kHz). Solid-state dispersion-corrected density functional theory calculations with BLYP-D3 confirm the correctness of the crystal structure and support the NH-tautomer. The NH hydrogen atom forms an intramolecular resonance-assisted N-H•••O hydrogen bond to the neighbouring indandione moiety.

Figure 1. Possible tautomeric forms of P.Y. 138.

Selecting the Most Appropriate NMR Experiment to Access Weak and/or Very Long-Range Heteronuclear Correlations

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Heteronuclear long-range NMR experiments are well established as essential NMR techniques for the structure elucidation of unknown natural products and small molecules. It is generally accepted that the absence of a given $^2$J$_{CH}$ correlation in an HMBC or HSQMBC spectra, would automatically place the proton at least four bonds away from the carbon in question. This assumption can, however, be misleading in the case of a mismatch between the actual coupling constant and the delay used to optimize the experiment, which can lead to structural misassignments. Another scenario arises when an investigator, for whatever reason, needs to have access to very long-range correlations to confirm or refute a structure. In such cases, a conventional HMBC experiment will most likely fail to provide the requisite correlation, regardless of the delay optimization. Two recent methods for visualizing extremely weak or very long-range connectivities are the LR-HSQMBC¹ and the HSQMBC-TOCSY² experiments. Although they are intended to provide similar structural information they utilize different transfer mechanisms, which differentiates the experiments making each better suited for specific classes of compounds. Here we have sought to examine the considerations implicit in choosing the best experiment to access weak or very long-range correlations for different types of molecules.

<table>
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<tr>
<th>Accessing weak and/or very long-range heteronuclear correlations</th>
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<tr>
<td><strong>HSQMBC-TOCSY</strong></td>
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Optimizing \( J \)-modulated ADEQUATE experiments through homonuclear decoupling (HD) and non-uniform sampling (NUS)

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Homonuclear \(^{13}\text{C}-^{13}\text{C} \) couplings at natural abundance can be measured using the \( J \)-modulated ADEQUATE experiment. To somewhat ameliorate F1 digitization requirements, a scaling factor was incorporated into the original ADEQUATE pulse sequence\(^1\,^2\). We have introduced BIRD-based homonuclear decoupling (HD) analogous to that described for the 1,1-HD-ADEQUATE and 1,n-HD-ADEQUATE experiments\(^3\) and evaluated the combination of NUS and HD on the measurement of both \( ^1J_{CC} \) and \( ^nJ_{CC} \) homonuclear \(^{13}\text{C}-^{13}\text{C} \) coupling constants. A significant improvement in the measurement of \( ^nJ_{CC} \) coupling constants, and time savings, were realized with the modified experiment.

\( \text{J-mod-1,1-ADEQUATE} \)

Abstracts for posters in session "Solid-state NMR techniques"
Signal Enhancement by Multiple-Contact Cross-Polarization under Magic-Angle Spinning

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Hartmann-Hahn (HH) cross-polarization (CP) of rare spins with a low gyromagnetic ratio such as $^{13}\text{C}$ and $^{15}\text{N}$ from abundant spins with a higher gyromagnetic ratio (e.g., $^1\text{H}$) is a popular way of obtaining high sensitivity NMR spectra of solids. In particular, the multiple-contact (MC) CP technique\(^1,2\) (Figure 1) has been shown recently to yield quantitative NMR spectra with a significant gain in the signal-to-noise ratio as compared to single-contact HHCP and state-of-the-art techniques such as adiabatic passage through the HH condition CP (APHH-CP)\(^3,8\). In this work, we show that the MC-CP technique requiring no pulse-shape optimization provides higher signal enhancements than optimized APHH-CP for all types of protonated carbons when the magic-angle spinning (MAS) frequency is comparable to the heteronuclear dipolar coupling(s), i.e., when APHH-CP through a single sideband matching condition is impossible or difficult to perform. Moreover, the MC-CP matching conditions under fast MAS are found to be much broader than the single-contact HHCP matching conditions over the whole range of contact times so that MC-CP is much less sensitive to radiofrequency (RF) field inhomogeneity than single-contact HHCP. It is then concluded that MC-CP is a simple and robust method applicable to systems characterized by a short spin-lattice relaxation time in the rotating frame ($T_{1\rho}$).

References:

\( \text{Figure 1. The MC-CP pulse sequence.} \)
New Approaches for $^1$H-detection in complex Membrane Proteins

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$^1$H-detection can much enhance spectral sensitivity in biological solid-state NMR. However, the general requirement to perdeuterate proteins critically curtails the potential of $^1$H-detection by the loss of non-exchangeable $^1$H. This is particularly severe for membrane proteins, which only partly back-exchange and hence can only be studied in fragments. We will present two complementary approaches, which allow $^1$H-detecting entire membrane proteins at high resolution and in complex, heterogeneous membrane.

We introduce a labelling scheme for $^1$H-detected ssNMR, dubbed fractional deuteration, which gives high-quality spectra for both exchangeable backbone and non-exchangeable side chain $^1$H (upper Figure), and allows quantitative assignments and aids in probing interresidual contacts.\(^1\) This labelling scheme provides an excellent $^1$H-resolution in membrane proteins, which we use to study membrane topology, dynamics, and functionally important protein-water interactions of an ion channel.

Second we show a novel approach to $^1$H-detect water-inaccessible protein regions. This labelling scheme provides excellent spectra of the lipid-shielded parts of membrane proteins without relying on tedious and not generally applicable unfolding-refolding protocols (lower Figure). We will present this new approach on model proteins and apply it to the ion channel KcsA, a well-accepted model for channel gating, for which we show assignments along with topological and dynamical studies. We believe these new methods will substantially expand the applicability of $^1$H-detected ssNMR on membrane proteins.

\(^1\) Mance, D. et al., Angew. Chem, 2015, 54
Magic-angle spinning up to 130 kHz: Coherence lifetimes, linewidths, polarization-transfers, and assignment strategies for proteins

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Recent developments in probe technology have made magic-angle spinning (MAS) frequencies up to 130 kHz available. At high spinning frequencies, the resolution in the proton spectral dimension is sufficient to enable sensitive proton-detected experiments for deuterated and fully back-exchanged proteins [1,2,3], as well as for fully protonated samples. Our goal is to systematically assess possibilities with faster MAS and to find the most efficient experimental schemes for resonance assignment - the first step towards structure determination and site-specific analysis of dynamics - in this new regime.

Linewidths and coherence lifetimes ($T_2$' relaxation times) are important parameters not only to quantify spectral quality but also to allow predictions for even higher MAS frequencies. We compare the line widths and coherences lifetimes of model proteins at different spinning frequencies and investigate how MAS influences the decay time of coherences. In a site-specific analysis, we furthermore show how secondary structure elements of proteins can play a role.

While $T_2$' relaxation times govern the efficiency of scalar-coupling based transfers, the lifetimes of spin-locked magnetization affect the dipolar-coupling based transfers and are also dependent on the MAS frequency. In order to find the most efficient way to transfer magnetization we quantitatively assess each polarization-transfer step needed for typical backbone assignment spectra at 93 kHz MAS and compare $J$-coupling based transfers with dipolar-coupling based ones.

With the insights gained from this analysis, we propose a set of efficient triple-resonance 3D experiments for backbone assignment and demonstrate them on the resonance assignment of ubiquitin[5]. However, the performance of the sequences in different structural and dynamical elements in ubiquitin allow us to infer suitable choices for other proteins in the future as well.

Figure 1: 100% back-ex. UL-[1H,13C,15N](HET-s(218-289)) at 850 MHz.

Figure 2: Assignment map for deuterated and 100% back-exchanged proteins at MAS frequencies around 100 kHz.
Optimizing radio-frequency driven recoupling (RFDR) using transient compensation

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Radio-frequency driven recoupling (RFDR) is a widely-used homonuclear dipolar-recoupling technique in solid-state magic-angle spinning (MAS) NMR spectroscopy. The RFDR sequence is commonly used as a building block for multi-dimensional pulse sequences because it is easy to implement and has low requirements on the radio-frequency field amplitude [1,2].

Pulse transients can be a source of severe performance degradation in dipolar recoupling sequences which can be avoided by using phase-transient compensated pulses [3]. We analyze the influence of phase transients on the efficiency of RFDR recoupling and compare the performance of the sequence using hard pulses and phase-transient compensated pulses. Phase-transient compensated pulses lead to broader polarization-transfer conditions and significantly improved efficiencies in model systems, independent of the experimental setup. We extend our findings to proteins by incorporating transient-compensated RFDR recoupling in multi-dimensional pulse sequences. Furthermore, we investigate various phase-cycling schemes typically applied to the \( \pi \)-pulse train (XY-4, XY-8, XY-4,1) and discuss their benefits and drawbacks in the presence of phase-transients as well as their ability to suppress effective fields generated by pulse imperfections. The experimental data will be compared to numerical simulations and analytical calculations taking into account the effective fields generated by the phase transients.

![Fig. 1: A build up curve of a RFDR sequence demonstrating the comparison between compensated and uncompensated pulses as well as the setup dependence of the transfer. The duration of the applied \( \pi \)-pulse was 5 \( \mu \)s at a MAS frequency of 30 kHz. A XY-4 phase cycling scheme was applied and the \( \pi \)-pulses were compensated by predicting the input shape using linear response theory. The transfer condition as well as the efficiency becomes highly predictable with the compensation independent of the setup.](image)

References

Local measure of the electromagnetic field in Magnetic Resonance coils: how simulations are needed to check B₁ measurements?

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The development of probes for Nuclear Magnetic Resonance (NMR) spectroscopy of metabolites, biomolecules or materials requires the accurate determination of the radio-frequency (RF) magnetic field strength, B₁, at the position of the sample since this RF-field strength is related to the signal sensitivity and the excitation bandwidth. The Ball Shift (BS) technique is a commonly employed test bench method to measure the B₁ value. Nevertheless, the influence of the RF electric field on BS is often overlooked. Herein, we derive, from Maxwell equations, an analytical expression of the BS, which includes both the electric and magnetic contributions. This equation shows that the BS allows quantifying the B₁ field strength only in regions where the electric energy is negligible with respect to the magnetic one. Using numerical simulations of the electromagnetic (EM) field and experiments, we show that this condition is fulfilled at 100.5 MHz inside the coil of a double-resonance ¹H/X 4 mm Magic Angle Spinning (MAS) probe. Conversely, NMR experiments show that the contribution of the electric energy to BS becomes significant when the X channel of this probe is connected to a frequency splitter. In that case, BS is less reliable to quantify the B₁ value because the coil is no longer balanced. We also demonstrate the high accuracy of the EM field simulations by comparing their results with experimental ones. Moreover, the simulations also provide additional information about the dependence of the EM field with respect to the position inside the coil ¹.

Figure 1: Density of the Magnetic a) and Electric b) Energies inside the RF coil, where a balanced loading produces a node of electric field at the middle of the sample space. The inner black box delimits the sample space, and the outer one represents the rotor wall.
Multi-technique approach to study dehydration of endomorphin crystals

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Hydrate formation of pharmaceutically important solids is increasingly one of the most prominent concerns of pharmaceutical industry. Often such hydrates easily undergo dehydration processes, which can influence the solubility, stability and bioavailability of a crystal. Therefore, understanding and describing the mechanism of these processes at a molecular level is of crucial meaning during the development of a new drug formulation. On the other hand, following dehydration in the solid matter is not an easy task.

In the current work we present the power of complementary approach combining advanced solid-state NMR and crystal structure prediction calculations to study reversible dehydration in a crystal of EM2-OH, a tetrapeptide, which is a free acid derivative of endogenous endomorphin – the strongest known agonist of the μ-opioid receptor.

EM2-OH crystallizes as a heptahydrate with two independent molecules in an asymmetric part of the unit cell. These two molecules (A and B, Figure 1) exhibit slightly different conformations, mainly due to the differences in the hydrogen bonding pattern with the water molecules. In molecule A the intermolecular hydrogen bond is formed between COO– and NH₃⁺ residues, whereas in molecule B both groups form hydrogen bonds with water molecules. These differences are clearly recognized by ¹³C and ¹⁵N CPMAS experiments (Fig. 1). Upon dehydration the ¹³C and ¹⁵N CPMAS NMR patterns change, but still the differences between molecules A and B can be observed. The NMR data suggested that not all water is removed from the crystal during dehydration. Indeed, the elemental analysis confirmed that after the process, three water molecules remain in the crystal lattice. Basing on the 2D-PASS, inv-HETCOR and CP-VC data, a hypothesis concerning the location of the lingering water molecules was made. To confirm it crystal structure prediction calculations employing GIPAW approach were performed. The computations proved the correctness of the earlier assumptions: two water molecules remain in the pockets made by the formation of the pseudo-cyclic conformations, and one forms a bridge between both EM2-OH molecules.

Figure 1. Comparison of two different conformers of EM2-OH heptahydrate and changes in the ¹⁵N CPMAS spectra of EM2-OH upon dehydration.

¹ The authors are grateful to the Polish National Center of Sciences (NCN) for financial support, Grant No. 2014/13/B/ST4/0350
The β-sheet Core of Recombinant Fibrillar Human IAPP Includes the Central FGAILS Segment

Franziska Weirich [1,2], Lothar Gremer [1,2], Ewa A. Mirecka [2], Stephanie Schiefer [2], Wolfgang Hoyer [1,2], Henrike Heise [1,2]

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Amyloid deposits formed from IAPP are a hallmark of type 2 diabetes mellitus and are known to be cytotoxic to pancreatic β-cells (1, 2). The molecular structure of the fibrillar form of IAPP is subject of intense research, and to date, different models exist (3-5). We present results of solid-state NMR experiments on recombinantly expressed and uniformly 13C, 15N-labeled human IAPP after fibrillation. Complete sequential resonance assignments and resulting constraints on secondary structure are shown. A single set of chemical shifts is found for most residues, which is indicative of a high degree of homogeneity. The core region comprises three to four β-sheets. We find that the central FGAILS segment is part of the core region and forms a β-strand. The eight N-terminal amino acid residues of IAPP, building a ring-like structure due to a disulfide bridge between residues C2 and C7, appear to be well defined but with an increased degree of flexibility as compared to the β-strands. This study supports the elucidation of the mechanistic and structural basis of IAPP amyloid formation by demonstrating that the highly amyloidogenic FGAILS segment can in fact constitute a β-strand in IAPP fibrils.

Ultrafast acquisition of solid state 2D NMR experiments

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Multidimensional (nD) solid-state NMR experiments provide a wealth of information on the structure and dynamics of a large variety of samples, ranging from inorganic materials to biosolids. The acquisition of nD spectra is very time consuming, prohibitively so for samples with a very long longitudinal relaxation time. The “ultrafast” (UF) NMR approach, proposed in 2002 by Frydman and co-workers, provides a dramatic acceleration of nD experiments, as UF 2D spectra can be obtained in a single scan.1 However, UF NMR relies on a spatial encoding of NMR interactions, which is not straightforward to combine with a fast rotation of the sample in magic-angle-spinning (MAS) experiments.2

Here we demonstrate the possibility to record two widely used solid-state 1H-1H 2D NMR experiments in an ultrafast fashion: BABA and RFDR. Using a MAS probe equipped with a microimaging system, and building on our earlier work in HR-MAS4, we show that careful calibration of the alignment of the gradient axis with the rotor axis, together with the synchronisation of the MAS period and the duration of the acquisition gradient, yields good quality spectra in a single scan. As proof-of-concept of the so called UF-BABA and UF-RFDR experiments, spectra were recorded on a sample of natural rubber (Figure 1), using 8 scans in the case of UF-BABA to observe the least intense cross peak. We also show that RFDR build-up curves, which are a central tool in structure determination by solid-state NMR, can be recorded in an ultrafast fashion. Ultrafast solid-state NMR experiment will prove particularly useful for samples that yield a high sensitivity per scan but have a very long longitudinal relaxation time.

Figure 1. UF-BABA (a) and UF-RFDR (b) pulse sequences. Folded UF-BABA (c) and UF-RFDR (d) spectra of a sample of natural rubber.

References:
Low-power broadband solid-state MAS NMR of $^{14}$N

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We propose two broadband pulse schemes for $^{14}$N solid-state magic-angle-spinning (MAS) nuclear magnetic resonance (NMR) that achieve (i) complete population inversion, and (ii) efficient excitation of the double-quantum coherence using low-power single-sideband-selective pulses. We give a comprehensive theoretical description of both schemes using a common framework that is based on the so-called jolting-frame formalism of Caravatti et al. 1, and which has been previously shown to be successful for developing low-power excitation pulse schemes for paramagnetic systems. 2-4. This is a remarkably powerful description that summarizes the complex spin dynamics in a straightforward way, and enables us to design new pulse schemes for different applications.

Firstly we use this formalism to explain the conditions under which we may obtain complete spin population inversion using single-sideband-selective adiabatic pulses ($S^3$APs). In the best cases it is shown that complete inversion of the spinning-sideband manifold is obtained for a $C_Q$ that is more than an order of magnitude greater than the radiofrequency (RF) field amplitude.

We then exploit the full power of the jolting-frame formalism for $^{14}$N to develop a pulse scheme that excites $^{14}$N double-quantum coherences using low-power single-sideband-selective pulses. Excitation efficiencies of 30—50% are obtained using RF field amplitudes that are an order of magnitude lower than the $C_Q$. The scheme easily can be incorporated into other pulse sequences.

These pulses and irradiation schemes are evaluated numerically using simulations on single crystals and full powders, as well experimentally on ammonium oxalate at moderate (20 kHz) MAS, and glycine at ultra-fast (111 kHz) MAS.

Investigation of the Li ion conduction behavior in the solid electrolyte Li$_{10}$GeP$_2$S$_{12}$ by two-dimensional $T_1$–spin alignment echo correlation NMR

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All-solid-state batteries promise a higher cycle life and to be safer than today’s Li-ion battery systems. The biggest challenge to overcome is the relatively slow lithium ion migration in solid-state electrolytes [1]. Li$_{10}$GeP$_2$S$_{12}$ (LGPS) is the fastest known Li-ion conductor to date and therefore an interesting candidate as solid-state electrolyte in Li-ion batteries [2]. This contribution reports on the correlation of the $^7$Li spin lattice relaxation time constants, $T_1$, with the decay rate of the spin alignment echo (SAE), $\tau_c$, [3-5] to link lithium migration mechanisms with different structural features of the LGPS powder sample. For this purpose a pulse sequence with two independently varied evolution times was developed and the obtained multidimensional NMR correlation data was processed with an algorithm for discrete Laplace inversion that does not use a non-negativity constraint. This facilitates the observation of negative relaxation components, which cannot be ruled out in a system that potentially shows exchange contributions on the NMR timescale [6]. The obtained 2D spin lattice – spin alignment correlation map (Figure1) provided different signal components that were assigned to different morphologies or regions in the LGPS structure. The correlation of $T_1$ and $\tau_c$ facilitates the distinction between relaxation-controlled and mobility-induced components in the $\tau_c$ distribution. In area B of Fig.1 the SAE decay rate overlaps with the limit set by the relaxation rate of quadrupolar order, $T_{1,Q}$, hence no direct mobility information is contained in this case. Area B contains the maximum of the distribution, which is consistent with the high Li ion conductivity of the material. Areas A and C show $\tau_c<T_{1,Q}$, thus $\tau_c$ represents a correlation time that is a direct measure of mobility. The dotted region D shows a
Local measure of the electromagnetic field in Magnetic Resonance coils: how simulations are needed to check $B_1$ measurements?

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The development of probes for Nuclear Magnetic Resonance (NMR) spectroscopy of metabolites, biomolecules or materials requires the accurate determination of the radio-frequency (RF) magnetic field strength, $B_1$, at the position of the sample since this RF-field strength is related to the signal sensitivity and the excitation bandwidth. The Ball Shift ($BS$) technique is a commonly employed test bench method to measure the $B_1$ value. Nevertheless, the influence of the RF electric field on $BS$ is often overlooked. Herein, we derive, from Maxwell equations, an analytical expression of the $BS$, which includes both the electric and magnetic contributions. This equation shows that the $BS$ allows quantifying the $B_1$ field strength only in regions where the electric energy is negligible with respect to the magnetic one. Using numerical simulations of the electromagnetic (EM) field and experiments, we show that this condition is fulfilled at 100.5 MHz inside the coil of a double-resonance $^1$H/X 4 mm Magic Angle Spinning (MAS) probe. Conversely, NMR experiments show that the contribution of the electric energy to $BS$ becomes significant when the X channel of this probe is connected to a frequency splitter. In that case, $BS$ is less reliable to quantify the $B_1$ value because the coil is no longer balanced. We also demonstrate the high accuracy of the EM field simulations by comparing their results with experimental ones. Moreover, the simulations also provide additional information about the dependence of the EM field with respect to the position inside the coil 1.

Figure 1: Density of the Magnetic a) and Electric b) Energies inside the RF coil, where a balanced loading produces a node of electric field at the middle of the sample space. The inner black box delimits the sample space, and the outer one represents the rotor wall.

Influence of Chemical Shift in RESPIRATION\textsuperscript{CP}

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We present a theoretical analysis of the influence of chemical shifts on the Rotor Echo Short Pulse IRrAdiation mediated Cross-Polarization (RESPIRATION\textsuperscript{CP})\textsuperscript{1} heteronuclear dipolar recoupling experiments in solid-state NMR spectroscopy. By going into the rf interaction frame and employing a quintuple-mode operator-based Floquet approach\textsuperscript{2}, we describe how chemical shift offset and anisotropic chemical shift affect the efficiency of heteronuclear polarization transfer. We discuss the validity of this approach and turn to quaternion\textsuperscript{3} calculations to determine the effective resonance conditions in a combined rf field and chemical shift offset interaction frame transformation. Based on the latter approach, we derive a broad-banded version of the RESPIRATION\textsuperscript{CP} experiment, called BB-RESPIRATION\textsuperscript{CP}. The influence of chemical shift was simulated through numerical simulations and verified experimentally for simultaneous acquisition of NCO and NCA spectra.

References:

Figure 1. Schematic representation of the new BB-RESPIRATION\textsuperscript{CP} transfer element with a periodic time of $2\tau_e$ which is repeated $M$ times for the entire sequence. The rf field strengths are denoted by $\omega_1^{(q)}$, the lengths of the short flip pulses by $\tau_p^{(q)}$, and the lengths of the offset compensating pulses by $\tau_{\text{com}}^{(q)}$, where $q = I, S$ for the I- and S-spin rf channels, respectively.
Adiabatic Radio-Frequency-Driven Recoupling in Solid-State NMR

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The homonuclear radio-frequency driven recoupling (RFDR) experiment[1,2] is commonly used in solid-state NMR spectroscopy to gain insight into the structure of biological samples due to its ease of implementation, stability, and low radio-frequency requirements. We demonstrate improved transfer efficiency for the RFDR experiment by generating an adiabatic passage[3] through the zero-quantum (ZQ) recoupling condition. The adiabatic passage is achieved by changing the position of the π pulses during the mixing time, hereby exploiting the evolution due to chemical-shifts which are otherwise refocused. A full theoretical description of the new experiment is presented. Additionally, we demonstrate improved polarization transfer performance through numerical simulations and finally we present supporting experimental results.

References:
A Single-Crystal NMR Kit for Your Existing NMR Probe

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Solid-state NMR allows characterization of orientation-dependent nuclear spin interactions (mathematically described by tensors), e.g., dipole-dipole- and quadrupolar-couplings, and the chemical shift anisotropy. Powder samples may readily yield relative tensor orientations in the molecular frame1, but otherwise need special experiments2 for a full orientation characterization. Single-crystal (SC) NMR allows full tensor characterizations from spectra acquired at different orientations of the crystal. Costly and specialized probes has prevented many laboratories from utilizing SC NMR for pursuing complete tensor descriptions.

We present a simple, inexpensive SC NMR kit that was developed to fit standard wide-bore NMR probes. With this kit, a web-based drag’n’drop software (webASICS) was released for SC NMR data analysis.

The SC NMR kit, Fig. 1, constitutes a piezo-crystal motor sufficiently small to sit next to the radio-frequency (RF) coil. The motor is held by a 3D printed scaffold on three brass poles connected to the NMR probe. The single-crystal is to be glued to a tenon; the tenon is slid into one of three mortices in a goniometer; the goniometer sits in a holder on the motor axle. The motor is driven by a control board connected via USB to the spectrometer host computer, and the single-pulse program sends an orientation (angle) request before each experiment. Spectra are recorded for a range of angles for each position of the tenon in the goniometer. Spectral peak positions are fitted in webASICS to theoretical models. The fitting routine provide correction parameters that reveal misalignment discrepancies.

We have tested our SC NMR kit with a single-crystal of $^{23}$NaNO$_3$ and compared our results with a dataset previously acquired on a dedicated SC NMR probe. We have found a good reproducibility in the experimental procedures and a quadrupolar coupling constant of $338.6\pm0.8$ kHz corresponding well with the literature3.

Figure 1. (Left) the SC NMR kit. (Right) a screenshot from webASICS

A home built probe for basic and advanced Magic Angle Turning experiments

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Magic Angle Turning is a powerful tool to achieve high resolution in NMR spectra without the use of fast spinning. It was discovered in the 1980s and has never played a major role in NMR spectroscopy, due to the fact that the low spinning speeds needed cannot easily be realized with commercially available hardware\textsuperscript{[1]}.

We present an in-house built Magic Angle Turning probe, in which the rotation is achieved through a step motor and a set of shafts and gears\textsuperscript{[2]}. A Hall probe is incorporated in the top of the probe and is used to control the Magic Angle\textsuperscript{[3]}. This setup allows for constant and precise rotation in the range of 20 to 100 Hz, as is needed for Magic Angle Turning experiments.

First 2D spectra recorded with this probe and a five pulse projection MAT\textsuperscript{[4]} pulse sequence are shown and compared to spectra from commercial probes. Furthermore, dipolar methods that have been combined with MAT are discussed as well as heating techniques that can be integrated in the setup.

\textbf{Figure 1.} 3D CAD design of the probe. The insert shows a close-up of the top part, where the arrangement of gears and bevel gears allows the probe to operate under a precisely varied angle between sample and magnetic field.

\textbf{Figure 2.} Non-sheared (top) and sheared \textsuperscript{31}P-MAT spectrum of a 55:45 K\textsubscript{2}O:P\textsubscript{2}O\textsubscript{5} phosphate glass, recorded with our custom built probe. Slices representing different CSA tensors as well as the projection into the indirect dimension are shown next to the spectrum in the bottom right.

\textbf{Figure 3.} First prototype of the probe. Spectra shown in fig. 2 were recorded with this setup.

**In Situ Solid-State NMR Investigation of Competitive Adsorption and Reaction in Zeolite Micropores**

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The reaction mechanism of etherification of \( \beta \)-citronellene with ethanol in liquid phase over acid zeolite beta catalyst is revealed by *in situ* solid-state \(^{13}\)C NMR spectroscopy. Comparison of \(^{13}\)C Hahn-echo and \(^1\)H–\(^{13}\)C cross-polarization NMR characteristics is used to discriminate between molecules freely moving in liquid phase outside the zeolite and molecules adsorbed inside zeolite pores and in pore mouths. In the absence of ethanol, \( \beta \)-citronellene molecules enter zeolite pores and react to isomers. In the presence of ethanol, the concentration of \( \beta \)-citronellene inside zeolite pores is very low because of preferential adsorption of ethanol. The etherification reaction proceeds by adsorption of \( \beta \)-citronellene molecule from the external liquid phase in a pore opening, where it reacts with ethanol from inside the pore. By competitive adsorption, ethanol prevents the undesired side reaction of \( \beta \)-citronellene isomerization inside zeolite pores. \( \beta \)-citronellene etherification on zeolite beta is suppressed by bulky base molecules (2,4,6-collidine and 2,6-ditertiarybutylpyridine) that do not enter the zeolite pores confirming the involvement of easily accessible acid sites in pore openings. The use of *in situ* solid-state NMR to probe the transition from intracrystalline catalysis to pore mouth catalysis depending on reaction conditions is demonstrated for the first time. The study further highlights the potential of this NMR approach for investigations of adsorption of multicomponent mixtures in general.

**Figure 1.** \(^{13}\)C\{\(^1\)H\} solid-state MAS NMR spectra acquired with a Hahn-echo (in red) and CP (in black): (a) \( \beta \)-citronellene \(^{13}\)C liquid-state NMR spectrum; (b) dehydrated zeolite beta mixed with \( \beta \)-citronellene; (c) dehydrated zeolite beta mixed with \( \beta \)-citronellene and ethanol (molar ratio of 1:10).