Book of abstract

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In-cell structural biology using NMR: overview and latest developments to depict IDPs at 310K

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In-cell structural biology by NMR is appealing in many regards: It proposes, among others, to investigate conformational equilibria or ligand binding/processing in very relevant conditions, *i.e* in cells [1,2]. We will briefly describe the past and present experimental conditions exploited in the field, and give an overview of the contributions and limits of in-cell NMR.

The approach comes with a number of challenges, among which i) the many difficulties in sample production, and ii) important signal losses due to promiscuous, transient interactions with cellular entities, which, in turn, urges to use (too) high concentrations of the studied proteins. We will show how we and others are trying to facilitate in-cell NMR studies, using new production methods in situ, new labeling schemes, and better adapted pulse sequences.

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Pulse(d) dynamic nuclear polarization

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

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

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

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5. Redrouthu, Kumar, Mathies Under review

THE BATTLE FOR SILVER BINDING: HOW THE INTERPLAY BETWEEN THE SILE, SILF AND SILB PROTEINS CONTRIBUTES TO THE SILVER EFFLUX PUMP

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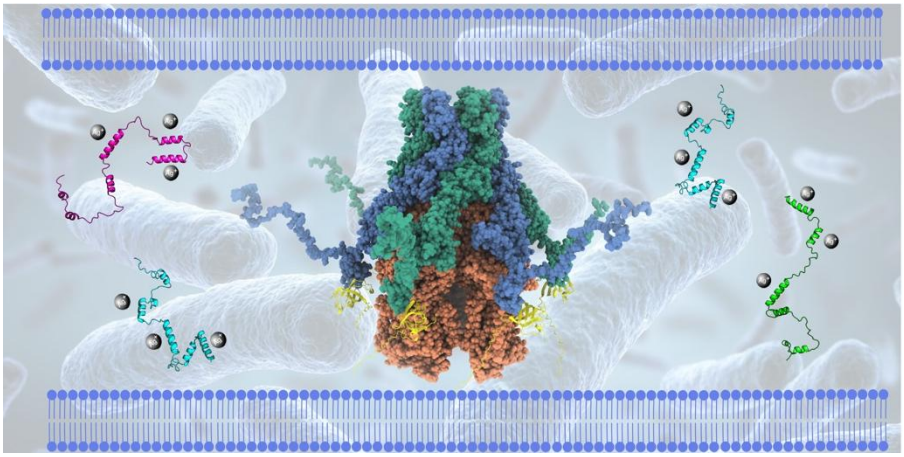
ABSTRACT

The antimicrobial properties of silver ions have extensively been used for thousands of years and it is still used today in several formulations in medicine[1]. Unfortunately, a silver resistance system from *Salmonella* strains was isolated after the death of patients in a burn unit in Massachussets in 1975[2]. Particularly, the resistance of Gram-negative bacteria to silver ions is mediated by a silver efflux pump, which mainly relies on a tripartite efflux complex SilCBA, a metallochaperone SilF and an intrinsically disordered protein SilE. However, the precise mechanism by which silver ions are extruded from the cell, and the different roles of SilB, SilF and SilE remain poorly understood. To address these questions, we employed Nuclear Magnetic Resonance and Mass Spectrometry to investigate the interplay between these proteins. We first solved the solution structures of SilF in its free and Ag^+-bound forms and we demonstrated that SilB exhibits two silver binding sites at its N- and C-termini. Conversely to the homologous Cus system, we evidenced that SilF and SilB interact without the presence of silver ions and that the rate of silver dissociation is eight times faster when SilF is bound to SilB, indicating that silver ions are transferred from SilF to SilB. Finally, we have shown that SilE does not bind to either SilF or SilB, regardless of the presence or absence of silver ions, further corrobarating that it merely acts as a buffer that prevents the cell from being overloaded with silver. Indeed, we have previously shown that SilE can interact with 6 to 8 silver ions[3].



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Side-chain proton detection and assignment at 160 kHz MAS and 1200 MHz magnetic field

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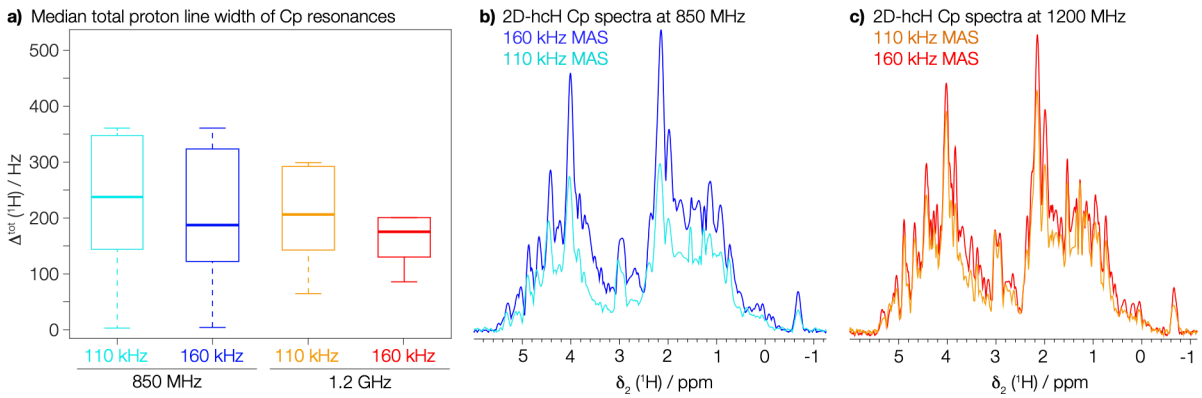
We investigated the benefits of fast magic-angle spinning (MAS) at 160 kHz, using a 0.5 mm probe (Prof. Ago Samoson, Darklands OÜ, Estonia), to enhance the resolution of side-chain resonances in proton-detected spectra of protonated proteins. These resonances are often part of a strongly coupled network of protons since many of them have similar chemical shifts. This leads to strong-coupling effects and additional line broadening when compared to amide or Hα protons. This induces severe overlap of many of these resonances, preventing their assignment, even at 110 kHz MAS. However, these resonances form most of the essential contacts that define the protein fold and are therefore crucial for determining protein structure and characterizing biomolecular interactions by their localization at the interface. We first show on a model system (ortho-phospho-L-serine) that a reduction of the homogeneous linewidth by a factor two can be achieved when comparing data recorded at 110 and 160 kHz MAS. A combination with high magnetic field (1.2 GHz) allows to further reduce line widths by increasing the chemical shift dispersion on a Hz-scale. In an application to the core protein (Cp), forming the 4 MDa Hepatitis B virus capsid, we could assign 60 % of the aliphatic protons using a combination of hCCH TOtal through Bond correlation SpectroscopY (TOBSY) and hNCH experiments. These experiments also benefit from the lengthening of the transverse relaxation time T_2' with faster spinning, which could as well allow observing flexible residues in the solid-state using INEPT-based pulse sequences often inefficient at slower MAS due to too-fast relaxation. The availability of side-chain protons for structure determination and protein-protein interaction studies will open new possibilities for the studies of complex proteins that are often produced in small quantities.



BIOPHYSICAL INSIGHTS INTO GHRELIN RECOGNITION AND SIGNALING REGULATION BY SODIUM IONS

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Ghrelin is a human post-translationally-acylated peptide hormone recognized by a single G protein-coupled receptor (GPCR), the growth hormone secretagogue receptor (GHSR). Ghrelin and its receptor play a central role in controlling processes such as growth hormone secretion, food intake and glucose metabolism. While better understanding signal transduction by GPCRs attracts a lot of interest, more data are needed to elucidate the recognition of complex natural ligands and the regulation of receptor activity by allosteric modulators. Deciphering ghrelin signaling and its regulation allows us to expand our understanding of GPCR functions and could facilitate the design of new therapeutics.

Using a combination of NMR in solution, coarse-grained molecular modeling and functional assays, we observed that ghrelin recognition by GHSR is accompanied by conformational changes of the peptide hormone (1). Our data with acylated and nonacylated ghrelin pointed to a model whereby the formation of a defined hydrophobic core structure within the peptide promotes access to the receptor ligand-binding pocket. Our results also pinpoint the importance of interrogating the role of peptide ligand conformational dynamics in interacting with GPCRs.

We also investigated the modulation of ghrelin – GHSR signaling by sodium ions (2), which have been proposed to play a substantial role in regulating the activity of the most studied GPCRs. Combining ^{23}Na NMR, molecular dynamics, fluorescence and bioluminescence, we observed that sodium binds to a conserved allosteric site and shifts the conformational equilibrium of the ghrelin receptor, thereby decreasing GHSR-mediated G protein activation. All together, these data point to sodium as an integral component of the ghrelinergic signaling system and provide a framework to explore sodium ion interactions with GPCRs.

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Assembly mechanisms of the plant protein remorin into membrane nanodomains

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ABSTRACT

Plant-specific REMORINs (REMs) are crucial proteins involved in plant defense against viral propagation by regulating cell-to-cell connectivity. They are tightly associated with the clustering of nanodomains at the plasma membrane, driven by specific protein-protein and protein-lipid interactions. REMs can be classified into 6 groups, containing a membrane-associating C-terminal anchor (REMCA), neighboring a coiled-coil domain that is followed by an intrinsically disordered N-terminal region (IDR). We have recently contributed to understanding the precise underlying mechanisms of nanodomain clustering by REMs, involving interactions of REMCA with specific phosphatidylinositol phosphates (PIPs) (1)(2). Moreover, our data have revealed that StREM1.3's nanodomain clustering depends on REM's oligomerization behavior (3) and on the phosphorylation status in the IDR (4). We now address the role of the structural divergence between the different REM groups and found that REMs rely on diverse sequence motif arrangements and REMCA sequences. We investigate REM's structural and dynamic organization based on domain-specific analysis and their positioning in the context of the three-domain protein. Bioinformatics analysis suggests implications of motif distribution in the regulation of nanodomain clustering. Based on 3D structure determination of REMCAs of different REM groups by NMR, we discovered the REMCA structural diversity, further highlighting the role of sequence adaptation and structure modulation to control membrane association and nanodomain formation.

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CARACTERISATION DE L'ETAT DE LIAISON DU SEL DANS LES PRODUITS ALIMENTAIRES PAR RELAXOMETRIE RMN DU 23Na

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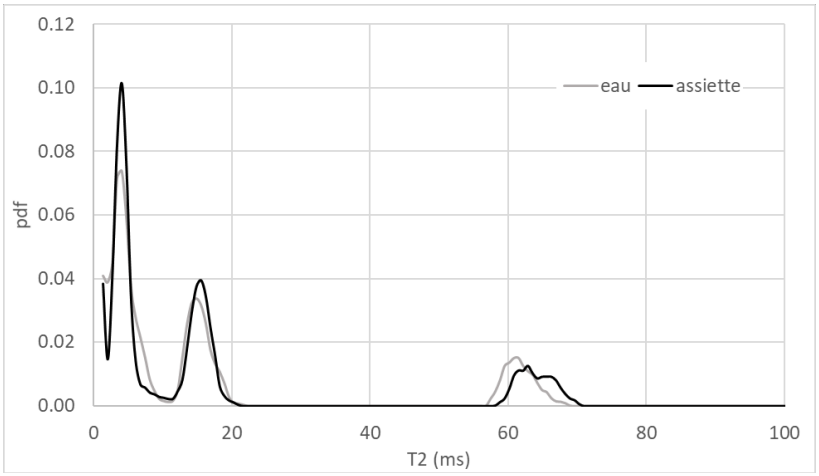
La quantité sel dans notre alimentation reste trop élevée et les organismes de santé publique recommandent sa diminution. Le projet ANR Sal&Mieux cherche des leviers de diminution à l'échelle domestique. Les travaux présentés ici visent à comprendre les liens entre type de salage et sensation salée, l'objectif étant de saler moins sans perte du goût salé. Les études sensorielles montrent que le salage à l'assiette fait ressortir le goût salé, la relaxométrie RMN a pour objectif de nous faire comprendre pourquoi en fournissant des informations sur l'état de liaison du sodium à la matrice alimentaire.

Les résultats présentés ici ont été obtenus sur i. la carotte entière et ii. les pâtes alimentaires, pour 2 types de salage : eau (salage dans l'eau de cuisson) et assiette (salage après cuisson). La relaxométrie RMN a été réalisée à 400 MHz (Bruker UltraShielded widebore magnet) avec une sonde volumique 1H/23Na 5 mm large bande (BBO, Bruker BioSpin). La relaxation du sodium dans les échantillons est caractérisée à partir d'une séquence mettant en évidence les cohérences double quanta (mesure à τ_{opt}=2.5ms, TR=1s, NS=4096), et d'une CPMG à phase alternée (TE=140µs, TR=1s, NS=4096). L'analyse des données de la CPMG est réalisée par un justement multi-exponentiel, sans apriori (NNLS), de la décroissance avec une régularisation fixe standard (λ=0.02). L'analyse statistique des données s'appuie sur un modèle linéaire généralisé intégrant l'ensemble des prédicteurs potentiels.

Un effet salage significatif est visible sur la répartition des T2 pour les deux aliments, comme l'illustre par exemple la figure ci-dessous pour les pâtes. De plus, un signal double quanta est détecté sur les 2 aliments quel que soit le type de salage.



Répartition des temps de relaxation transversaux pour les pâles alimentaires en fonction du type de salage.

Ces travaux sont financés par le projet Sal&Mieux ANR-19-CE21-0009.

NMR investigation of miRNA dynamics by ^{19}F relaxation dispersion experiments

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ABSTRACT

Over the last decades, a diversity of non-coding RNA have been revealed such as micro-RNAs (miRNA), that play an essential role in RNA induced gene silencing and target up to 60% of protein-coding genes in humans.[1] As RNA conformational changes can trigger their functional diversity, the investigation of RNA dynamics is a key feature to improve the understanding of their mechanism of action.[2]

NMR spectroscopy is an efficient tool to investigate dynamic processes. However, obtaining an accurate description of RNA conformational landscape remains long and complex. In particular, spin system assignment and measurements of dynamic parameters remain often limited by significant signal crowding and low intensities at dynamic sites. To overcome this bottleneck, a fluorine was site-specifically introduced in the miRNA sequence using chemical synthesis to facilitate the investigation of miRNAs interactions and dynamics at the molecular level, by providing unambiguous, well-resolved signal measurable within simple 1D NMR experiments.

We particularly looked into the dynamical interaction of let-7, a miRNA presents in all organisms and shown to be down-regulated in several forms of cancers, with two relevant mRNA targets: lin28a and lin41.[3] To probe these interactions at the atomistic scale, fluorine atoms were site-specifically introduced in let-7 as NMR probes. We show that the introduction of fluorine in let-7 allows for a fast screening of the different complexes and their secondary structures. Moreover, ^{19}F based relaxation dispersion experiments were recorded to investigate the conformational states of these highly dynamical biomolecular complexes at the micro- to milli-second timescale.[4] R1rho on-resonance experiments allowed to characterize the presence of chemical exchange of these complexes and off-resonance experiments were recorded to provide further information on these excited states.

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Solid-State Dynamic Nuclear Polarization NMR Spectroscopy, A Gateway to Establish the Spatial Distribution of Polymorphic Phases in a Solid Material

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ABSTRACT

In the context of organic molecular materials, the phenomenon of polymorphism arises when a given type of molecule can form two or more solid phases with different crystal structures. In the context of pharmacology, sample formulation can be the key of the posology and toxicity of a chemical compound and have to be understood. In particular: is a polymorphic form stable? Does it transform to another form? How does this transformation take place, and can we avoid it? Nuclear Magnetic Resonance can very efficiently determine which polymorph is present in a given sample, since two polymorphs have different chemical physical properties and nuclear spin environments. However, conventional solid-state NMR methods are impacted by two main roadblocks. First, NMR is an insensitive technic that struggles to detect low abundance forms. Second, solid-state NMR average the information over the all sample, and is general not in capacity to determine the localization of a given polymorph within the NMR sample.

In this study, we use a peculiar form of NMR called “Dynamic Nuclear Polarization Magic Angle Spinning” (a.k.a. DNP-MAS)[1] to kill two birds with one stone. First, this technique can be used to amplify drastically solid-state NMR signal by one or two orders of magnitude, allowing an increase by 2 or 4 orders of magnitude the time needed to perform an NMR experiment. Second, this technique can be used to gain spatial resolution in NMR and allow to localize the spectroscopic information within the sample. We applied this technique to the 3-Aminobenzoic acid, also known as meta-aminobenzoic acid or MABA).[2] Indeed, this molecule is known to possess several polymorphs, with the polymorphic Form I hard to characterize by PXRD. By applying our methodology to this compound, we manage to determine that what is believed to be Form I is a mixture between form I and III, with form III almost undetectable to the PXRD spectrum of form I. A geometrical model of the form III within the form I has been determined with a death star-shaped geometry.

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AN INNOVATIVE SOLVENT-FREE POLYMER SAMPLE PREPARATION METHOD FOR DNP SSNMR

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ABSTRACT

Solid-state NMR (SSNMR) is highly suitable for analyzing polymers but has low sensitivity. This limitation can be overcome using dynamic nuclear polarization (DNP), a technique that enhances NMR sensitivity by transferring the electronic spin polarization of polarizing agents to surrounding nuclei[1]. However, mainly soluble polymers can be analyzed by DNP SSNMR because efficient methods for preparing the samples for DNP usually require initial polymer solubilization[2]. In this work, we will present an innovative solvent-free sample preparation method based on the use of supercritical CO_2 ($scCO_2$) technology. More precisely, $scCO_2$ is used as a plasticizing agent to lower the polymer glass transition/melting points, allowing thus an efficient mixing of polarizing agents (Pas) within the resulting soften/molten polymer matrix, similarly to a conventional polymer extrusion process but at moderate temperatures. This method was applied to incorporate either TEKPol[3] or AMUPol[4] as polarizing agents within different polymers. Preliminary results related to the use of $scCO_2$ for polymer impregnation will also be presented. In this second case, $scCO_2$ is used as a solvent and this method allows polymers to be homogeneously loaded with PAs. The resulting samples were characterized by CW EPR and DNP SSNMR. The results were compared to conventional polymer sample preparation methods (Fig.1) highlighting the potential of the proposed processes as new and efficient methods to prepare polymer samples for DNP[5].

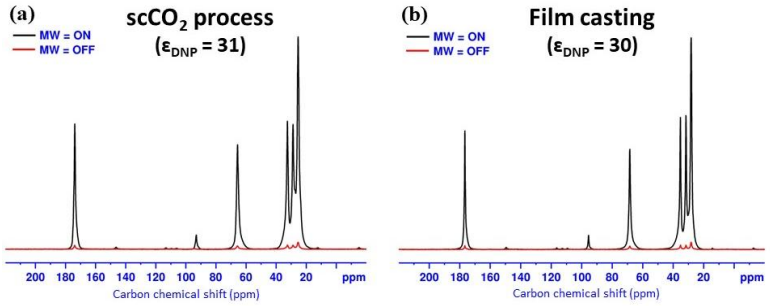

Fig.1. ^{13}C CPMAS DNP SSNMR spectra of a polycaprolactone sample obtained by (a) supercritical preparation method, (b) film casting preparation method

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Investigation of the cooperative Spin-Crossover in a mononuclear manganese(III) complex via solid-state NMR crystallography

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Spin-Crossover (SCO) complexes are an emergent class of materials, defined by a reversible transition between two electron spin states upon applying an external stimulus such as temperature, light or electric field, that are highly promising as multifunctional molecular switches. Despite all efforts to date, the link between structural changes in the coordination of individual atoms and the distribution of electron spin density throughout the molecular complex remains unclear, hindering an understanding of the exact nature of the phase transition. Here, using high-resolution, variable-temperature solid-state paramagnetic NMR on the SCO complex $[MnL1]^{+}PF_6^{-}$ [1], we were able to correlate paramagnetic NMR shifts to the structural and electronic cooperative changes that the Mn(III)-mononuclear complex undergoes, and map the electron spin density changes across the SCO transition upon cooling/heating at atomic scale.

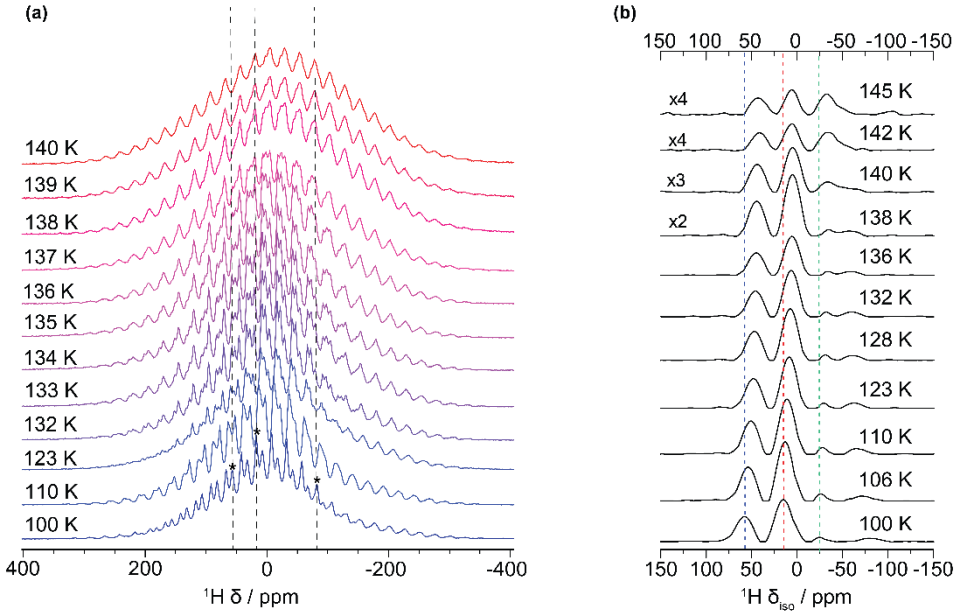

Figure 1. Variable Temperature ^{1}H LTMAS NMR spectra of the compound $[MnL1]^{+}PF_6^{-}$.

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MEASURING TREE WATER CONTENT *IN-SITU* WITH A PORTABLE, UNILATERAL MAGNET

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Keywords: portable MRI, low-field NMR, imaging, plants, trees

The use of MRI in the plant sciences has traditionally been limited due to the immobility of the devices, and restrictions with regard to sample size and shape. To overcome these limitations and to be able to study plants directly in their natural environment, we evaluated the capacities of a portable, unilateral magnet: The Nuclear Magnetic Resonance Mobile Universal Surface Explorer (NMR-MOUSE), designed by Blümich *et al*. [1]. This device permits measuring the water NMR signal in a 100-micrometer slice, and by means of a built-in lift, the magnet can be moved to record an NMR profile within a depth of approximately 25-millimeters from the surface of the device. We tested its capacity to measure tree water content by following the dehydration dynamic of thirty cut branches from six different species and two different functional types. We also tested the device on four *in-situ* trees. There was a linear correlation between the integral of the MRI profiles obtained and the water content of the branches and trees. This correlation was present regardless of tree species or functional type. Using the profiles obtained on the branches, we then tested the capacity of the device to differentiate the conductive tissues, i.e. the xylem and phloem fluxes. The MRI profiles of the branches presented distinct peaks which corresponded to the xylem and phloem tissues, whose location was validated with x-ray microtomography imaging. In conclusion, the NMR-MOUSE is a promising candidate for measuring tree water dynamics in the field.

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

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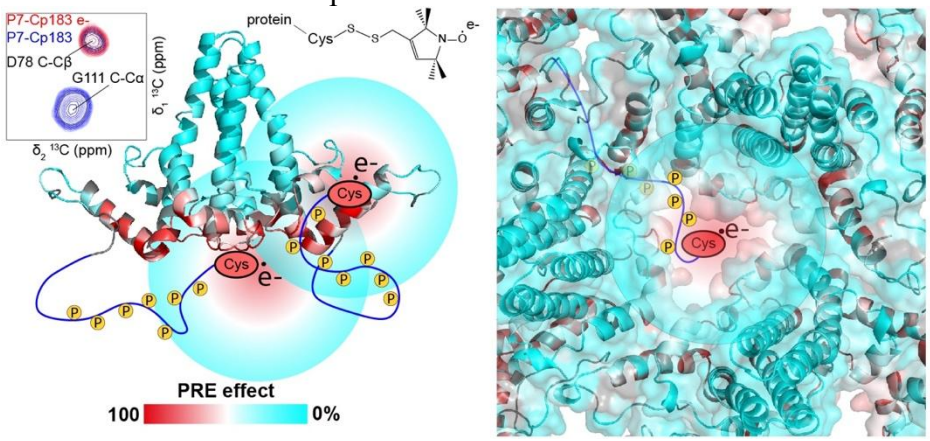
The hepatitis B virus consists of a capsid that surrounds the viral genome and is composed of 120 core protein dimers (Cp). Here, we investigate the functional C-terminal domain (CTD) of Cp, which for instance interacts with partner proteins for cellular trafficking. To do this, it has to expose its signal sequence on the outside of the capsid shell. How this is regulated by phosphorylation remains largely unknown, mainly because this 34-amino acid peptide is invisible in cryo-EM, X-ray diffraction, or classical solid-state NMR (ssNMR) experiments due to its short proton T1ρ, which prevents efficient transfer during cross-polarisation times. Here we use a spin-labelling strategy in which we introduce a nitroxide radical onto the C-terminal cysteine of Cp after mutating all other cysteines. We then use a combination of proton and carbon-detected ssNMR to obtain paramagnetic relaxation enhancements (PRE) by comparing the 3D peak intensities of paramagnetic and diamagnetic samples. This allowed us to obtain site-specific distance information for over 90 % of the residues of Cp, which we used to compare the excursions of the CTD under three different conditions: in the unphosphorylated, RNA-filled capsid; in the phosphorylated, empty capsid; and in the presence of a capsid assembly modulator that induces abnormal capsid assembly. We then used our PRE restraints as distance constraints in molecular dynamics simulations of Cp. Our results show that in all capsid forms, the spikes are completely unaffected, indicating that the CTD remains largely inside the capsid. However, in phosphorylated empty capsids, the data suggest that the CTD would protrude in a "loop-out" conformation, displaying one nuclear localization signal through the triangular pores of the capsid. We therefore identify the triangular pores as the main site of protrusion, contrary to what was previously postulated. In the presence of the CAM, the CTD appears to be less constrained in the inside of the capsid, and appears to protrude even more from the triangular pores. Our work provides, for the first time, a molecular view of the CTD localisation in different Cp forms and more generally positions paramagnetic tags and PREs as important tools for elucidating flexible domains in complex assemblies such as viral capsids.



COMBINATION OF SOLUTION AND SOLID-STATE NMR AT FAST MAS FOR THE STRUCTURAL CARACTERIZATION OF SARS-COV-2 ORF6 MEMBRANE PROTEIN

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ABSTRACT

By using fast MAS solid-state NMR we propose a structural model for ORF6 that differs from the proposed AlphaFold2 models.

While X-ray diffraction and cryo-EM are effective techniques for determining the structures of large molecular systems, smaller systems can only be accurately determined using NMR. However, membrane proteins that are small in size present a challenge for solution state NMR due to the size of the detergent micelles required for their solubilization. In addition, fewer structures of this protein group have been experimentally solved, resulting in less accurate predictions by AlphaFold2. ORF6 is a 61-amino acid, membrane-associated accessory protein of SARS-COV-2, which is found to be the most cytotoxic protein of the virus by interfering with nuclear pore transport causing an interferon inhibition. However, the relationship between its membrane localization and the nuclear pore remains unclear.

We aim to decipher the structure and oligomeric state of ORF6 protein using detergent micelles or lipid membranes. To better understand the oligomerization behavior of ORF6, we employed a range of biophysical techniques, including SEC-MALS, analytical ultracentrifugation and NMR. A combination of solution- and solid-state NMR was used to gain insight into its structure and dynamics.

Our data show that the micelle-associated ORF6 in detergent have a not defined oligomeric state from monomer to tetramer, which depends notably on the protein to micelle ratio. This suggests that detergent micelles cannot mimic the native membrane. The combination of solution and solid-state NMR data allowed us to get a complete picture of the protein in different environments, with solution-state NMR spectra acquired in detergent micelles allowing to specifically assign the C-terminal flexible amino-acids, and solid-state NMR data allowing the detection of more rigid residues in lipid membranes, conditions which are closer to the native conditions. Specific amino-acid labeling and paramagnetic relaxation enhancement allowed us to assign the structured and rigid region of ORF6 and to identify significant structural differences from the models obtained by AlphaFold2.

With this comprehensive approach, we can propose a structural model of ORF6 that contribute in the understanding of its interactions with the membrane and other proteins involved in SARS-CoV-2 infection.

Conformational heterogeneity in the chromophore pocket of mEos4b impacts the observed photoswitching and photoconversion properties

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Photoconvertible fluorescent proteins (PCFPs) are an essential tool for PALM-type super-resolution microscopy. Upon application of UV light, the initial green fluorescence of PCFPs irreversibly turns red caused by a light-induced extension of the chromophore conjugated electron system. One of the main factors limiting the maximal achievable resolution in PALM is the limited photoconversion efficiency (PCE) of the PCFPs, i.e. only a fraction of the molecules typically reaches the red state. In order to improve PCE, detailed mechanistic knowledge of the photoconversion process is crucial, which remains actively debated till date. Using NMR spectroscopy coupled with *in-situ* light-illumination and fluorescence microscopy we studied the Green-to-Red photoconversion mechanism of mEos4b, a popular PCFP. As PCFPs also show pronounced reversible photoswitching between their green state and a metastable dark state, we also studied this photoswitching mechanism. We discovered that mEos4b (as well as other PCFPs) exhibits a well-defined two-state heterogeneous population in its green form. We found marked differences in these two states in terms of their structure and local chemistry around the chromophore pocket. We studied the conformational exchange between the two states, notably under UV light. I will describe how this hitherto undetected conformational partitioning impacts photoconversion and reversible photoswitching of mEos4b.

Biomolecular MAS NMR: some good reasons for spinning faster

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ABSTRACT

The switch from the traditional MAS NMR approaches with ^{13}C and ^{15}N detection to ^{1}H has accelerated the site-specific analysis of complex immobilised biological systems and opened the way to samples of higher molecular weight and available in limited amounts.

We will take the moves from a critical analysis of recent literature data, share our first results on a prototype Bruker 0.4mm probe capable of rates exceeding 150 kHz and discuss the expected impact of fast MAS on resolution and sensitivity of different NMR experiments on different classes of biomolecular samples.

Characterizing histidine side chains in an improved PETase

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ABSTRACT

We have assigned the NMR (Nuclear Magnetic Resonance) signals of all imidazole ring nuclei of the six histidines in an optimized PETase derived from the Leaf-branch Compost Cutinase (LCC) enzyme, and could thereby determine the dominant tautomeric structure for each histidine. A pH titration experiment yielded pKa values for all histidines, showing a value of 4.7 for the catalytic His242, whereas all other histidines are characterized by an even lower pKa value. Comparison between the S165A inactive variant and the active enzyme detail the hydrogen bonding pattern of the catalytic histidine. We finally show that the hydrolysis activity towards a soluble substrate bis-(hydroxyethyl) terephthalate (BHET) follows the same titration curve, validating the protonation state of the catalytic histidine as an important factor for its activity.

The C-terminal helical extension of MAX60 modifies the folding pathway of the common structural core shared by MAX effectors.

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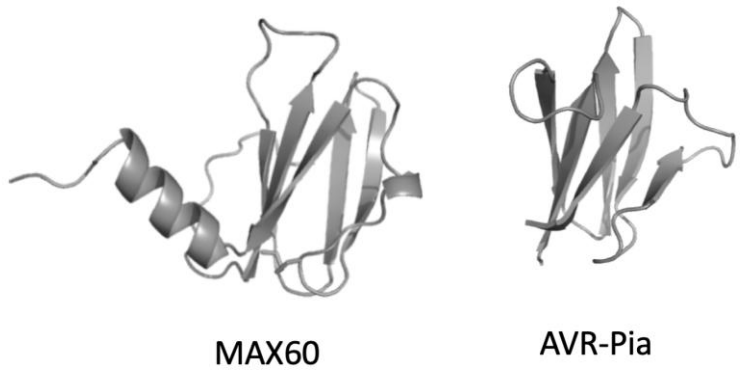
If we know from Anfinsen laws that all the information needed for the 3D structure of a protein is contained in its sequence, the paths taken by the polypeptide to fold in a well-defined native structure are far to be well understood. This issue is particularly important in the case of proteins having a similar 3D fold, but very divergent sequences. MAX effectors are a family of proteins widely distributed in the phytopathogen fungus *Magnaporthe oryzae* that share a conserved common 6-stranded ß-sandwich fold despite low sequence identity. These effectors family of almost 100 members constitute the molecular arsenal for the fungus infection. We analyzed the folding pathway of MAX60, a newly identified MAX effector, and compared it to the folding pathways already described for AVR-Pia and AVR-Pib. To this aim, we used High-Hydrostatic Pressure NMR (HHP-NMR), that has proven to be particularly well suited to decipher the folding pathway of a protein. In particular, this technique allows the identification of folding intermediates that could be otherwise rubbed out by harsher method as chemical denaturation.



Ribbon 3D structures of MAX60 and AVR-Pia

MAX60 shows the common core shared by all MAX effectors, but displays an additional decoration: a C-terminal helical extension. In a previous study, we found that AVR-Pia and AVR-Pib, despite their low sequence identity, displays a similar folding intermediate, made of the ß3ß4 ß-sheet.

In the case of MAX60, the C-terminal α-helix and the ß1ß6 ß-sheets are the first elements of structure formed during the folding process, a striking difference to what observed for AVR-Pia and AVR-Pib. Possibly, the origin of this difference is due to the establishment of stabilizing interactions between the ß1ß6 sheets and the first turns of the C-terminal helix, that stabilize this folding intermediate. Thus, proteins with similar topology might present similar folding pathway, but in the frame of a similar structural context. In the case of MAX60, a C-terminal extension that makes close contacts with the protein core seems able to change the folding pathway adopted by the ß-sandwich.

Understanding the signal transduction mediated by Mincle receptor

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ABSTRACT

Mincle is a C-type lectin receptor that plays key roles in the innate immune system by acting as a sensor of pathogen-associated molecular patterns. Mincle is a transmembrane protein that consists of a short cytosolic N-terminal sequence and an extracellular domain including a C-terminal carbohydrate recognition domain (CRD). The extracellular and cytosolic domains are connected by a single-pass transmembrane domain. The extracellular binding of trehalose dimycolate, a glycolipid from *Mycobacterium tuberculosis*, to human Mincle leads to intracellular activation of NF-κB via the Syk-Card9-Bcl10-Malt1 pathway[1,2]. Signal transduction requires FcRγ, a single-pass transmembrane protein bearing cytosolic motifs that are phosphorylated by Src family kinases in the first step of this signalling pathway[3] (Fig. 1).

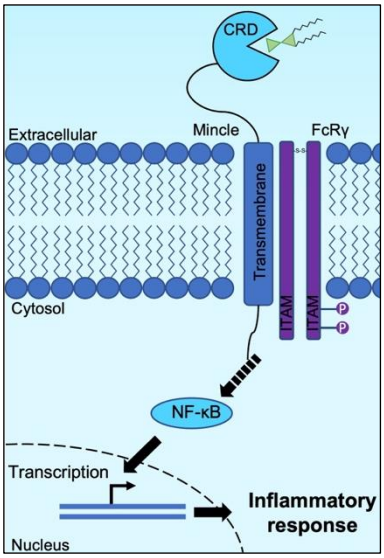

Figure 1. Schematic representation of the signaling pathway mediated by Mincle receptor.

We aim to express the isotopically labelled protein using constructions of various sizes, including the whole protein and its partner in a membrane context using lipid nanodiscs. We are currently carrying out ligand interaction studies and characterisation of the dynamic properties of Mincle CRD through ^{15}N relaxation measurements. In parallel, a theoretical approach employing multi-scale molecular dynamics is used. To capture various conformations of the protein, an adaptive sampling protocol has been employed in combination with the Amoeba polarizable force field.

The philosophy behind this project is to implement an integrative approach involving these multiscale simulations and a range of biophysical techniques in order to understand the molecular mechanisms underlying this signalling event and, potentially, to contribute to the development of new therapies against tuberculosis.

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The interplay between the SilF and SilB proteins contributes to the silver efflux pump mechanism

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ABSTRACT

Silver is well known for its biocide properties, effective against more than 650 microorganisms including positive and negative bacterias, fungis, and viruses[1]. The excessive use over the years has led to the development of a resistance system against silver in bacteria[2].

The silver resistance was discovered for the first time in the Massachusetts General Hospital in 1975[3].The resistance create for Gram-negative bacteria is the efflux pump part of the superfamily Resistance-Nodulation- Division (RND) composed of nine proteins. (figure 1)

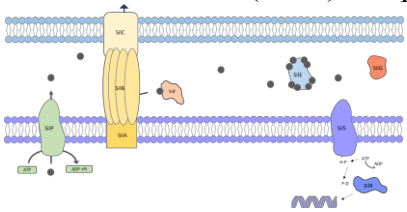

Figure 1: Schema of the silver efflux pump with its 9 proteins implied in silver resistance in Gram-negative bacteria.

The resistance of Gram-negative bacteria to silver ions are mediated by a silver efflux pump, which mainly relies on a tripartite efflux complex SilCBA, a metallochaperone SilF and an intrinsically disordered protein SilE. However, the precise mechanism by which silver ions are extruded from the cell, and the different roles of SilB, SilF and silE remain poorly understood.

To address these questions, we employed Nuclear Magnetic Resonance and Mass spectrometry to investigate the interplay between these proteins. We first solved the solution structures of SilF in its free and Ag+ -bound forms and we demonstrated that SilB exhibits two silver binding sites at its N- and C-termini. Conversely to the homologous Cus system, we evidenced that SilF and SilB interact without the presence of silver ions and that the rate of silver dissociation is eight times faster when SilF is bound to SilB.

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Dissolution DNP on hydrophobic molecules using organic solvents opens new perspectives for the study of complex organic mixtures

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Abstract: Dissolution DNP has proven to be a very powerful NMR technique enabling exceptional increase in sensitivity in the liquid state, up to four orders of magnitude [1]. Over the last two decades, numerous developments and research on d-DNP have been carried out in various fields (biology, biochemistry, medicine, metabolomics, ...)[2,3]. Most of these works were based on the study of rather hydrophilic, aqueous solutions. However, fewer d-DNP developments have been made on hydrophobic solutions[4,5], thus challenging the availability of the technique in fields such as the energy industry, lipidomics or organic chemistry. Several reasons can explain why d-DNP on hydrophobic matrices is difficult to envision, such as the necessity to use more dangerous solvents/compounds, the incompatibilities with hardware components, or having to deal with molecules displaying short T_1. Yet, overcoming these difficulties could allow to expand the scope of d-DNP applications to different kind of liquid matrices. Here, we present our first d-DNP results on ^{13}C labelled benzaldehyde prepared in a toluene glassing matrix, hyperpolarized through cross-polarization on our Bruker prototype DNP polarizer, and rapidly dissolved with isopropanol and injected in a 14 T spectrometer. These first experiments allowed us to obtain signal enhancement over 70 000 with 5° pulse acquisitions, corresponding to 11% of ^{13}C polarization in the liquid state on a labelled molecule displaying a T_1 in the order of 10 to 20 sec.

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Local structure determination of lanthanide-based materials through solid-state paramagnetic NMR and computational studies.

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ABSTRACT

Solid state NMR spectroscopy in the presence of paramagnetic lanthanide ions has been gaining a lot of interest for local structure determination while the long-range structure can be provided by XRD. However, due to the paramagnetic effects on the NMR spectra, experimental studies often require computational supports for a proper interpretation of the data.

Large spin-orbit coupling and strong electronic correlation of the lanthanide ion limit the use of DFT for a proper description of the local electronic structure. Therefore, magnetic properties were obtained with first-principle calculations on chemical model complexes, on lanthanide-dopped YAG and cerium-based photoanode. The local structures were first optimized by solid-state DFT calculations and Monte-Carlo simulations starting from the host crystal structure.

The first principle magnetic properties are in agreement with the available experimental data and the computed pseudo-contact shifts characterize some of the NMR signals. The nuclei nearby the lanthanide ion are subject to a non-negligible contact contribution that can be rationalized with DFT hyperfine coupling calculation. Finally, the experimental quadrupolar parameters and the DFT calculations suggest a local structure deformation due to the dopant lanthanide ion.

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Sustainable and cost-effective MAS DNP-NMR at 30K with cryogenic sample exchange

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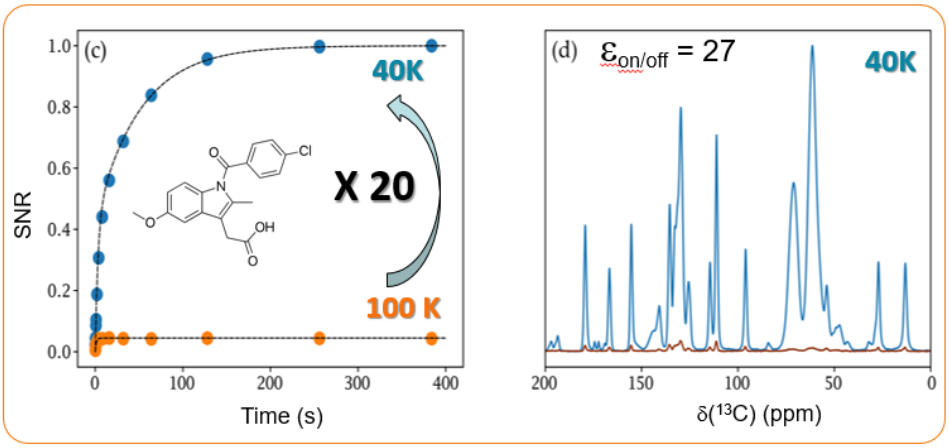
ABSTRACT

Dynamic Nuclear Polarization (DNP) has revolutionized solid-state NMR spectroscopy by overcoming the sensitivity limitations of the technique. However, despite impressive performance improvements, sensitivity enhancements achievable with magic angle spinning (MAS) DNP are still far from the theoretical maximum. In addition, fast-relaxing systems remain difficult to polarize under current MAS-DNP temperature conditions (100K),which strongly restrict its range of applications.

One way of addressing these limitations is to access lower temperature regimes. In this purpose, we have developed a new hardware system for sustainable cryogenic Helium MAS-DNP experiments. This system includes a closed-loop cryostat (SACRYPAN) that can reach 30 K with a single cryocooler and a single compressor, and a triple-channel cryogenic NMR probe (PAVLOT) with excellent RF/MW performance, spinning stability, and cryogenic feedthroughs to adjust the MAS and channel matching and tuning. It also features a cryogenic sample exchange system that enables sample changing within few minutes.

It can perform 100 kHz 1H decoupling at 60W without arcing and 50 kHz on the 13C with 30W and exhibits excellent spinning frequency stability up to 15 kHz at 50 K. Moreover, the probe can be warmed up and disconnected in just 2 hours, and the full system shows days to weeks of sustainable operation. The fact that it only takes 15 minutes to reach 30 to 40K after sample insertion allows for intensive use, on various and diverse applications.

In terms of concrete results, the system has shown significant improvements compared to conventional MAS-DNP systems, with a signal-to-noise ratio improvement up to a factor 23 between 40 and 100K on proton-dense organic microcrystals doped with the latest generation of polarizing agents, which are believed to be the most performing. We hope that this will open up new possibilities for applications that are currently out-of-reach at 100K in a next future.



Ultrahigh-sensitivity ^{19}F DNP NMR for Pharmaceuticals

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ABSTRACT

Dynamic Nuclear Polarization (DNP) is an active research domain, helping to improve sensitivity of NMR. In combination with fluorine ^{19}F NMR, it should have a great potential to analyze fluorinated active pharmaceutical ingredients APIs. Yet, few studies combining DNP and ^{19}F NMR have been published (1-3). ^{19}F is a 100 % abundant nucleus, spin ½, with a Larmor frequency close to ^{1}H. In this work we tested different DNP formulations and polarizing sources, starting from radicals established for 1H cross effect DNP, we tested fluorinated versions of these radicals to compare and optimize enhancements on ^{19}F nuclei, as well as varying degrees of Fluorination of the matrix. Results on those fluorine radicals are encouraging, leading to better enhancement, shorter build up time, higher signal to noise ratio and therefore a better sensitivity for the analysis of fluorine nuclei than their protonated version. Considering the increasing number of fluorinated drug compounds on the market and the low amount of active ingredients in a formulation, these fluorinated radicals bear a high future potential for the analysis of drugs.

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New Polarizing Matrices for High-Field DNP Enhanced NMR Spectroscopy

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Even though the principle of Dynamic Nuclear Polarization (DNP) was understood 70 years ago, it is only recently that this approach become routinely applied to boost the sensitivity of solid-state NMR spectroscopy under Magic Angle Spinning (MAS). DNP MAS NMR requires specific strategies to formulate the sample. The most common one consists in incorporating radicals containing free electrons – the polarizing agent (PA) – in a matrix – typically a solvent forming a glass at cryogenic temperatures – and the analyte. However, complications may raise in case of a direct contact between the PA and a reactive analyte that will reduce the radical, which will in turn drastically cut the DNP enhancement factor. Several alternative sample formulations were proposed such as embedding the PA in dendrimeric structures (1). However, none of them are fully versatile.

In parallel, solid polarizing matrices such as mesoporous silica-based materials incorporating nitroxide radicals have recently been investigated as a solution to separate the electron sources from the substrates to analyze (2-6). Such solids could potentially be exploited to polarize reactive substrates. Here, we will present preliminary DNP MAS NMR data recorded at 9.4 T and 100 K, obtained using highly porous silica xerogels containing TEMPO-based biradicals and synthesized through a sol-gel process.

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LOW FIELD NMR APPLIED TO THE CHARACTERIZATION OF POLYMER DYNAMICS: WHAT YOU CAN DO WITH ONLY 20 MHz?

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ABSTRACT

Solid state NMR has been a reference technique for the characterization of polymers in order to analyze and determine their structure and dynamics all along the processing steps as in the final material used for application. But it is well known the resolution and sensitivity issues inherent to the technique, which at times hinder to obtain the desired levels of precision and performance. Ultra-fast rotation, DNP, HR-MAS, and special pulse sequences have been used to overcome such issues in order to get the desired information with this technique. But the lack of specialized equipment and lack of know-how limit their use in current common research and, above all, in the industry.

Time-Domain NMR or Low field NMR is a low maintenance, relatively low cost, low sample preparation time and robust technique which can offer a significative wide range of dynamic information on solid or gel like samples. Highlight and quantification of dynamic fractions in semicrystalline materials or polymer blends, reticulation ratio in elastomers and mobility of water molecules inside polymeric networks are some examples of the information that TD-NMR can provide.

In this work we will show different practical applications of TD-NMR to the polymer science, using pulse sequences as Magic Sandwich Echo, CPMG and Double Quantum experiments and only a 20MHz spectrometer.

Novel Polarizing Agents for High-Field and Fast MAS DNP Solid-State NMR

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ABSTRACT

Dynamic Nuclear Polarization (DNP) has proved to be a valuable technique to enhance the sensitivity of MAS solid-state NMR experiments by up to two orders of magnitude. It permits to transfer the higher electron polarization to the nuclei of interest. At the heart of the technique are the polarization agents (PA), paramagnetic molecules that represent the source of unpaired electrons. They also determine the polarization transfer mechanism and the overall efficiency of the hyperpolarization process. At 9.4 T, PA such as the di-nitroxides AMUPol (1) and TEKPol (2) lead to enhancement factors of up to about 250 at 100 K (3). Recently, the introduction of PA with stereo-controlled conformation around the nitroxide radical, like HydroPOLs (4), which promotes a better solvent accessibility, have shown enhancements as high as 330.

Here we present new bis-nitroxide radicals developed to be highly efficient at 18.8 T with enhancements far above 100. They are derived from the previously introduced TinyPols (5) and are expressly suited for DNP MAS NMR at high magnetic field (>14 T) and fast MAS frequencies (>40 kHz). We present a further major improvement in the radical design, optimizing a molecular geometry promoting a better ^{1}H-^{1}H spin-diffusion, and incorporating also recent concepts such as the stereo-controlled conformation and an efficient radical-solvent interaction, showing a further development of these highly performant PAs. In particular, M-TinyPol(OH)$_4$ shows enhancements up to about 200 even at 65 kHz of MAS frequency and 18.8 T.

These systems provide high overall sensitivity that place them at the highest values among the PA developed in aqueous media at 18.8 T. These performances open the way to high field DNP NMR in biological systems and they can help the development of very fast-MAS ^{1}H-detected DNP enhanced experiments.

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STRUCTURAL INVESTIGATION OF LIPID NANOPARTICLES BY DNP MAS NMR

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ABSTRACT

RNA-based therapies are expected to revolutionize the treatments of a large number of diseases. The potential of RNAs and especially messenger-RNA (mRNA) has been remarkably illustrated by recent development of vaccines against SARS-Cov-2. To deliver mRNA in cells, lipid nanoparticles (LNPs) are the most advanced platform, protecting mRNAs from nucleases, allowing their efficient uptake by the cells and their escape from endosomes into the cytosol, where they are translated into the antigenic protein.[1] The structure of mRNA-LNPs plays a key role in the stability of mRNA and its expression in cells, but is still largely unknown. Dynamic Nuclear Polarisation (DNP) NMR techniques can be applied to better understand the architecture of these vaccine formulations and to study the arrangement of their different components.[2] Notably, the propagation of the polarization as a function of micro-wave irradiation time can provide information about the geometry of the investigated particles.[3] Here, we will show preliminary DNP MAS NMR data acquired on LNPs in vaccine-like formulations and present how key structural information can be extracted.

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Interaction dynamics of ySERF protein and TAR complex from the RNA perspective

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SERF is a small RNA binding protein, presenting an intrinsically disordered region which plays a crucial role as a regulator of age-related proteotoxicity. SERF disordered N-terminal region is conserved in homologs of the protein which are between 60 to 80 residues in length. As an RNA binding disordered protein, SERF forms droplets with RNA in a concentration dependent manner. TAR is a powerful candidate to be a model partner for SERF as a well-characterized, stable and dynamic RNA, in order to investigate the basic biophysical principle underlying such interaction.

In order to observe the interaction, unlabelled TAR has been titrated to labelled ySERF and the interaction has been observed in the disordered N-terminal region. Moreover, a variety of other biochemical experiments shows that SERF interacts with TAR and forms a compact complex.

On the other hand, it is very interesting to investigate the interaction from the RNA point of view, which is a complementary perspective to understand the behaviour of the complex. Hence, titration experiments of unlabelled ySERF into ^{13}C and ^{15}N labelled TAR has been performed with the ratio of from 0.1 to 5 ([SERF]/[TAR]). Canonical Watson-Crick base-pairs of TAR helices are observed using ^{15}N HSQCs throughout the titration, demonstrating that the secondary structure of TAR is largely conserved. Besides that, it is possible to locate the interaction interface on TAR by using ^{13}C HSQC experiments. High chemical shift perturbations on the bulge region of TAR are pointing towards a principal interaction site in and around the bulge.

In this study it's been shown that TAR RNA is interacting with ySERF protein. While TAR secondary structure is conserved during this interaction, the bulge region of TAR is crucial and additional experiments will be needed to determine how this process affects the overall interhelical motion of TAR in presence of ySERF.

Probing surface chemistry of halogenated perovskite nanocrystals using NMR and Dynamic Nuclear Polarization

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Abstract:

In the development of colloidal nanocrystal synthesis, the discovery of $CsPbBr_3$ nanocrystals in 2015 and their unique light-emitting properties have generated significant research interest.[1–3] Like for most semiconductor nanocrystals, the ligands are key to control both the growth (size and shape), but also the colloidal stability of halogenated perovskite nanocrystals (PNCs). In addition, the ionic nature of the core (Figure 1a) makes them very different from other covalent semiconductor NCs (CdSe, ZnO, ZnS, etc.), and particularly prone to degradation from factors such as light, humidity, and heat. Understanding the surface chemistry of these systems is thus mandatory to further improve rationally both the reproducibility of hot-injection synthesis (using the so called native ligands), and the colloidal stability through ligand-exchange. Native ligands (Figure 1b) for $CsPbBr_3$ NCs are typically composed of long chain alkylammonium (e.g. oleylammonium) and carboxylates (e.g. oleate). Recently, several research groups have used theoretical and experimental approaches to study the surface termination of these systems and the role of passivating ligands. This includes the use of both liquid and solid-state NMR approaches.[4–6]

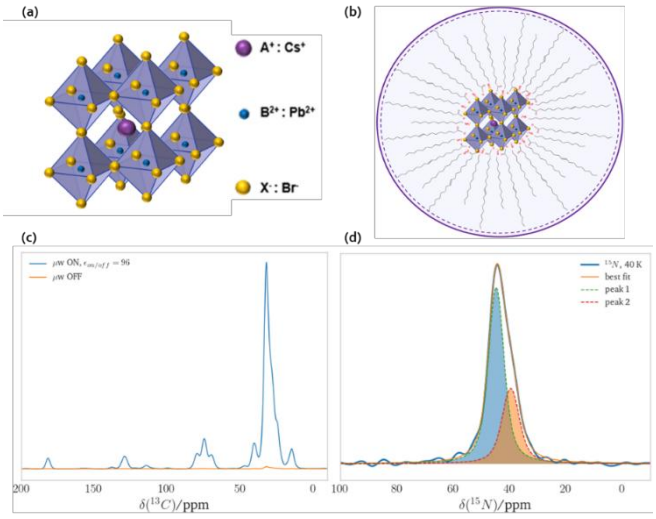

Figure 1. a), b) Scheme of the inorganic core (a) surrounded by the organic ligand shell (b) of perovskite ($CsPbBr_3$), c) Direct detected ^1H-^{13}C CP MAS-DNP spectra of $CsPbBr_3$ NCs with UDPA at 40K (c-AsymPolTEK), d) ^{15}N CP MAS-DNP spectra of $CsPbBr_3$ NCs with UDPA at 40K (c-AsymPolTEK)

Despite these very interesting contributions, there are still many open questions to be addressed, to rationally improve the surface chemistry of these key systems. In this work, we discuss the results obtained on $CsPbBr_3$ NCs, where we used a combination of liquid-, solid-state NMR, and Dynamic Nuclear Polarization (DNP) experiments. During the course of this work, we modify a few steps of the hot injection[6] synthesis recently reported to improve the quality and the stability of the NCs, and obtain monodisperse cuboidal NCs. Then, we raise the question of the best procedure to prepare PNCs for solid-state NMR measurements, given that the native ligands are extremely labile and that the purification and sample preparation steps might significantly modify the ligand/core interface and possibly even the NC surface termination. The synthesized NCs were characterized using UV-Vis absorption/photoluminescence, SEM, AFM, TEM to complement the NMR data. Finally, we discuss the implementation and the challenges associated with conducting DNP experiments on PNCs. We report for the first time efficient DNP experiments on $CsPbBr_3$ NCs, which is crucial for improving the atomic scale description of the QD surface. Notably, we compare the DNP efficiency that can be achieved using TEKPol[7] and AsymPol-TEK[8] (that we recently developed) polarizing agents at 100 and 40 K (Figure 1c and d) using a homebuilt closed-cycle helium spinning system and DNP probe.[9]

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New MAS-DNP NMR methods towards the characterization of extremely dilute surface species in platinum-based heterogenous catalysts.

Thomas C. Robinson, Zhuoran Wang, Laura A. Völker, Alexander Yakimov, Domenico Gioffré, David Gajan, Aaron Rossini, Christophe Copéret, Anne Lesage

Platinum is a metal center at the core of many heterogeneous catalysts that can be probed by ^{195}Pt MAS NMR spectroscopy, providing detailed structural information on its molecular environment. Despite the favorable spectroscopic properties of ^{195}Pt, which has a spin quantum number I=1/2 and a relatively high receptivity, until recently, platinum NMR was rarely performed to study catalytic surfaces. Indeed, the large CSA experienced by ^{195}Pt spins combined with the low concentration of sites make this technique extremely insensitive. It has been previously shown that this sensitivity limitation could be overcome with DNP.[1,2] As an alternative approach, the ^{195}Pt NMR signature of surface metal centers can be indirectly detected through sensitive spy nuclei such as ^{1}H or ^{31}P.[3]

We have recently introduced a novel DNP Surface Enhanced NMR (DNP SENS) methodology under fast MAS frequencies (~40 kHz) to indirectly detect ^{195}Pt NMR parameters using neighboring insensitive spy nuclei by taking advantage of through-bond *J*-couplings. This approach has been developed on a Pt-N-heterocyclic carbene material[4], a prototypical example of isolated Pt sites on catalytic surfaces and is now being extended to a platinum-nitrogen molecular complex as well as on single atom catalyst, namely platinum deposited on carbon nitride, a challenging new class of materials [(5)]. Double resonance ^{13}C{^{195}Pt} and ^{15}N{^{195}Pt} experiments are implemented that allow one to detect the full CSA pattern of the Pt nuclei in a site-specific way, provided new insights into the coordination sphere of the metal center.

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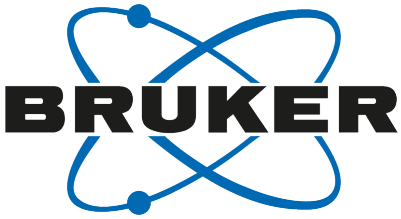
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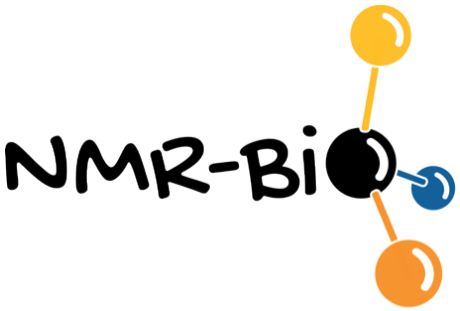
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