
Premières journées
Grand-Sud de RMN
FACULTÉ DE MÉDECINE
Programme &
Livre des résumés
30 et 31 mai 2016, Montpellier

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Programme

Journées Grand-Sud de RMN
(30-31 Mai 2016)
(Amphithéâtre d'Anatomie, "ancienne" Faculté de Médecine, Montpellier)

Lundi 30 mai

16h00 – 19h00 : Accueil des participants et dégustation de produits régionaux* dans le cadre exceptionnel de l'ancienne Faculté de Médecine de Montpellier (XII^{ème} siècle)

19h00 : **Ouverture des journées** (**Christian Roumestand**, CBS, Montpellier)

Session "Mas Daumas Gassac", modérateur : C. Roumestand

19h15-19h55 : **Michel Zanca** (BioNanoNMRI, CHU Montpellier)
Looking for the functional brain: can we read in mind using functional magnetic resonance imaging?

Mardi 31 mai

Session "Pic Saint-Loup", modérateur : (à définir)

8h30 - 8h50 : **Jean-Marie Bonny** (AgroResonance, INRA, St-Genès-Champanelle)
Mitigation of functional sensitivity variations within the human brain for studying food behaviour by MRI.

8h50 - 9h10 : **Gilles Silly** (ICGM-UMR 5253, Montpellier)
^{1}H, ^{19}F, ^{13}C NMR investigation of fluoropolymers

9h10 - 9h30 : **Anne Lesage** (Institut des Sciences Analytiques, Villeurbanne)
Dynamic Nuclear Polarization at 40 kHz Magic Angle Spinning

9h30 - 9h50 : **Guilhem Pages** (AgroResonance, INRA, St-Genès-Champanelle)
Low- and high-field NMR spectrometry: complementary in the struggle against counterfeiting

9h50 – 10h20 : **pause café (30 min)**

**(à consommer avec modération)*

Session "Terrasses du Larzac", modérateur : (à définir)

10h20 - 10h40 : **Guy Lippens** (LISBP CNRS UMR 5504, Insa Toulouse)
NMR meets Tau: insights in its function and pathology

10h40 - 11h00 : **Antoine Loquet** (CBMN UMR5248, Pessac)
Functional amyloid fibrils controlling cell death investigated by solid-state NMR

11h00 - 11h20 : **Elise Delaforge** (IBS, Grenoble)
Large-Scale Conformational Dynamics Controls H5N1 Influenza Polymerase PB2 Binding to Importin α

11h20 - 11h40 : **Tiago Cordeiro** (CBS, Montpellier)
Protein disorder in retinoic acid receptor heterodimer regulation

12h00 - 13h00 : **pause repas (1h)**

13h00 - 15h00 : **session poster (2h)**

Session "Picpoul de Pinet",(modérateur : (à définir)

15h00 - 15h20 : **Erick Dufourc** (CBMN UMR5248, Pessac)
Magnetic and fluorescent phospholipids: enhanced properties for liposome science

15h20 - 15h40 : **Jia-Ying Guan** (IBS, Grenoble)
NMR and EM studies of a 1 MDa chaperonin in action

15h40 - 16h00 : **Kristaps Jaudzems** (CRMN, Lyon)
De novo 100 kHz MAS structure determination of a viral nucleocapsid and insights from high field DNP

16h00 - 16h20 : **Denis Lacabanne** (IBCP, Lyon)
Efficient and stable reconstitution of the ABC transporter BmrA for solid-state NMR studies

16h20 - 16h40 : **pause café (20 min)**

Session "Saint Saturnin", modérateur : (à définir)

16h40 - 17h00 : **Rémy Sounier** (IGF, Montpellier)
Conformational landscape of μ-opioid receptor bound to biased opiates.

17h00 - 17h20 : **Giulia Mollica** (ICR UMR7273, Marseille)
Off-MAS NMR for dipolar coupling measurements in multiple spin systems

17h20 - 17h40 : **Virginie Gervais** (IPBS, Toulouse)
NMR studies of a novel family of DNA Binding Protein: The THAP proteins

17h40 - 18h00 : **clôture**

Conférences

(Amphithéâtre d'Anatomie)

Looking for the functional brain: can we read in mind using functional magnetic resonance imaging?

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Current methods used for brain functional imaging in Nuclear Medicine give access to cerebral perfusion and metabolism levels, allowing the diagnosis of different pathologies like Alzheimer dementia for instance. In the same purpose, but essentially for physiological explorations, BOLD fMRI allows to detect the organization of sensory perception or action, or even to surprise the thought in rest condition. This became possible due to the non-invasiveness and the high spatial resolution magnetic resonance imaging (MRI) allows to obtain, both from 2D or 3D views, of the inside of an alive body, vegetable, animal, human being. The MRI technique indeed supplies at present great performances, as the best compromise between spatial high resolution, wealth and diversity of contrasts, easiness of use and a relative harmlessness, what makes it practically inescapable in the study of the alive.
It is so, among the numerous offered possibilities by the technique that the BOLD functional MRI allows to explore the human cerebral activity in situation of rest or action. Its use became almost inescapable if we try to understand the physiological and\or pathological mechanisms involved in the motor, neurosensory and cognitive behaviors. The technique became reliable and reproducible enough to be used in clinical routine where it allows exploring the brain in various domains of study, from the healthy physiological behavior to the pathological one, including psychology and even "neuromarketing" sensitivity for example. It allows more and more faithful modeling of the functioning of certain stages of the perceptive and creative thought.
The display of some physiological and pathological examples will allow illustrating some of the involved mechanisms and could let glimpse the scale of the offered possibilities.
Examples will be given in motor control, with application to abnormal movements surgery[1,2], in language[3], with application to epilepsy surgery, in audition, with application to schizophrenia, in olfaction, with application in wine testing[4,5] and in vision, with applications in thought spying[6,7] and in neuro-marketing.
Conclusion will open horizons as wide as dream allow, for instance in robotics, augmented man and…

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Mitigation of functional sensitivity variations within the human brain for studying food behaviour by MRI

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The susceptibility differences between air and soft tissues, and also between soft tissues themselves, lead to magnetic field inhomogeneities in the human brain, especially at high static magnetic fields. These inhomogeneities can be roughly categorized as a function of their sizes (micro-,meso- and macroscopic). Microscopic and mesoscopic inhomogeneities can be useful for many structural applications. Macroscopic ones induce various artifacts in images reconstructed from gradient-echoes combined with echo-planar spatial encoding, which is still the reference method for blood-oxygen-level-dependent functional MRI (BOLD fMRI). This is why the amplitude and orientation of the magnetic field gradients (MFG) must be known accurately, either for predicting BOLD sensitivity or for optimizing the acquisition parameters to avoid an excessive decrease in sensitivity.

To this end, MFGs were mapped in the human brain using a cohort of healthy volunteers at 3 T. We show that the vector symmetry of MFGs with respect to the inter-hemispheric plane was salient, mostly resulting to an antisymmetry of the left-right component. This antisymmetry can be explained by the usual position of the head with respect to the static main magnetic field and because most of the brain structures are mirrored along the inter-hemispheric plane. If MFGs are affected by significant inter-individual variability, this variability is substantially higher for magnitude than for direction. A conceptually important point is to link such consistent antisymmetry of MFG to the idea that this could possibly lead to inter-hemispheric differences in BOLD-sensitivity. This hypothesis remains speculative at this stage and further work is needed to test it (1).

In parallel to these findings regarding the gradient orientations, our results on MFG amplitudes confirmed that the temporal, frontal and occipital regions are the most affected by MFGs. A straightforward solution to mitigate BOLD-sensitivity modulations due to the most intense MFGs is to reduce the voxel volume. Accordingly we compared activations elicited by viewing food pictures in two scanning conditions differing only in the voxel volume. We found that high-resolution acquisition led to a better detection of activations when viewing food pictures (2). The activations detected by high spatial resolution were notably consistent with food-responsive regions. Furthermore, these regions were found activated bilaterally, in contrast with previous findings obtained with the conventional coarse voxel volume.

Several important food-responsive areas of the brain are known to suffer to different degrees from susceptibility artifacts, such as occipital lobe, orbitofrontal cortex, amygdala or insula. It is critical to correct BOLD-sensitivity modulations to obtain activation maps closer to the real network of neural responses. Our work addressed this issue by further questioning, but also by proposing generic solutions which are now used in our daily practice.

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^{1}H, ^{19}F, ^{13}C NMR investigation of fluoropolymers

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Fluoropolymers can take on an amazing variety of characteristics depending on the details of their molecular structures. Modern methods of polymer synthesis have been adapted to provide tremendous flexibility in designing fluoropolymer structures so that materials can be prepared for a variety of applications. On one hand understanding the microstructures of these polymers is essential to understand the reaction mechanism and to modify synthetic strategies in order to obtain the desired microstructure and on the other hand it allows to probe their structure–property relationships and to improve the overall performance of fluoropolymer materials.
Fluoropolymers have three NMR active nuclei (^{1}H, ^{19}F, and ^{13}C) that are ideally suited for creating useful NMR experiments (c.f. Tab. 1). These three NMR active nuclei provide three separate probes for studying their structures in simple 1D-NMR experiments.

Tab. 1 : Basic NMR Characteristics of ^{1}H, ^{19}F, and ^{13}C

Nuclei	Spin, I	Natural abundance (%)	Frequency (MHz, at 9.4T)	Receptivity relative to ^{13}C	Reference compound	Shift range (ppm)
^{1}H	1/2	99.9885	400	5.87×10^{3}	Me_4Si	15
^{19}F	1/2	100	376.3	4.90×10^{3}	$CFCl_3$	> 300
^{13}C	1/2	1.07	100.6	1	Me_4Si	> 250

Tab. 2 summarizes typical J-couplings found in the organic polymers. It appears that the fluorocarbon-based polymers also have some unique NMR characteristics because of the ^{19}F nuclei which produce special homo- and heteronuclear J-couplings. Hence taking advantage of the enormous flexibility of modern NMR instruments to control pulse timing events, an elaborate array of multidimensional NMR experiments can be implemented for complex structure determination.

Tab. 2 : Ranges of Typical $^{n}J_{XY}$ Couplings in Fluoropolymers

n	$^{n}J_{CH}$ (Hz)	$^{n}J_{CF}$ (Hz)	$^{n}J_{HF}$ (Hz)	$^{n}J_{FF}$(Hz)	$^{n}J_{HH}$(Hz)
1	125-175	250-350	-	-	-
2	0-5	30-50	30-50	200-300	10-20
3	3-8	5-10	8-15	5-15	3-8
4	-	-	-	10-90	-
5	-	-	-	2-30	-
6-7	-	-	-	1-7	-

The main difficultly in implementation of sophisticated pulse sequences comes from the large chemical shift range of ^{19}F which implies to make intensive use of composite and adiabatic pulses either for decoupling or inversion/refocusing purposes. However thanks to the high natural abundance and sensitivity of both ^{1}H and ^{19}F, good signal to noise ratios are easily obtained. Then simple 2D correlations experiments designs as HETCOR or HETERO-COSY[1] which became obsolete because of their poor sensitivity in $^{1}H\{^{13}C\}$ experiments can be re-imagined.

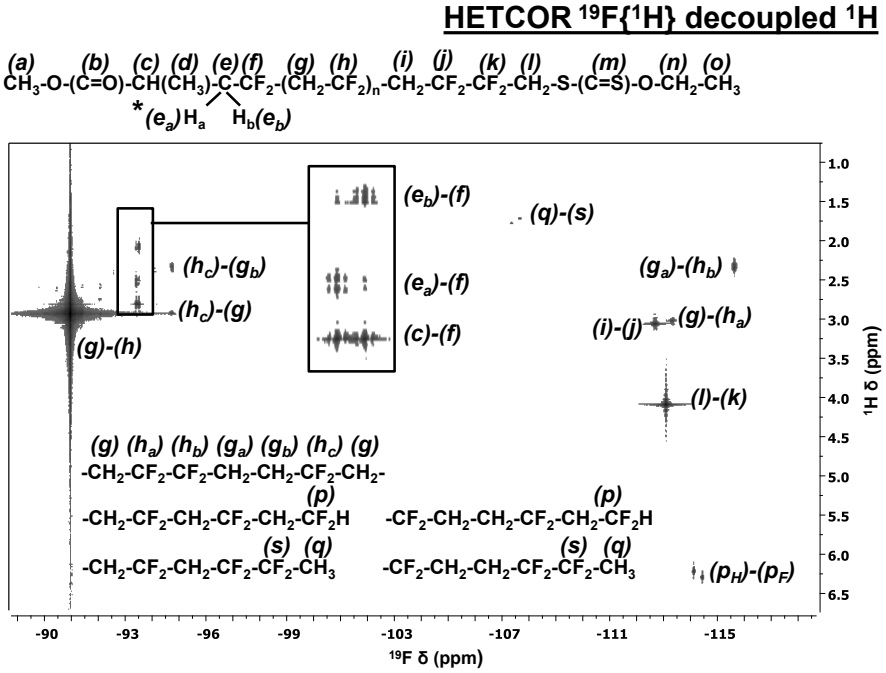

Fig. 1 : $^{19}F\{^{1}H\}$ HETCOR spectrum of PVDF51-XA in $(CD_3)_2CO$. Vertical axis: ^{1}H NMR chemical shift; horizontal axis: ^{19}F NMR chemical shift decoupled ^{1}H.

The aim of this publication is to present how advanced ^{1}H, ^{13}C and ^{19}F NMR spectroscopy experiments using decoupling strategies allow obtaining a precise description of fluoropolymers. For instance the microstructure of the PVDF chains synthesized using RAFT polymerization as recently been investigated[2]. In particular a very detailed description of the different α- and ω-chain-ends of PVDF51-XA is obtained and an uncommon NMR heteronuclear coupling between the proton of the stereocenter of the CTA R-group and the fluorine atoms of the CF_2 moiety of the first added VDF unit is revealed.

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Dynamic Nuclear Polarization at 40 kHz Magic Angle Spinning

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Solid-state NMR spectroscopy has become one of the most important anaytical techniques for structure elucidation and dynamic studies for biological samples as well as for a wide range of 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 Overhauser Effect (OE). Many aspects of these mechanisms have received much attention in order to improve the performance of DNP. Thus the design of optimized binitroxide polarization sources made it possible to enhance the NMR signals by more than 2 orders of magnitude at 9.4 T and 100 K (1).

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 CE, simulations predict a decrease of the enhancements with increasing MAS rate (2-5). Using a prototype 1.3 mm MAS probe operating at 18.8 T 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 report enhancements of around 60 over a range of sample spinning rates from 10 to 40 kHz (6). We will present new investigations on the MAS frequency dependence of other DNP mechanisms. The overall sensitivity enhancement factor in this spinning regime will be discussed and new applications will be presented.

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Low- and high-field NMR spectrometry: complementary in the struggle against counterfeiting

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Due to their biological activity, drugs and dietary supplements (DS) are products for which a strict quality control must be performed. DS contain food-related products identified in traditional medicine as having therapeutic activities and these food supplements can be considered in between medicines and conventional foods. Both medicine and DS counterfeits are increasing; however, the counterfeiting is different for both products. For classical drugs, the counterfeiting is more often based on the excipients added to the drug, while for the DS counterfeiting consists in adding a synthetic molecule. The classical gold standard analytical method to detect counterfeiting is based on LC/MS. While its sensitivity is excellent, its specificity for detecting unknown adulteration can be problematic. NMR analysis has the advantage to analyse the product as a whole and does not do any assumption on the counterfeit type.

To analyse a complex mixture by NMR, diffusion NMR is the technique of choice. Complex mixtures often contain molecules with different sizes leading to significantly different diffusion coefficients. This is the case for medicines which contain small molecular weight excipients (SDS, triethyl citrate …) as well as macromolecules (povidone, starch …). In this case, it is difficult to observe simultaneously both types of molecules with an appropriate resolution. To improve the diffusion maps, we developed a new gradient sampling strategy based on a sigmoid shape. This shape allows sampling a significant number of points at both low and high gradient strengths. We demonstrated the proof of concept of our approach by deformulating two drugs, one of known and the other one of unknown composition [1].

DS counterfeiting, especially for products bought on the Internet, is frequent. Due to its identification and quantification capabilities, NMR is the technique of choice for detecting counterfeiting. Unfortunately, inside a quality control laboratory, high-field NMR spectrometers are not present (cost, skills…). We demonstrated that sexual enhancement DS counterfeits can be detected on a low-field cryogen-free benchtop NMR. After recording reference spectra, the manufacturer added molecule(s) was (were) identified inside the adulterated DS. The quantification of the added molecule was performed on one DS. We found similar values as those measured on a high-field NMR spectrometer [2].

In conclusion, we demonstrated the interest of both low- and high-field NMR spectroscopies to analyse medicines and DS. Using diffusion NMR with a new gradient sampling ramp at high-field we partially deformulated a drug of unknown composition. Low-field NMR can be used as a counterfeiting screening method. Thanks to its compact size and user-friendly interface, this type of spectrometer can readily be used inside quality control laboratories.

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Guy LIPPENS

NMR meets Tau: insights in its function and pathology

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The tubulin associated unit (Tau) is a remarkable protein. First discovered as a protein factor that promotes the assembly of tubulin into microtubules (MTs) [1], it has gained further notoriety when it was identified as the principal component of the tangles that characterize neurons of Alzheimer's diseased (AD) patients [2–5]. It was equally one of the first recognized examples of an intrinsically disordered protein (IDP) [6], but how exactly it exerts its function(s) is still a point of debate. Other functions than MT stabilization in the neuronal axons have more recently been described, and concern both synaptic [7] as nuclear [8,9] localizations. The possible spreading of (toxic) Tau forms from one neuron to another is another tantalizing aspect that might be at the origin of the spatio-temporal hierarchy of AD [10,11], but a clear molecular definition of the propagating species – monomer [12,13], dimer [14] or oligomers [15–17] is not yet available . Finally, post-translational modifications (PTMs) seem to regulate its function or dysfunction in a most complex manner. Phosphorylation is the most studied PTM, and AD Tau is often described as a hyper-phosphorylated form [18], although neither a list of exact sites or their stoichiometry are associated with this term. Equally, its implication in the spreading of Tau is still subject of debate [19]. Recently, other modifications such as lysine acetylation [20,21] or proline isomerization [22] have also been linked to its dysfunction, but the field lacks mechanistic insights in how these modifications might interfere with its function and/or promote its aggregation. Therefore, despite it being around for nearly forty years, Tau still mobilizes extensive research efforts.

Over the last ten years, we and others have applied biophysical approaches including high-resolution NMR spectroscopy to both the functional and pathological aspects of Tau. The assignment of the ^{1}H, ^{15}N HSQC spectrum has been a long-time technical effort that we started ten years ago, [23–27] and we have been joined in this effort by other research groups [28–30] to such a point that the full spectrum has been completely assigned.

In this presentation, I will not consider the technical progress that has led to this full assignment, but rather focus on what we have learned from these NMR studies, considering both the mechanistic details of the Tau:tubulin relationship and of the aggregation process. Mechanistically, we understand better how Tau through its flexibility can exert its function.

Solid-state NMR results of Tau on full-length stabilized microtubules confirm this remarkable flexibility for such a tight complex.

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Antoine LOQUET

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.

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Elise DELAFORGE

Large-Scale Conformational Dynamics Controls H5N1 Influenza Polymerase PB2 Binding to Importin α

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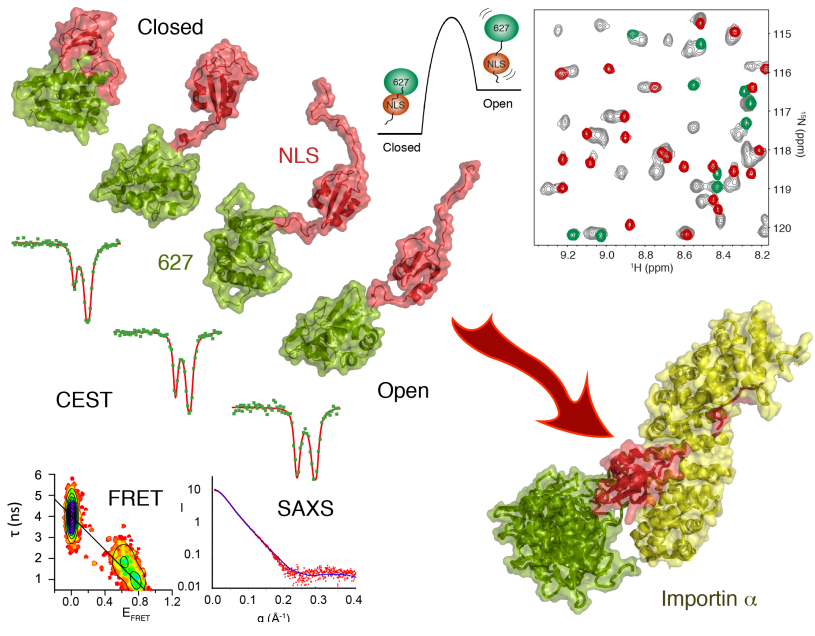
Influenza A RNA polymerase is formed from three components, PA, PB1, and PB2. PB2 is independently imported into the nucleus prior to polymerase reconstitution. All crystallographic structures of the PB2 C-terminus reveal two globular domains, 627 and NLS, that form a tightly packed heterodimer that 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.[1]

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.

1. Large Scale Conformational Dynamics Control H5N1 Influenza Polymerase PB2 Binding to Importin α. Delaforge et al ***J Am Chem Soc*** 137,15122-34 (2015).



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.

Tiago CORDEIRO

PROTEIN DISORDER IN RETINOIC ACID RECEPTOR HETERODIMER REGULATION

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Nuclear receptors are engaged in transcription regulation of a myriad of human genes in response to specific ligand binding via allosteric activation of the C-termini helix (referred to as helix H12) of their ligand-binding domain. These receptors transduce signals from ligand binding to regulate gene expression by recruiting co-regulator proteins that modify the chromatin and the associated transcriptional complex. Herein, the human regulatory complex of the retinoic receptor heterodimer (RAR/RXR) bound to the intrinsically disordered co-repressor N-CoR was studied by integrating solution-state NMR and small-angle X-ray scattering. We show that N-CoR binds to the heterodimer as a multi-site fuzzy complex. The conformational distribution of the complex is modulated by cognate ligands, point mutations or the removal of the flexible C-terminus of RXR. Paramagnetic relaxation enhancements reveal that transient long-range contacts are present between sequentially distant functional regions of N-CoR. Herein we show that in the absence of ligand, helices H12 of RXR and RAR are disordered. Selective RAR agonist, Am580, induces folding of H12, whereas in the presence of the inverse agonist BMS493, H12 stays largely disordered. These results substantiate a link between the structural dynamics of H12 and RXR–RAR heterodimer biological functions, and highlights disordered-to-order transition as an essential mechanism for retinoic acid mediated regulation. Intrinsic disorder is substantially embedded in the synergetic regulation of RXR–RAR.

This work has been carried out with financial aid of the Agence Nationale de la Recherche (SPIN-HD ANR-CHEX 2011).

Erick DUFOURC

Magnetic and fluorescent phospholipids: enhanced properties for liposome science

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Lipids are essential components of cell membranes and may also serve in pharmacological or cosmetic issues when they are under the form of liposomes. Understanding the mechanical properties of membranes and tuning their deformability is of great interest in biology and medicine. We introduce a phospholipid that contains a biphenyl function in one of its fatty acyl chains and is capable of forming micrometre multilamellar vesicles. Besides the very powerful fluorescence brought by the biphenyl unit the liposomes are also magnetically deformable into oblates in magnetic fields greater than 10 Tesla. We show that the deformation obeys the theory of membrane elasticity, as it is dependent on the magnetic susceptibility of the molecule, the membrane fluidity and thickness and on the strength of magnetic fields. As a control, liposomes made of saturated chains lipids are not fluorescent and shown to deform into prolates under magnetic field action. We also discovered a very interesting property, the deformation remnance, *i.e.*, the biphenyl liposomes stay deformed for several hours after having been removed from the magnetic field influence. Our findings might inspire the developments of fluid materials that can be tracked by fluorescence and deformed by magnetic fields.

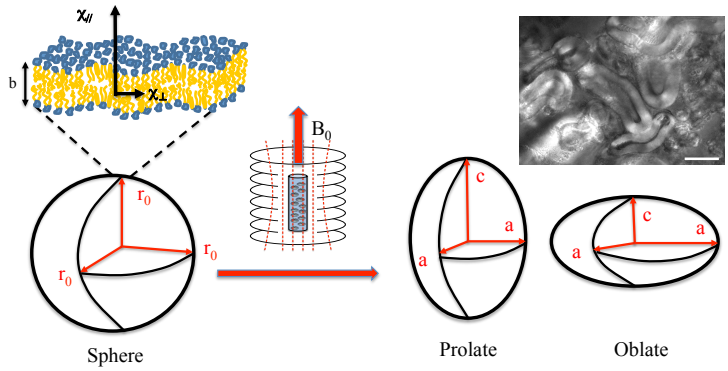

Figure 1. Schematic representation of the deformation of multi-bilayered liposomes into prolates or oblates when placed into a magnetic field. The deformation measured as the difference between the ellipsoid axes (c-a) depends on magnetic field strength B_0, bilayer thickness, b, curvature elasticity, k_C, lipid magnetic susceptibility anisotropy, $\Delta\chi = \chi_{\parallel} - \chi_{\perp}$, as predicted by Helfrich [1,2]: $c - a = -f\frac{r_0^3 \Delta\chi b B_0^2}{\mu_0 k_C}$, where f is a constant r_0 the radius of the spheroidal liposome before deformation and m_0 the magnetic permittivity. On the upper right of the figure a phase contrast microscope image illustrates the deformation (scale bar 1mm).

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Jia-Ying GUAN

NMR and EM studies of a 1 MDa chaperonin in action

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Chaperonins are huge ATP-dependent chaperones (~1 MDa) that are essential for proper cellular protein folding, and have shown links to many human diseases, including neurodegenerative disorders and cancer. These protein assemblies form giant "folding cages" to isolate and mediate the refolding of misfolded proteins. Atomic details of the mechanism of these nanomachines and interaction with substrate proteins remain limited due to the huge size and the dynamic feature of the complexes.

NMR spectroscopy offers a unique ability to monitor structural and dynamic changes at atomic resolution. Recent developments in specific isotope labeling of methyl groups in a perdeuterated protein have significantly extended the frontier of NMR in the study of challenging biomolecules.[1, 2] We use a combination of strategies, including advanced isotope labeling for NMR[3–5] integrated with complementary electron microscopy (EM) to probe the modes of actions of a 1 MDa chaperonin with its substrate protein.

We observed the structural rearrangement corresponding to the different states during the functional cycle of this large biological machinery processing its substrates at atomic resolution. Interestingly, we observed direct evidence for the unfoldase activity of chaperonins in absence of ATP, and characterized the dynamical state of proteins encapsulated in the folding chamber. Binding of ATP triggers the release and refolding of the substrate protein in the closed cavity of the chaperonin.

We demonstrate that the interaction between the chaperonin and the substrate protein can be studied by a combined approach using NMR and EM. This combined approach allows us to characterize MDa-large functional protein complexes in a time- and atomic-resolved manner.

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

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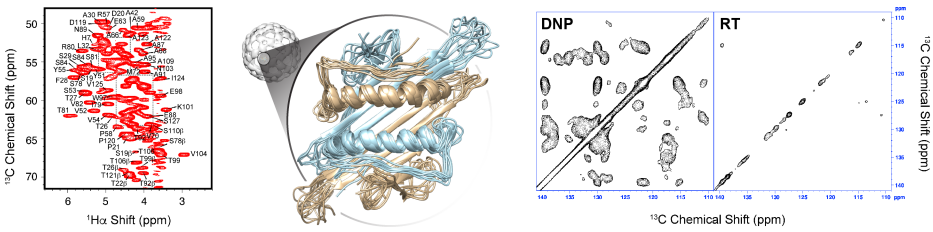
The atomic-level characterization of large viral particles is one of the greatest challenges of modern structural biology, as well as a fundamental step for the design of effective antiviral treatments. Over the last decades, solid-state NMR (ssNMR) has developed into a powerful structural tool for studying 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 ^{1}H-^{1}H 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.



Hα-Cα region of a ^{13}C-^{1}H CP-HSQC of AP205 at 100 kHz MAS (left). ssNMR structure of the dimeric capsid subunit (middle). Comparison of the aromatic region of the 800 MHz ^{13}C-^{13}C 2D NMR correlation spectra of AP205 nucleocapsids using DNP at 100 K and using conventional NMR at 280 K (right).

Denis LACABANNE

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.

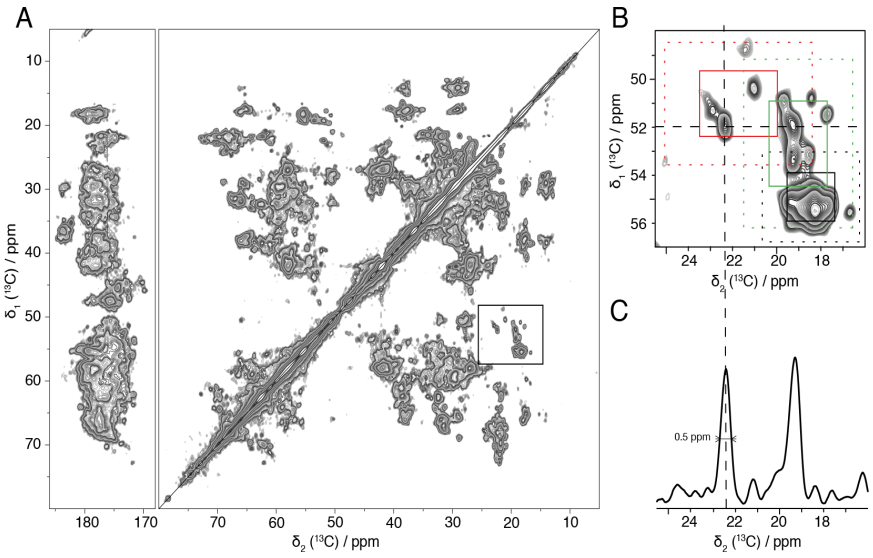

Figure 1. *(**A**) ^{13}C-^{13}C two-dimensional solid-state NMR correlation experiment of the protein BmrA (**B**) Extract of the alanine Cα-Cβ correlation, secondary structure regions for β-sheet (red), α-helical (black) and coil (green) from alanine signals are indicated. Solid lines correspond to the mean value with the standard deviation and dashed lines to the mean value with 2 times the standard deviation (**C**) 1D trace from the two-dimensional experiment at 52.1 ppm, the peak width at half-height is 0.5 ppm.*

Rémy SOUNIER

Conformational landscape of μ-opioid receptor bound to biased opiates.

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Opioids are the most widely used and effective analgesics for the treatment of pain. The most commonly used opioids for pain management act on μ-opioid receptors (μOR). μOR are activated by a structurally diverse spectrum of natural and synthetic agonists including endogenous endorphin peptides, morphine and methadone.

We recently described the structures of the μOR in inactive[1] and agonist-induced active states[2] providing snapshots of the receptor at the beginning and end of a signalling event. However, much remains to be learned about the mechanisms by which different agonists can induce distinct levels of G-protein activation and/or arrestin recruitment upon activation of μOR. In addition, we used liquid-state NMR spectroscopy to examine the process of μOR activation by monitoring signals from methyl-labelled lysines. Assignment of resonances is achieved by a mutagenesis approach. Our results show that there is a weak allosteric coupling between the agonist-binding pocket and G-protein-coupling interface (transmembrane segments 5 and 6). Furthermore, our analysis provided clues on the successive structural events leading to the full active conformation of μOR[3].

To go further, we propose to analyze the conformational landscape of the μOR in distinct pharmacological conditions. We carefully analysed the efficacies of several opioids using cell-based molecular pharmacology approaches relying on advanced fluorescence technologies and were able to find opiates presenting distinct efficacies in G-proteins activation, β-arrestins recruitment and μOR internalization. We are currently using these particular ligands in our NMR assays. In particular, we have developed a double labelling scheme to monitor signals from distinct μOR sub-domains. Our goal is to provide a mechanistic understanding of opioid receptor activation upon binding of ligands presenting distinct efficacy and/or biased signalling properties.

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Giulia MOLLICA

Off-MAS NMR for dipolar coupling measurements in multiple spin systems

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The dipolar coupling is one of the most important spin interactions in NMR as it provides a means for determining intermolecular distances, and from these, the geometry and conformation of molecules. In solid-state NMR, dipolar couplings are usually eliminated by spinning the sample around an axis oriented at the magic-angle with respect to the external magnetic field, but they can be purposely reintroduced during selected periods of the experiment using carefully designed pulse sequences.

In particular, the possibility of accessing weak homonuclear dipolar couplings, corresponding to long-range interactions, is of the utmost importance for crystal structure determination since they provide unique information about the conformational and crystal packing properties of the material. However, the measurement of weak homonuclear couplings remains difficult in the case where the sample contains clusters of many magnetic nuclei, for example carbon nuclei in uniformly labeled molecules, or proton nuclei in almost any organic material [1], because of the concomitant presence of stronger couplings dominating the spin dynamics.

Here we show that, combining off-magic-angle spinning, frequency-selective pulses and multiple quantum filtering, not only ^{13}C-^{13}C, but also ^{1}H-^{1}H dipolar couplings can be estimated in multiple-spin coupled networks [2,3]. The use of such couplings as constraints for NMR crystallography of small powdered organic molecules is discussed.

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Virginie GERVAIS

NMR STUDIES OF A NOVEL FAMILY OF DNA BINDING PROTEINS: THE THAP PROTEINS

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Thanatos-Associated proteins (THAP) define a large family of DNA-binding proteins sharing an evolutionary conserved C2CH zinc-coordinating domain (THAP domain) and controlling the expression of genes involved in diverse cellular processes.
THAP proteins use the THAP domain to bind to the DNA target sequences and subsequently recruit binding partners to achieve a desired regulatory effect. Outside the conserved THAP domain, THAP proteins differ in length and predicted motifs/domains in the remaining part of their sequences, including coiled-coils motifs.
Since the discovery of THAP proteins in the early 2000s (1), a few structural studies performed by us and others provided a number of high-resolution structures of THAP domains in their free state and in complex with DNA (2-4). We will present recent advances in the knowledge of the THAP domains structures and their interaction with DNA, obtained by NMR. We finally report the 3D structure of the coiled-coil domain of human THAP11, a recently characterized human THAP protein with important functions in transcription and cell growth in colon cancer (5).

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Affiches

(Salle Dugès)

Classement des affiches :
(Salle Dugès)

Numérotation croissante

P1	A.	Duconseille
P2	T.	Le Marchand
P3	A.	Bertarello
P4	D.	Cala-De Paepe
P5	K.	Sanders
P6	D.	Lalli
P7	L.	Lecoq
P8	T.	Schubeis
P9	J.	Stanek
P10	C.	Laguri
P11	E.	Colas-Debled
P12	L.	Andreas
P13	M.	Feracci
P14	F.	Ziarelli
P15	B.	Grenier
P16	T.	Texier Bonniot
P17	S.	Viel
P18	K.	Ainouch
P19	P.	Barthe
P20	K.	De Guillen
P21	H.	Demene
P22	A.	Gontier
P23	A.	Larcher
P24	M.	Vanheusden
P25	E.	Cahoreau
P26	Y.	Coppel
P27	G.	Ferre
P28	A.	Baudin
P29	M.	Berbon
P30	D.	Martinez
P31	C.	Rossy
P32	J.	Tolchard

Ordre alphabétique

K.	**Ainouch**	P18
L.	**Andreas**	P12
P.	**Barthe**	P19
A.	**Baudin**	P28
M.	**Berbon**	P29
A.	**Bertarello**	P3
E.	**Cahoreau**	P25
D.	**Cala-De Paepe**	P4
E.	**Colas-Debled**	P11
Y.	**Coppel**	P26
K.	**De Guillen**	P20
H.	**Demene**	P21
A.	**Duconseille**	P1
M.	**Feracci**	P13
G.	**Ferre**	P27
A.	**Gontier**	P22
B.	**Grenier**	P15
C.	**Laguri**	P10
D.	**Lalli**	P6
A.	**Larcher**	P23
T.	**Le Marchand**	P2
L.	**Lecoq**	P7
D.	**Martinez**	P30
C.	**Rossy**	P31
K.	**Sanders**	P5
T.	**Schubeis**	P8
J.	**Stanek**	P9
T.	**Texier Bonniot**	P16
J.	**Tolchard**	P32
M.	**Vanheusden**	P24
S.	**Viel**	P17
F.	**Ziarelli**	P14

Effect of the exposure of gelatin to various environmental conditions on its chemical composition

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The gelatin is an animal protein that comes from collagen skin or bone. It is used in pharmaceutical industry to make hard capsules. The variations of environmental conditions of production applied to the collagen and therefore gelatin may have an impact on its chemical composition and, as a consequence, on the capsules properties (1). Indeed, previous studies revealed that the gelatin tends to form cross-links between its amino acids in high temperature and humidity conditions or in the presence of chemical compounds as aldehydes (sugars, lipids, oxidations) (2). To understand the impact of the processing on the chemical composition of the gelatin, pig skins gelatin produced under different environmental conditions were analyzed in High-Resolution Magic Angle Spinning NMR (HRMAS). 1D proton NMR spectra were acquired and analyzed using PCA (Principal Components Analysis). The environmental conditions of production were discriminated and the spectral zones contributing to separate them showed differences on the amino acids composition and their structural arrangement. However, the abundance of amino acids masked the possible presence of other molecules. In our conditions, the HRMAS, alone, did not allow identifying all the molecules as the cross-links, sugars or lipids, but suggested the formation of Desmosine-type cross-link under one environmental condition of production.

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Investigation of amyloidogenic properties of D76N beta-2 microglobulin by Solid-State NMR

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β-2-microglobulin (β2m), is a 99-residue protein responsible for dialysis-related amyloidosis, and is recognized as a molecular archetype for the study of folding and amyloid transition processes. Fibrils of a newly described mutant of β2m (D76N), have been discovered in kindred patients suffering from gastrointestinal syndromes and autonomic neuropathy. The aptitude of this mutant to form fibrils in vitro has been revealed to be spectacularly higher than for the wild type protein. Understanding how a single point mutation can induce such a change in reactivity would provide a better understanding on the mechanism of formation of fibrils.

Using a combination of ultra-fast (>60 kHz) spinning rates with 100% NH re-protonation in a perdeuterated background and high magnetic fields, we acquire spectacularly resolved ^{1}H-detected correlations allowing resonance assignment of both native D76N β2m in microcrystalline form, and of the fibrils.

On one hand, the good resolution of the CP-based HSQC spectra of microcrystalline D76N and WT β2m allowed us to investigate the backbone dynamics by ^{15}N relaxation experiments. The analysis of R_1 and $R_{1\rho}$ data with a 1D Gaussian Axial Fluctuation (GAF) model gives site-specific information about nano- to milli-second backbone motions. The comparison of the dynamical behavior of the two proteins highlights regions of the protein where the D76N mutant is particularly destabilized, shedding light on the mechanism of formation of the fibrils.

On the other hand, the influence of growth conditions on D76N fibrils morphology was investigated. The comparison of fingerprint spectra and resonance assignment performed on two different samples of D76N fibrils brought to light a structural difference at the molecular level. For a further investigation of this highlighted polymorphism, homonuclear recoupling based experiments were set to find long-range ^{1}H-^{1}H contacts. The results provided strong evidence for a polymorphism occurring not only on the assembly of the protofilaments as previously observed for other kind of fibrils but at the level of the beta-sheets organization.

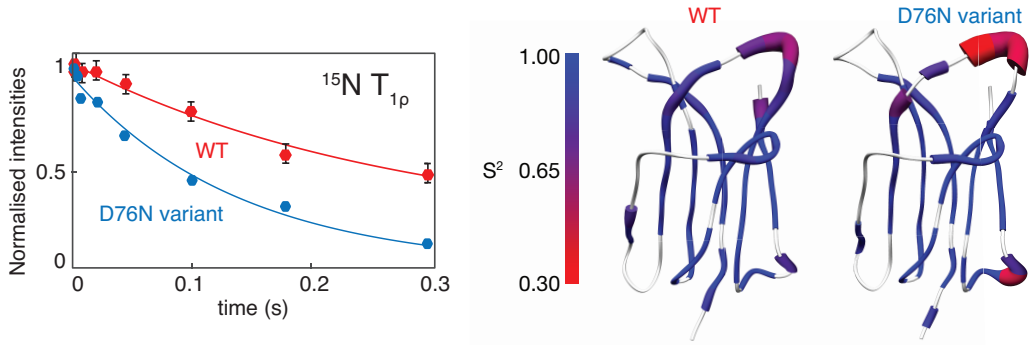

Figure 2: a) ^{15}N $R_{1\varrho}$ relaxation curves for residue E77 of microcrystalline D76N and WT beta-2 microglobulin. b) Comparison of the dynamics of D76N and WT beta-2 microglobulin: projection of the order parameters (S^2) onto the structure of WT b2m (PDB code: 2d4f).

P3 : A. BERTARELLO

Paramagnetic metalloproteins and fast magic-angle spinning: theory and experiments meet at the metal center

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We show how the application of a new set of NMR experiments, recently developed for the study of complex paramagnetic inorganic battery materials[1], can be adapted to the solid-state NMR analysis of paramagnetic metalloproteins, and can be used to improve the information obtainable from these systems. These experiments combine ultra-fast (60-111 kHz) magic-angle spinning frequencies and short high-powered adiabatic pulses (SHAPs)[2], and are applied to ^{13}C,^{15}N-labelled microcrystalline, metalloenzyme superoxide dismutase (SOD), which has two high-affinity binding sites for metal cations[3]. Here, by the use of the aforementioned experimental setup and first-principle pNMR calculations[4,5], we are able to detect, characterize and assign ^{1}H, ^{13}C and ^{15}N signals from residues directly coordinating the metal centers. The present work represents a robust approach to the NMR study of paramagnetic metalloproteins, opening a new avenue for the study of the structure and the reactivity of metal centers in complex insoluble systems.

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Faster probes and higher proton contents: when resolution meets sensitivity in biomolecular MAS NMR

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Proton dilution by perdeuteration is one of the most efficient ways to achieve narrow ^{1}H linewidths in biomolecular solid-state NMR. This is typically achieved by expressing a protein in a deuterated medium, and then reprotonating the amide groups by exchange in water buffers with controlled H_2O/D_2O ratios during the purification stage. This approach however impacts the sensitivity, prevents the observation of ^{1}H in the side-chains, and is not viable for samples that do not unfold reversibly. Fast MAS at high magnetic field is an additional key tool to narrow ^{1}H linewidths, allowing to record spectra with resolved ^{1}H lines from samples with progressively higher ^{1}H contents. In this work, we report the ^{1}H linewidths on a set of model microcrystalline protein samples spinning at rates from 20 kHz to 111 kHz. By investigating proteins with variable protonation levels, we show that fast MAS rates on small rotors conjugates the availability of resolved ^{1}H-^{15}N (backbone) and ^{1}H-^{13}C (side-chains) correlations with high sensitivity.

High Resolution solid-state NMR of paramagnetic systems using 111 kHz magic angle spinning

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Many important new technologies in chemistry, biochemistry, and materials science today, such as catalysts and battery electrodes, are composed of paramagnetic materials. Solid-state NMR studies of these materials suffer 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. These effects often lead to conventional solid-state NMR experiments failing to result in any observable signal under moderate MAS rates.

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. 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 side-band free ^{31}P MAS spectrum can be achieved in 1D on the very paramagnetic cathode material $LiFe_{0.25}Mn_{0.75}PO_4$, where large paramagnetic shifts and shift anisotropies span more than 4000 ppm (Figure 1).[1]

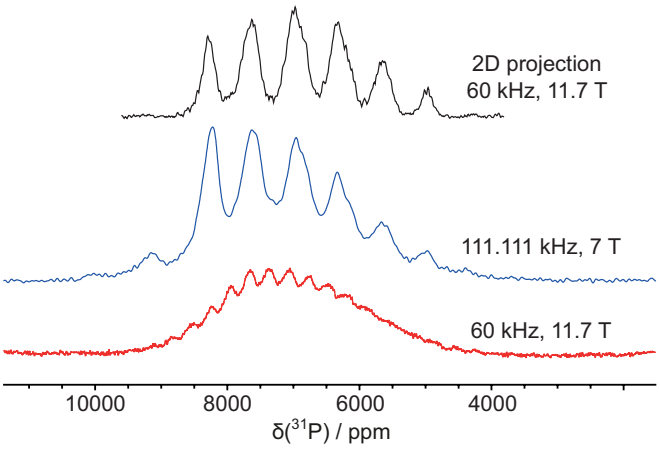

***Figure 1**. Comparison of rotation rate and field strength on $LiFe_{0.25}Mn_{0.75}PO_4$. The MAS spectrum in blue has nearly identical resolution to the isotropic projection of a 2D adiabatic MAT, without signal losses due to the short T_2 and without biased intensities due to differential relaxation effects at the various ^{31}P sites.*

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 very similar to single-sideband-selective adiabatic pulses (S^3APs),[3] however 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.

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P6 : D. LALLI

Understanding Structure and Function of the Membrane Protein Proteorhodopsin in Nanostructured Silica Host Materials by ss-NMR

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Nanostructured silica-surfactant membranes that incorporate high concentrations of the light-driven proton pump proteorhodopsin (PR) are novel materials promising for solar-to-electrochemical energy conversion. Importantly, the efficiency at which PR converts solar energy into a proton gradient is highly correlated with its structure. Optical absorbance measurements show a retained photo-activity function when the protein is incorporated in synthetic silica hosts, suggesting a retained global folding. High-resolution proton-detected ssNMR spectra of PR encapsulated in silica nano-channels conducted at high magnetic fields and ultra-fast magic angle sample spinning allow for structural studies. In particular, a comparison of multidimensional spectra of PR guests in native-like lipid environments and silica-surfactant hosts provides evidence that the protein retains its structure, explaining its retained functionality. Moreover, the spectra analysis yields a molecular characterization of the protein interactions within the nanostructured silica channels. These atomic-level insights provide important information for designing materials that effectively exploit PR for solar energy conversion.

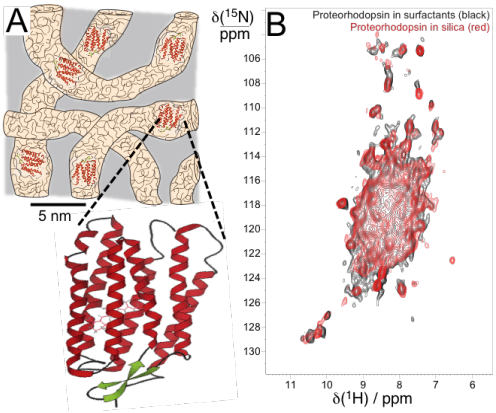

Fig. (1): (A) Schematic diagram of nanostructured silica-surfactant materials that include surfactant nanochannels (yellow), silica network (grey), and PR guest species (red). (B) Solid-state proton-detected 2D ^{1}H-^{15}N correlation spectra acquired on PR in nanostructured silica-surfactant materials (red trace) and in native-like surfactants (black trace).

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 Tfb1 subunit of TFIIH ($Tfb1_{PH}$) 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 $Tfb1_{PH}$ 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 $Tfb1_{PH}$ 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.

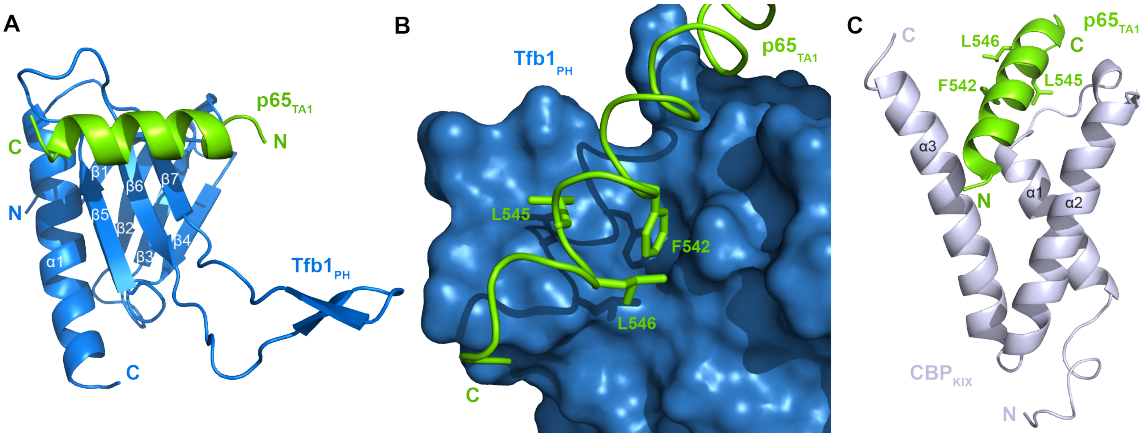

Figure 1: (A) *NMR structure of the complex between $p65_{TA1}$ (in green) and the Tfb1 subunit of TFIIH (in blue).* ***(B)*** *The three hydrophobic residues from the ΦXXΦΦ motif of p65 are involved in crucial interactions in the binding pocket.* ***(C)*** *HADDOCK model of the complex between $p65_{TA1}$ (in green) and CBP_{KIX} (in grey).*

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In-membrane solid state NMR of metalloenzymes and metal transporters

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Membrane proteins in lipid bilayers are one of the most exciting target for solid-state NMR since they are not easily accessible by other methods traditionally used in structural biology. In the most common approach, proteins are purified with detergents and reconstituted into lipids (e.g. liposomes). However, the use of detergent often induces structural inhomogeneity, and overcoming this step would be favorable. Indeed, recent studies have demonstrated the possibility to investigate proteins in their native membrane environment. Low sensitivity, though, presents a serious challenge in this approach.

The possibility to increase spinning speeds by using very small magic angle spinning (MAS) rotors is enabling the measurement of proton detected solid state NMR of very small amounts of sample. This methodology has been successfully applied to a range of proteins including membrane systems and paramagnetic metalloproteins. The latter ones particularly benefit from fast MAS since paramagnetic effects can be detected with high sensitivity and even exploited for obtaining long range structural information.

Based on this, we are planning to apply fast MAS solid state NMR to a lipid embedded membrane protein capable of binding paramagnetic metal ions. We chose the XXX and the inner membrane divalent cation transporter CorA from *Escherichia coli*. Initial steps aim at the measurement of well resolved spectra of 13C,15N-enriched proteins. We will present our first results on the optimization of protein overexpression and membrane isolation. The presented data tackle various aspects of sample preparation for in-membrane solid state NMR measurements. Furthermore, sensitivity based shortcomings and biochemical approaches to overcome them will be discussed

Aliphatic Resonance Assignment in Fully-Protonated Proteins with 100 kHz Magic-Angle Spinning NMR

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Sequence-specific assignment of resonances is a critical, and often time consuming step on the path to determination of site-specific protein structural and dynamical information.

Under magic-angle spinning (MAS), sequence-specific resonance assignment is accomplished primarily by inter-residue matching with ^{13}C shifts, either using ^{13}C detection or, more recently, ^{1}H detection. With the progressive development of both labeling schemes and of hardware capable of higher MAS frequencies, resonance assignment with ^{1}H detection has matured into a methodology that significantly reduces the acquisition time while also improving confidence in the result. This approach requires pairs of spectra to establish inter-residue correlations, ideally making use of six H^N-N spectra simultaneously correlating CO, Cα and Cβ resonances to resolve degeneracies for sequential connectivities.(1)

Here we introduce a new approach for resonance assignment that is enabled by very fast (100 kHz and above) MAS at magnetic field of 1 GHz. Under these experimental conditions, narrow (<150 Hz) amide and aliphatic ^{1}H resonances can be recorded in fully protonated molecules. Additionally, long ^{13}C and ^{15}N coherence lifetimes can be accessed under low-power ^{1}H decoupling, enabling several consecutive J-mediated coherence transfers along the protein backbone.

These two features are exploited with the design of four new ^{1}H-detected experiments, which correlate either the amide ^{15}N-^{1}H pair or the ^{13}Cα-^{1}Hα pair of a central residue *i* with the amide ^{15}N of residue *i*+1 and *i*-1, respectively. Sequential alignment is obtained by joint analysis of signals from pairs of 3D datasets, and sequence-specific alignment results from matching with ^{15}N frequencies,(2) contrary to traditional ^{13}C matching (Figure 1). These new datasets contribute to a high level of redundancy for establishing sequential connectivity, allowing extension of ^{1}H-detected methods to fully protonated targets of considerable size, of importance in the cases where protein deuteration and amide back-exchange are not feasible.

The different datasets can be simultaneously recorded in a single experiment, introducing a significant savings in experimental time. This is achieved by exploiting the largely independent sources of magnetization provided by $^{1}H^N$ and ^{1}Hα in a protonated protein, adjusting the RF irradiation so to follow different (up to 8) independent pathways,(3) and simultaneous detecting $^{1}H^N$ and ^{1}Hα signals (Figure 2).

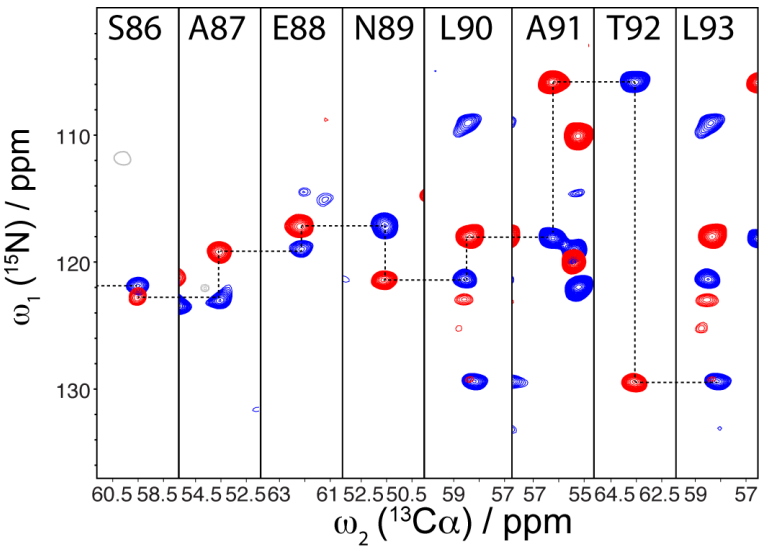

Figure 1 (left). 2D ^{15}N-^{13}Cα planes (strips) from two novel complementary ^{1}Hα–detected experiments for sequential assignment, 3D (H)NCAHA (*in blue*) and (H)N(CO)CAHA (*in red*), acquired on a ^{13}C,^{15}N-labelled microcrystalline sample of nucleocapsid AP205 (90 repeats of a swap-dimer, 90 x 2 x 131 aa, MW=2.5 MDa) in a 0.7mm rotor spinning at 100 kHz and 1 GHz spectrometer.

The approach is demonstrated with both microcrystalline and sedimented fully protonated proteins, with as little as 0.5 mg protein sample. Notably, this novel solid-state NMR methodology yields resonance assignments in the hexameric DnaB (454 aa, 6 x 50.6 kDa) of *G. Stearothermophilus*, and allows chemical shift perturbation studies following binding to the DnaG partner protein.

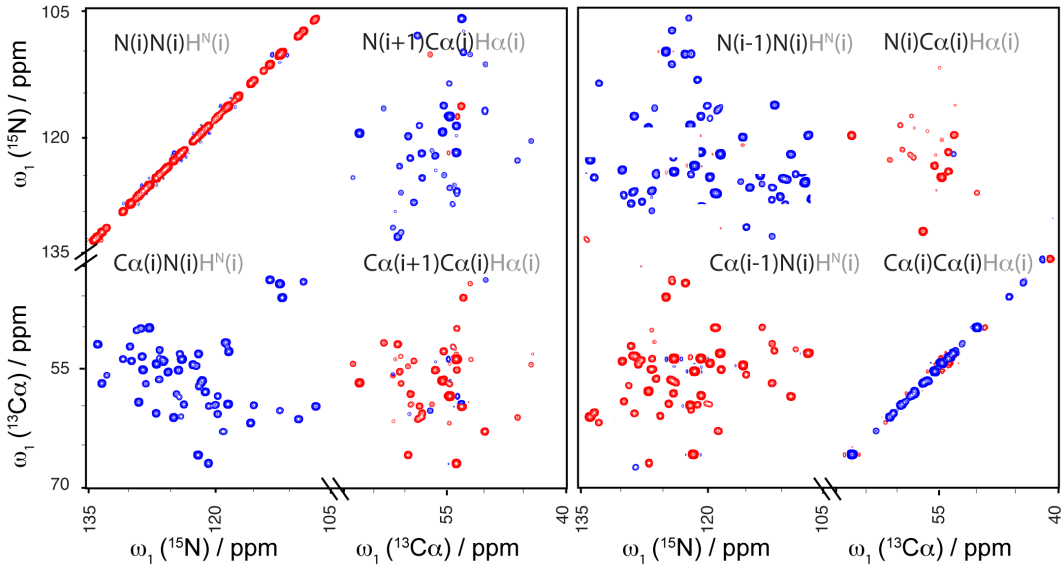

Figure 2. 2D ^{13}Cα-^{15}N, ^{13}Cα-^{13}Cα and ^{15}N-^{15}N projections from 3D (H)N(CACO)NH, (H)CA(CO)NH, (H)NNH, (H)CANH, (H)N(CO)CAHA, (H)CA(CO)CAHA, (H)N(CACO)CAHA and (H)CA(CO)CAHA simultaneously acquired on a ^{13}C,^{15}N-labelled microcrystalline GB1 sample in a 0.7mm rotor spinning at 100 kHz and 1 GHz spectrometer.

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Investigation of LipoPolySaccharide and its transport pathway by combination of liquid and solid-state NMR

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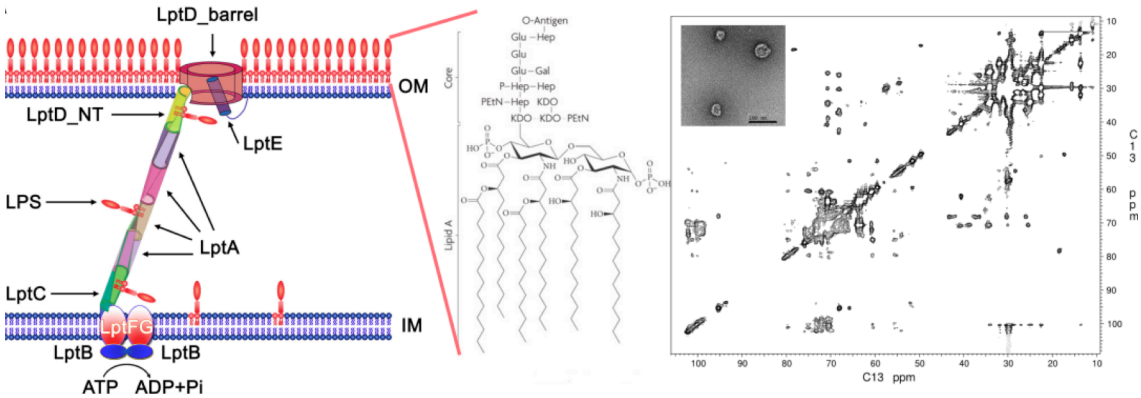
Gram negative bacteria possess two membranes separated by a periplasmic space. Their outer membrane (OM), in contact with the outside medium, is atypical as its inner leaflet is made of phospholipids and the outer leaflet of LipoPolySaccharides. These complex glycoconjugates are composed of Lipid A, several lipid chains linked to a phosphorylated disaccharide, a core oligosaccharide and an O-antigen, an highly variable oligo- to polysaccharide. The role of LPS is crucial in maintaining the bacteria physical integrity. Its lipid part constitutes an impermeable barrier and protect cells against osmotic pressure and the entry of outside compounds (e.g. antibiotics) and its variable O-antigen composition, can help bacteria evade the host immune system during infections.

The amphiphile nature of LPS and its intrinsic heterogeneity greatly complicates the study of its composition, macromolecular organisation and network of interactions. LPS are then usually studied in fragmented form following chemical treatments. Here we have used a combination of liquid and solid-state NMR to study intact ^{13}C labeled LPS from *E. coli* and the pathogenic bacterium *Pseudomonas* aeruginosa. Magic Angle Spinning NMR is an ideal technique to study insoluble compounds, such as LPS which has a high tendency to assemble into bilayers.



Localisation of LPS and its transport system (Lpt) in E. coli. Right Schematic view of LPS and Left 13C MAS ssNMR spectrum

On the other end, O-antigens and core oligosaccharides, which are more flexible can be observed by liquid state NMR. The combination of both techniques allows the study of the LPS molecule in its intact state but also analysis of the interaction with some of its ligands such as antibiotics.

In the bacteria the LPS molecules have to be constantly produced and exported to the outer-membrane (OM) in particular to ensure the division of bacteria. The LPS transport system is composed of seven proteins located in the inner, outer membrane and in the periplasmic space [1]. Here we focused on the study of two LPS transport proteins LptC and LptA that are responsible for the export of LPS across the periplasm in *E. coli* [2]. These two proteins interact with LPS and assemble into a complex that allows LPS transfer. The assembly of the transfer complex has been studied by liquid state NMR using Methyl specific labeling and protein mutants unable to oligomerise, as well as by Small Angles X-ray Scattering. Binding of the individual proteins, and complexes have been studied with intact LPS and model detergents to probe the interaction sites. These data all together shed light on the LPS transfer mechanism and opens perspectives for the discovery of new active molecules targeting the LPS transport system.

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NMR & Biochemical Characterization of the interaction between a 1MDa chaperonin and several substrates

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Chaperonins are molecular chaperones proteins involved in protein refolding processes. These macromolecules are organized in two rings piled on top of one another with a cavity in their respective centers. These rings can exhibit two different conformations, an open and a closed states. The transition between these two states involves a major conformational change, which is fueled by Adenosine Triphosphate (ATP) binding and hydrolysis. This conformational change allows the chaperonin to isolate and mediate the refolding of misfolded proteins. Amongst the different types of molecular chaperones, chaperonins are of particular interest because their mechanism of action is not yet totally understood.

NMR spectroscopy is a powerful tool to probe protein changes due to molecular interactions at atomic resolution. Especially, specific isotope labeling of methyl groups [(1-3)] is a technique of choice to study huge protein assemblies like chaperonin because they overcome the NMR size limitation (1-2). We use a combination of different biophysical techniques to complement the liquid and solid state NMR data, such as electron microscopy (EM) to probe the modes of actions of a 1 MDa chaperonin, along with different sizes and types of substrates.

To study the protein folding inside our model chaperonin, the thermosome, different model substrate of various sizes and biological functions were selected, such as aggregating and fibrilating proteins. Amylogenic proteins are of particular interest because they are involved in numerous human diseases. As they are unfolded, they are perfect candidates as thermosome ligands. We observed that our model chaperonin can prevent aggregation of different substrates and also influences fibrillation mechanisms.

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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 ^{1}H-^{1}H 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.

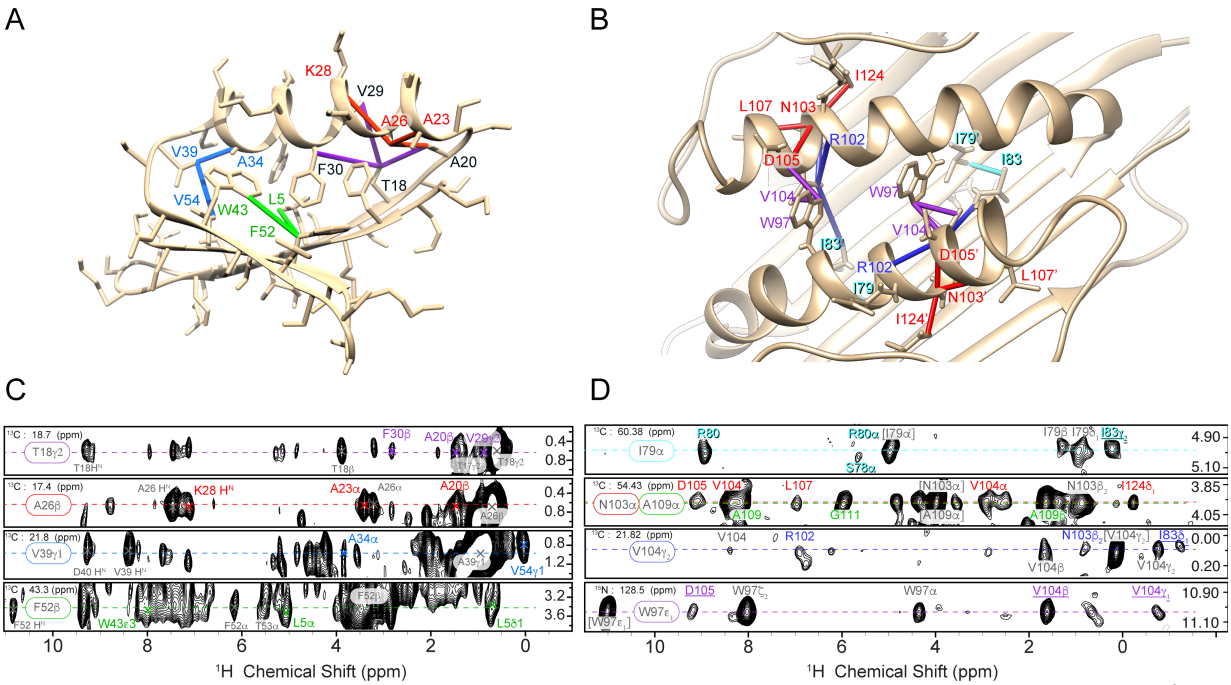

Figure 1. MAS NMR structures calculated from spectra encoding distances with 3D ^{1}H-^{1}H RFDR. A model protein (A,C) was used as a test case for the de novo structure determination of a dimeric 130 residue protein assembled in a 2.5 MDa particle (B,D).

Figure 1 shows the MAS NMR structures of a small microcrystalline protein (A) and a 130 residue nucleocapsid protein assembled in a ~2.5 MDa complex. Several of the long-range distances measured are indicated in the structure, and the corresponding strips from the ^{1}H-

^{1}H RFDR spectra are shown in panels (C) and (D). The structure calculation was performed using unsupervised protocols in UNIO-CANDID, which ensures an unbiased analysis.

Additionally, we find that narrower proton resonances, longer coherence lifetimes and improved magnetization transfer offset the reduced sample volume 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.

P13 : M. FERACCI

Structural basis of RNA recognition and dimerization by the STAR proteins T-STAR and Sam68.

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Sam68 and T-STAR are members of the STAR family of proteins that directly link signal transduction with post-transcriptional gene regulation. Sam68 controls the alternative splicing of many oncogenic proteins. T-STAR is a tissue-specific paralogue that regulates the alternative splicing of neuronal pre-mRNAs. STAR proteins differ from most splicing factors, in that they contain a single RNA-binding domain. Their specificity of RNA recognition is thought to arise from their property to homodimerize, but how dimerization influences their function remains unknown. Here, we establish at atomic resolution how T-STAR and Sam68 bind to RNA, revealing an unexpected mode of dimerization different from other members of the STAR family. We further demonstrate that this unique dimerization interface is crucial for their biological activity in splicing regulation, and suggest that the increased RNA affinity through dimer formation is a crucial parameter enabling these proteins to select their functional targets within the transcriptome.

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Suppressing solvent signals in Dynamic Nuclear Polarization (DNP) solid-state NMR *via* the Electronic Mixing-Mediated Annihilation (EMMA) method

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A simple procedure based on the so-called Electronic Mixing-Mediated Annihilation (EMMA) methodology is shown here to effectively suppress solvent signals in dynamic nuclear polarization (DNP) solid-state NMR. These signals are present when analyzing samples prepared by glass forming or incipient wetness impregnation, two common methods used in DNP solid-state NMR for adding polarizing agents (*e.g.* biradicals) to diamagnetic compounds. This becomes especially critical when the amount of solvent is large with respect to the sample under study, which may hamper proper analysis of the resulting CPMAS spectrum. Similarly to the ERETIC™ method, which uses an electronic signal as an internal standard for quantification, EMMA is based on an electronically generated time-dependent signal that is injected into the receiver coil of the NMR probe head during signal acquisition (fig. 1). More specifically, the line shape, width and frequency of this electronic signal are determined by deconvoluting the solvent signal in the frequency domain. This deconvoluted signal is then converted into a time-dependent function through inverse Fourier Transform, which is used to generate the shaped pulse that is fed into the receiver coil during the acquisition of the Free Induction Decay. The power of the shaped pulse is adjusted to match the intensity of the solvent signal, and its phase is shifted by 180° with respect to the receiver reference phase. We are presenting here preliminary data illustrating the potential of the EMMA method for DNP-enhanced solid-state NMR, using polymer materials as a case study.

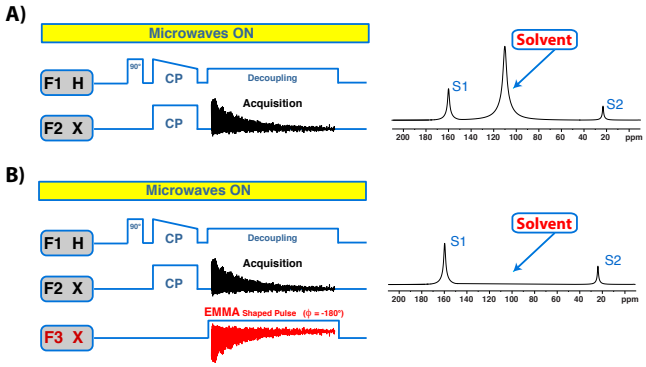

Fig. (1): *Schematic representation of a DNP-enhanced NMR ^{13}C CPMAS experiment without (A) and with (B) the use of the EMMA method for suppressing the solvent signal. (S1 and S2 refer to NMR signals of the sample).*

Azacalixarènes, Relation Structure/Activité : une approche théorique

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Les azacalixarènes sont des oligomères cycliques d'unités phenol reliées entre elle par des ponts azotés. Ces molécules comme on peut le voir sur la Figure 1 ci-dessous ont une forme de calice d'où leur nom de aza**calix**arènes. Par cette cavité ces molécules peuvent capter différents types de composés et sont utilisées dans le domaine de la chimie hôte-invité[1].

Une grande variété de substituants peut être fixés sur ces azacalixarènes[1,2] (Figure1) ce qui permet de créer des structures cycliques entièrement conjuguées dont les propriétés optoélectronique peuvent être chimiquement modifiées en changeant la nature de ces substituants. Ainsi il est chimiquement aisé de modifier la taille de l'anneau aromatique permettant de contrôler la taille et la dynamique conformationelle des cavités ainsi que son interaction avec son environnement direct. Expérimentalement on peut suivre facilement les effets des variations de structures au niveau des propriétés physico-chimique par la RMN (Résonnance Magnétique Nucléaire).

Afin de calculer les effets de différentes substitutions, Figure 1, mais également de quantifier l'effet de changements d'isomérisations sur les propriétés physico-chimique (densité électronique et encombrement stérique) un de nos buts est de simuler les spectres de RMN de différentes molécules modèles à l'aide de méthodes de DFT (Density Functional Theory en Anglais). Nous avons pour projet d'utiliser la dynamique moléculaire, pour en prenant en compte les effets de la température générer des structures qui seront utilisées comme point de départ pour des calculs de DFT. On pourra ainsi simuler le spectre RMN à l'aide de la méthode GIAO[3] (Gauge-Independent Atomic Orbital en Anglais). Dans un premier temps il nous faut valider la partie méthodologie DFT afin de voir comment les choix de fonctionnelles et de bases peuvent influencer les spectres obtenus. Nous pourrons ainsi prédire comment, en fonction de changements de structures, il est possible de faire évoluer les propriétés physico-chimiques et ainsi contrôler la capacité des azacalixarènes à jouer leur rôle chélateur.

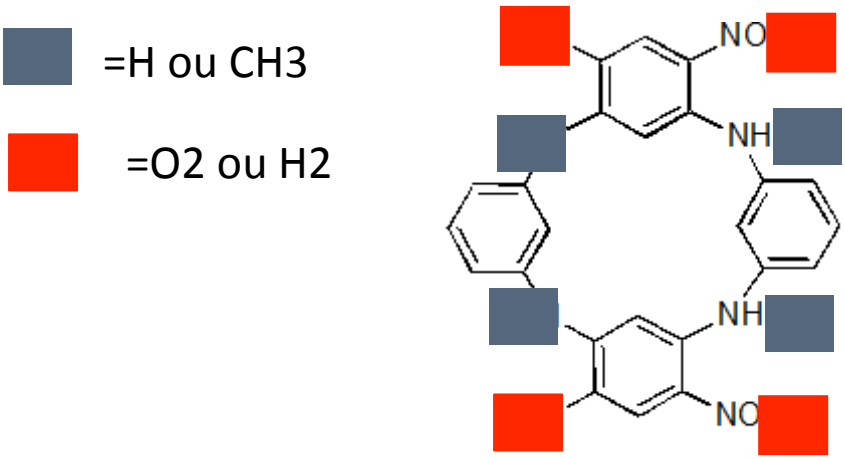

Figure1 : Substitutions réalisées sur les Azacalixarènes

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^{13}C-^{13}C single-bond correlations for organic molecules at natural abundance by solid-state nuclear magnetic resonance

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Solid-state NMR is a key technique for the structural characterization of organic solids, especially those that cannot be studied by conventional diffraction techniques. In this respect, the primary step of the structural investigation consists in determining the connectivity between carbon atoms. This procedure is usually achieved through carbon double-quantum (DQ) correlation spectroscopy experiments that rely on ^{13}C-^{13}C scalar *J*-couplings to transfer magnetization between ^{13}C nuclei separated by a single covalent bond.[1] These experiments usually lead to the assignment of ^{13}C resonances and are particularly valuable to investigate isotopically enriched samples. In the case of natural-abundance (NA) samples, however, the efficiency of such experiments is often limited due to the long evolution times required for DQ excitation/reconversion, which correspondingly reduce the sensitivity (especially for samples with broad ^{13}C linewidths). In contrast, correlation experiments based on ^{13}C-^{13}C dipolar couplings have a number of important advantages. One important feature is that the DQ coherences build up much faster than in experiments based on *J*-couplings, and hence could be used even for samples with relatively broad line widths. Here, we demonstrate that correlation spectroscopy based on the ^{13}C-^{13}C dipolar coupling leads to the unambiguous assignment of carbon resonances in NA powders having either narrow or broad ^{13}C linewidths. Our methodology is based on a pulse sequence that reintroduces the DQ dipolar interaction and which is nearly twice more efficient than standard recoupling pulse sequences. Consequently, using standard solid-state NMR hardware at room temperature and practicable spinning frequency, 2D ^{13}C-^{13}C DQ dipolar correlation spectra for NA samples can be obtained in a reasonable amount of time (2 to 3 days).

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Analyzing synthetic polymers by dynamic nuclear polarization solid-state nuclear magnetic resonance

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Description of microstructure/morphology/properties relationships in polymeric materials, including accurate structural elucidation of chain-ends, is classically required to finely tune their macroscopic properties. In this context, NMR is typically regarded as one of the techniques of choice but its low sensitivity usually precludes elucidation of subtle structural features in polymers. Dynamic Nuclear Polarization (DNP)[1-4] could potentially circumvent this difficulty by boosting NMR sensitivity. This communication describes our ongoing efforts in using DNP for the characterization of polymers by solid-state NMR at 9.4 T.[5-7] Experimental aspects regarding sample preparation methods (mainly glass forming and film casting, herein referred to as GF and FC, respectively) as well as polarizing agents efficiency will be discussed. Specifically, by investigating amorphous and semi-crystalline polymers of varying molecular weights, we show that GF with tetrachloroethane as the solvent provides larger sensitivity enhancements than FC. Moreover, while both methods yield comparable spectral resolution for amorphous polymers, FC is to be preferred for analyzing semi-crystalline polymers when spectral resolution is a priority, as the presence of the solvent in GF gives rise to deleterious inhomogeneous broadening due to conformational distribution of the polymer chains in the frozen solution. Interestingly, use of deuterated solvents in GF is shown to significantly reduce the intensity of the solvent signals in the DNP enhanced ^{13}C CPMAS spectrum, while preserving the sensitivity enhancement observed for the polymer signals. All together, the large absolute sensitivity ratios[8] provided by DNP (here between 10 and 40) allowed chain-end signals to be clearly identified in the NMR spectrum of functional, so-called *living* polymers of relatively high molecular weight, hence providing access to full structural characterization.[5,6] This unprecedented sensitivity improvement opens up new avenues for the characterization of synthetic polymers.

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P18 : K. AINOUCH

Differential Resistance to Vinca-Alkaloids of Human Malignant Melanoma : a Metabolomic NMR Study

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The human malignant melanoma is the most severe cutaneous tumor. Its propensity to develop acquired resistances quickly defeats all the treatments, including the new targeted therapies: MAPK inhibitors (MAPIs) that vemurafenib is the leader. The use of the classical chemotherapies, such as vinca-alkaloids (VAs), stays thus essential, even if they present the same limit of development of resistances.

To study these mechanisms in vitro, original models of human MM cell lines specifically resistant to the three major VAs (CAL1R-VAs), and the three MAPKIs approved in Europe and / or in the USA (A375R-IMAPKs), were established by long time continuous exposure of parental cell lines CAL1 and A375, respectively, to chemotherapies of interest. The objective of this project is to identify through an NMR metabolomic approach the metabolic perturbations related to the resistant phenotype of CAL1R-VAs.

The metabolic profiles of the various CAL1 cell-lines will be established by NMR. Then, a statistical analysis will allow us i) to compare the profiles of the resistant cell-lines to that of the parental cell-line, ii) to identify changes in their metabolism that can be correlated to the profiles of chemo-resistance. Molecular studies will be then conducted to validate the impact of these changes in chemo-resistance. Ultimately, identification of metabolic signatures predictive of drug resistance should lead to better understanding of drug resistance mechanisms of MM, to a personalized treatment based on tumor metabolic profiles, also to the identification of new therapeutic targets.

P19 : P. BARTHE

NMR, the perfect tool to solve *Mycobacterium tuberculosis* protein structure?

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The success of protein structure resolution depend on various factors. One of the first is the ability to get a stable and pure sample in relatively large amount (mg range). To do so, we extensively use bioinformatics to analyse protein sequence homology and perform extensive homology modeling. If X-ray crystallography is often consider as the technic of choice for structure resolution, it relies on the crystal structure obtention. From our experience, a lot of protein we study did not crystallize. The use of Nuclear Magnetic Resonance (NMR) allows us to exceed this problem ; 13 *Mycobacterium tuberculosis* protein structures were solved at the “Centre de Biochimie Structurale”.
Analysis of structure and dynamic of these proteins[1] revealed the different reason why the X-ray crystallography is not as effective and why NMR should be more considered. Moreover, the NMR enables us to study various changes, such as phosphorylations[2], interactions with ligands[3].

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P20 : K. de GUILLEN

Recognition of a fungal virulence effector by binding to an integrated decoy domain and additional, independent sites in the rice NLR immune receptor RGA5

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Nucletide-binding and leucine rich repeat domain proteins (NLRs) are important receptors in plant immunity where they recognize pathogen effectors in the host cytosol. In plants, Receptor-ligand interactions are generally characterized by high specificity and affinity. In plant immunity, this has been documented for cell-surface located pattern recognition receptors for which the structural bases of such highly specific recognition and the link to downstream signaling are beginning to be elucidated (Böhm et al., 2014; Zipfel, 2014). For cytoplasmic NLR receptors, much less is known. The recognized signals are cytoplasmic effectors that have frequently been identified but the molecular bases of recognition and the underlying mechanisms are largely unknown (Cui et al., 2015). This is in large part due to the lack of knowledge on the biochemical details of binding and the structures of the key players. In the present study, we obtained novel insights into the structural and molecular bases of the binding of the *M. oryzae* effector AVR-Pia to the rice NLR immune receptor RGA5 and in particular the role of the integrated RATX1 decoy domain. In this work, we investigated the molecular mechanisms underlying the AVR-Pia recognition by the rice NLR immune receptor RGA5 . We generated structure-informed AVR-Pia mutants and we tested them for their binding to RGA5, $RGA5_{RATX1,}$ $RGA5_{\Delta RATX1}$ and $RGA5_{C\text{-}ter}$ in the heterologous system *N. benthamiana* and yeast respectively. Then we evaluated the avirulence activity of the AVR-Pia mutants impairs to bind $RGA5_{C\text{-}ter}$ and $RGA5_{RATX1}$ in *M. oryzae* transgenic strains when inoculate them in rice plants confering Pia resistance. We identified the AVR-Pia surface that binds to $RGA5_{RATX1}$ domain. Nevertheless, we showed thatAVR-Pia mutants impaired in $RGA5_{RATX1}$ binding were able to strongly bind $RGA5_{\Delta RATX1}$ even though $RGA5_{\Delta RATX1}$ was disabled to recognize the AVR-Pia mutants. This study suggest that a cooperation among RGA5 domains could be required to bind AVR-Pia and uncover the main role of $RGA5_{RATX1}$ domain in effector recognition.

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P21 : H. DEMENE

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 aworrisome 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 ^{15}N relaxation and D_{HN} RDCs measurements

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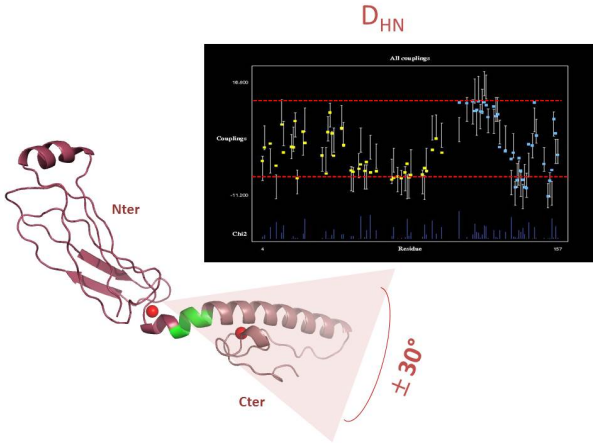

Figure 1.Relative independence of P1 zinc fingers as seen by RDC measurements.

Structural characterization of the C-termini of G Protein-Coupled Receptors by NMR : impact of their phosphorylation

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To design more effective drugs without side effects, it is essential to better understand the functioning of GPCRs (G Protein-Coupled Receptors) that are today the target of one third of drugs[1] on the market. Beside a common core of seven transmembrane helices (7TM), the GPCRs harbor N- and C-termini, that could not be crystallized due to their intrinsic flexibility. In particular, no structural data are available for the C-terminal regions of GPCRs, which are nevertheless crucial for signal transduction, desensitization and internalization or other cellular signaling functions (scaffolding). Some of these functions are mediated by the interaction with beta-arrestin, involved in important signaling pathways distinct from those activated by G proteins that were initially seen as the only cytoplasmic partners of GPCRs. This functional interaction is modulated by the phosphorylation of the C-terminal regions by several specific kinases associated to GPCRs (GRK)[2]. It's important to note that Intrinsically Disordered Proteins (IDPs) have become a growing interest in the field of cell biology for the past ten years. This study will reveal the structural and dynamic keys necessary for the interaction of the phosphorylated C-ter with β-arrestins, providing essential information to guide the rational design of peptide mimetics able to modulate specific signaling cascades. We are particularly interested in vasopressin (V2R)[3] and ghrelin (GHS-R1a)[4] receptors. This project will focus on their structural characterization in their phosphorylated and non-phosphorylated forms, using complementary spectroscopic techniques (NMR and SAXS)[5], followed by cellular assays. Then, structural characterization of these C-terminal domains will be integrated in the entire GPCR to describe them in their original biological context.

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Development of an Original NMR Method for the Study of the Complexation of Organoboron Molecules with Diols

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Boronic acids[1] and benzoxaboroles[2] (which are cyclic derivates of boronic acids) are emerging as an interesting class of molecules for pharmaceutical applications, in particular for the development of new drugs. Indeed, these molecules can bind to cis-diols, and are able to inhibit, for example, an enzymatic activity. This reactivity is also interesting for self-assembly and dynamic combinatorial chemistry applications.

Despite the increasing number of studies on complexation between boronic acids or benzoxaboroles with diols, the spectrofluorimetric method[3] usually used to investigate these interactions is controversial, especially in the case of multifunctional boronic acids and benzoxaboroles. In this context, NMR measurements in solution can provide complementary information and, to the best of our knowledge, have never been used for this purpose with benzoxaboroles.

The aim of the presentation will be to present ^{1}H and ^{19}F 1D NMR spectra, as well as 2D DOSY NMR experiments (Fig. 1), and to show how this allows the complexation to be studied. Then, the results will be compared with the data collected by the more standard spectrofluorimetric analysis.

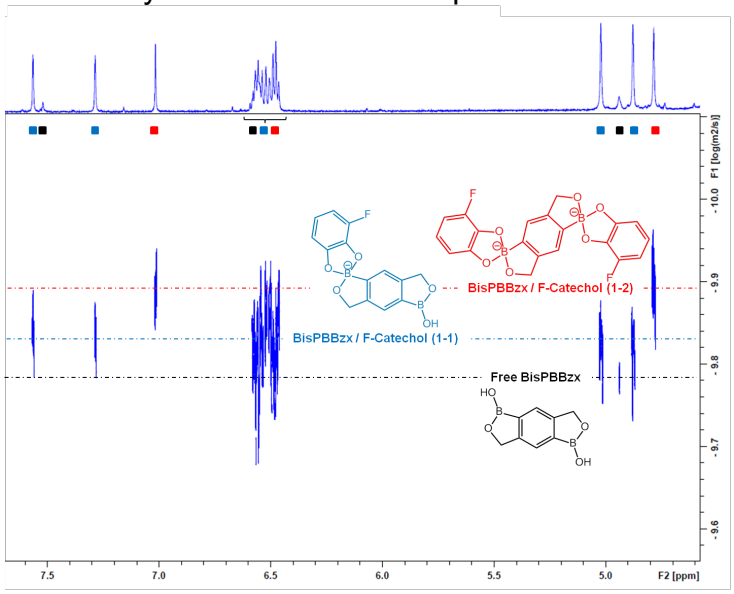

Fig. 1 : ^{1}H DOSY NMR spectrum (600 MHz) of the Phenylbisbenzoxaborole (BisPBBzx) / 3-Fluorocatechol (F-Catechol) system in DMSO-d^{6}/Buffer (60/40, v/v.).

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P24 : M. VANHEUSDEN

Exploring the folding pathway of titin domain I27 as a mono- and bimodule with high pressure NMR

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Titin is a giant, multidomain muscle protein that spans half of the sarcomere in vertebrate striated muscle. The titin molecule is divided into two segments for which the function and build up differs. While the A-band segment, anchored to the myosin thick filaments in the sarcomere, is largely invariant in its alternating fibronectin-III like and immunoglobulin like modules amongst different splicing forms, the I-band region of titin shows some variations. The in tandem arranged immunoglobulin like modules that build up this I-band segment can differ in length, just as the interspacing, less structured linker sequences (1). Since these variations correlate with the differences in passive tension along the different splicing factors, the I-band region of titin has attracted a lot of attention for the past decades. It is known to be involved in the generation of muscle elasticity and passive muscle tension, but the molecular basis of this phenomenon is not yet fully understood (2). In order to find a molecular explanation for this, researchers have tried to study the modules and their interphases that build up the I-band segment in isolation. Remarkable is that the stabilities of the different modules that build up titin differ in a position related manner, but that they are all quite stable, meaning that the complete unfolding of these domains can not be the basis of titin elasticity (3). Furthermore, the unfolding and thus extensibility of a part of titin's I-band region is no direct proof for it's elasticity and passive tension, since elasticity requires the thermodynamical property to be reversible, a criterion that is not necessarily fulfilled when a module unfolds and thus extends. Since refolding of proteins can be efficiently studied by high pressure NMR, we will try to follow the unfolding and refolding pathway that module I27 follows and track the possible folding intermediates that might be formed.
In this preliminary study based on 1D HP-NMR spectroscopy (4), we want to characterize the unfolding parameters (steady-state and kinetics) of the I27 module, alone or in a bi-module construct. It will be interesting to know if the two I27 modules behave independently in the bi-module construct, or if unfolding parameters differs suggesting unfolding cooperativity between the two modules.

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P25 : E. CAHOREAU

NMR-based strategies for high-throughput fluxomics

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Fluxomics (i.e. measurerement of intracellular metabolic fluxes from 13C-labelling experiments) is an unvaluable tool for the comprehensive investigation of metabolism and is growingly applied to a broad range of research fields, ranging from biotechnology to health. Fluxomics is originally a low throughput method, which limits the number of organisms (strains) and conditions which can be investigated. We have developed a fully integrated solution for high-throughput fluxome analysis which combines a robotic cultivation[1], sampling workstation and a set of 1D and 2D NMR analyses (zqf-TOCSY, HSQC and Heteo-Jres[2]). This platform was applied to a set of bacterial strains and allowed 48 fluxomes to be profiled per run.

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Liquid and solid state NMR studies of ZnO Nanocrystals stabilized by organic ligands.

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The synthesis and characterization of nanomaterial is a pluridisciplinary research domain involving several disciplines like physics, chemistry, biology and materials science. Chemistry methods allow access to well defined nano-objects. With solution procedures, nanocrystals (NCs) are synthesized using ligands that stabilized the NCs and allow the formation of colloids. In these cases, ligands play a role all along the synthetic process and interact with the surface of the final NCs. Notably, ligands turn to be responsible for the colloidal stability of the NCs in solution but also for modifications of the NC's chemical and physical properties.

Characterizations of the NC/ligand systems are difficult because of the complexity associated with chemical, structural and dynamic heterogeneities especially at the interfaces. Among all the characterization techniques allowing the access of information concerning the ligands grafted on the NC surface, NMR spectroscopy is a very powerful technique. Most of the studies performed to date used liquid state NMR spectroscopy.[1] Solid state NMR spectroscopic studies devoted to the characterization of NCs are increasingly used with the recent instrumental and methodological developments.[2] These NMR techniques can address structural and dynamic questions related to the organic capping molecules and/or the NCs themselves at different scales.

Few years ago, we established organometallic procedures for synthesizing ZnO NCs in the presence of different organic ligands that allows a good control of the size, the shape, the 2D or 3D organization and the chemical or physical properties of the ZnO NCs.[3] We already reported characterization of ZnO NCs stabilized by alkylamine, by a mixture of alkylamine/alkylacid or alkylthiol using liquid state NMR spectroscopy.[4] These studies evidenced the complexity of these systems.

We go one step further by exploring the possibility to use solid state NMR spectroscopy on ZnO NCs stabilized by organic ligands for obtaining structural and dynamic indications on the inorganic/organic interfaces. Multinuclear ^{1}H, ^{2}H, ^{13}C, ^{15}N, ^{67}Zn and ^{17}O MAS NMR investigations were undertook and additional detailed informations at the microscopic level on the ZnO NC/Ligand system were obtained. These studies highlighted the complementarities between liquid state and solid state NMR studies of nanocrystal/ligand systems.

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NMR Structure and Dynamics of the Agonist Peptides Bound to Opioid Receptors

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The human kappa opioid receptor (KOR) which belongs to the G protein-coupled receptor (GPCR) protein family is implicated in addiction, pain, reward, mood, cognition, and perception. Its activation by the agonist neuropeptide dynorphin is critical in mediating analgesia and tolerance. In order to elucidate the molecular mechanism underlying its biological function, the inactive structure of KOR bound to the antagonist JDTic has been elucidated by X-Ray crystallography (1). More recently, the active structure of the mu opioid receptor has also been obtained (2). But there was no structural information about endogenous opioid neuropeptides during their interaction with their cognate receptor. Furthermore, studying the structural dynamics of the GPCR-ligand complexes is of major importance to understand the activation process of those proteins.

Our solution NMR study of dynorphin A (1–13), the natural agonist of KOR, provided quantitative data on KOR-bound conformations and dynamics (3). The receptor was purified in detergent micelles and its functionality was verified by radioligand binding using the scintillation proximity assay technology. It displayed an intermediate affinity for dynorphin, with a Kd of 7.10^{-7} mol.L^{-1}, in agreement with the fast exchange rate observed in NMR experiments. Analysis of the KOR-bound peptide structure (using transferred NOE experiments) and dynamics (analysing ^{15}N transverse relaxation rates) revealed a central helical turn (from L5 to R9) and flexibly disordered N- and C- termini. An ensemble of receptor-peptide conformations was identified with molecular dynamics simulations. The insights from combining molecular modelling with NMR provide an initial framework for understanding the multi-step activation of a GPCR by its cognate peptide ligand.

Our current objectives are to characterize the high affinity states for agonist (i.e. in the presence of downstream signalling G-proteins) and to extend these results to other GPCR. Moreover we developed an expression protocol in the bacteria *E. coli* in order to label KOR with stable isotopes and thus study the receptor's dynamics itself.

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P28 : A. BAUDIN

Structural studies of peptidomimetic ligands of TRAIL complexed to pro-apoptotic Death Receptor 5

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Apoptosis, the programmed death of cells, plays a protective role against tumor formation. This phenomenon is either regulated by the intrinsic pathway or by the extrinsic pathway. The latter is stimulated through the activation of cell-surface death receptors (DR) by Tumor necrosis factor-Related Apoptosis Inducing Ligand, or TRAIL. The ligand binding leads to the trimerization of the receptors, which is a necessary step for the apoptosis answer. This TRAIL ligand has the interesting particularity to bind only the DR4 and DR5 receptors of tumor cells. This unique property is at the center of several therapeutic assays for cancer cell targeting.
The aim of this study is to use a structural approach in order to find new peptidomimetic ligands of TRAIL, or TRAIL$^{mim/DR5}$, that can enhance activation of the extrinsic pathway of apoptosis. Several of these different peptides have been produced through solid-phase synthetic chemistry in monomeric, dimeric or trimeric states, and have been shown to selectively bind to DR5 [**1**]. Some of these peptides have been studied with NMR spectroscopy and show a β-hairpin motif [**2**]. We plan to use two-dimensional and three-dimensional NMR spectroscopy to study the molecular details of the interaction between these new ligands and Death Receptor 5. We have designed a plasmid construct with a fusion protein (NusA) in order to optimize the yields of production and purification of the ^{15}N-labelled protein in minimal medium, and we plan to assign the backbone using a ^{13}C-^{15}N double-labelling strategy. The first 2D ^{1}H/^{15}N-HSQC spectra show that the protein is well-folded.
The other part of this project is to crystallize the complex formed by the receptor and either the monomeric, dimeric or trimeric peptides, then to use X-ray diffraction on the crystals obtained, in order to get atomic scale information. These data should help us to have a better understanding of extrinsic apoptosis, and thus designate a model candidate to trigger the TRAIL apoptosis pathway for cancer therapy.

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P29 : M. BERBON

Production of uniformly and selectively ^{15}N- and ^{13}C-isotope labeled functional amyloids for structural studies 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. The atomic structural features of this type of non-crystalline and insoluble protein assembly are inaccessible by conventional techniques such as X-ray or solution NMR. We therefore use the emerging technique solid-state NMR [1] to study the structure and assembly mechanism of functional amyloids involved in cell death signaling. We present the expression in *Escherichia coli*, the purification and self-assembly of functional amyloids for structural studies by solid-state NMR. The here-investigated functional amyloids transmit the signal in fungal programmed cell death cascades through amyloid formation [2]. A tool in structural studies by solid-state NMR is the use of different complementary selective ^{15}N and ^{13}C isotope labeling schemes optimised to access a maximum of information. We describe the set-up of the production (expression, purification and assembly) of uniformly and selectively ^{15}N /^{13}C labeled functional amyloid fibrils for solid-state NMR analysis [3]. Our data reveal the amyloid core in the fibrils as well as their structural features such as structural homogeneity and secondary structure.

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Interactions between carbon nanoparticles and biological membranes

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Organic solvent exposure poses serious threats to public health. Among the Volatile Organic Compounds (VOC) family, toluene is widely used in the production of paints, glue, gasoline and even in the pharmaceutical industry. People working in these areas are chronically affected by toluene [1]. One of the most preoccupant side effect of toluene inhalation is the disruption of the cell membranes, particularly at the central nervous system level. The lipophilic property of toluene suggests that membrane associated phenomena are integral to the mechanism of action of this solvent [2]. ROS formation and alteration of membrane fluidity [3] could be responsible of damages induced by toluene but at that time, a description of toluene behaviour in a membrane environment at the molecular level is still missing. On the other hand, there has been a resurgence of interest for Fullerene, a carbon nanoparticle so called « Buckminster ball ». It can be described as a pure carbon cage of 1 nm in diameter. Recent studies have shown anti-HIV activity, antimicrobial action and its possibility to inactivate intracellular free radicals [4]. Liposomes have been widely used as a bio-compatible carrier for Fullerene to target and treat deseases in biomedical approaches [5]. However, potential undesired biological side effects have been observed. The ability of Fullerene to be incorporated in biological membranes could alter their mechanical properties and potentially induce cytotoxic effects [6]. Here, we present the study on the effect of the incorporation of small hydrophobic compounds into lipid bilayers using Solid-State NMR. We were able to follow the changes of the mechanical membrane properties as well as the dynamics of the hydrophobic compounds in a membrane environment. The results indicate that they are located in the hydrophobic core of the lipids and destabilize membrane integrity. Surprisingly, we also observed that the more the environment is rigid, the more the nanoparticles are mobile.

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Shuttling motion in rotaxane systems studied by EXSY NMR

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The mechanically-interlocked hydrogen-bonding rotaxane systems[1] used in the present study are composed of a dibenzo-24-crown-8 macrocycle which can undergo a slow shuttling motion between two dibenzylammonium stations rotaxane **[1]**. The methylation of the triazole moieties of rotaxane **[1]** gives rotaxane **[2]** comprising two methyltriazolium stations. The rate of macrocycle motion between the two DBA stations was monitored by EXSY NMR in order to estimate the exchange constants K_1 and K_2.

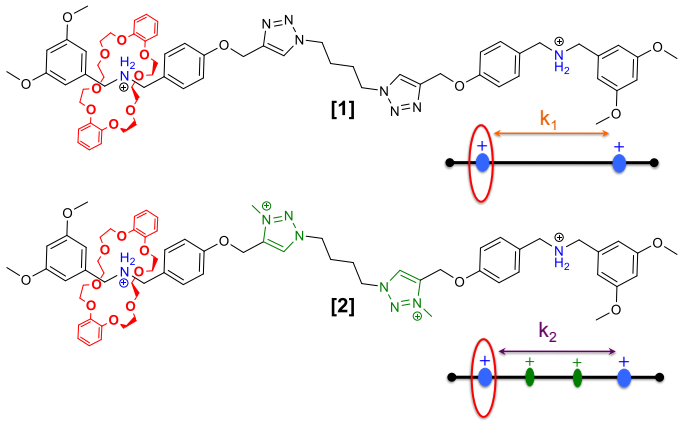

Fig. 1 : *Structures of rotaxane **[1]** and **[2]***

For each rotaxane, two sets of signals are observed suggesting the formation of supramolecular complexes between the chains and the macrocycles in which the motion is slow along the molecular axle on the NMR timescale. NOE correlations permit the unambiguous localization of the macrocycle between the two benzyl rings for **[1]** and **[2]**, as depicted in the scheme above.
Increasing the temperature of the samples **[1]** or **[2]** to 70 °C (343 K) did not induce the coalescence of each pair of signals. However, EXSY NMR spectra exhibit cross peak signals due to exchange suggesting that the ring is in motion along the chain. The analysis of the evolution of each cross peak signals observed between a pair of signals depending upon the chosen mixing time affords an estimation of the rate constants using the initial rate method.

The data obtained suggest that the introduction of two charged methyltriazolium stations drastically slows down the shuttling motion of DB24C8 between the two DBA stations.

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Initial steps in describing the F_1F_o ATP synthase dimer interface and modelling the small hydrophobic subunits of the F_o region with solution state NMR.

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The multi-subunit complex of F_1F_o ATP synthase is the principle source of cellular ATP throughout the biological kingdom. Although variability exists in the precise F_1F_o composition between species, the most studied protein is from *Saccharomyces cerevisiae* where the complex is comprised of 17 core subunits. Two of the Yeast F_1F_o subunits (e and g) and the N-terminal membrane domain of subunit 4, which all locate to the mitochondrial membrane F_o region, have recently been shown [1] to be absolutely required for the dimerisation of ATP synthase which facilitates the formation of the mitochondrial cristae [2]. Currently, no high-resolution structural information for the interface of dimeric ATP synthase exists and as a result, the interactions which stabilise the ATP dimer are unknown; with prior investigations typically hampered by poor yields of the membrane domain subunits.

To this end, we have optimised a home-made cell-free expression system for the production and isotopic enrichment of these subunits - with final yields >1 mg, which allows for investigation with nuclear magnetic resonance (NMR) spectroscopy. Initial studies, using unlabelled and ^{15}N-Ala samples, focussed on validating the incorporation of samples into LMPG micelles and optimising the quality of NMR spectra for an 83 residue (8.7 kDa) construct comprising the N-terminal region of subunit 4 from the Yeast F_1F_o ATP synthase (S4T). From synthesising single (^{15}N) and double (^{13}C, ^{15}N) labelled samples of S4T, two and three-dimensional NMR spectra (^{15}N and ^{13}C HSQC, HNCACB, CBCACONH, HNCA, HNCOCA and HCACO) were acquired at 800 MHz enabling us to assign 95% of the S4T backbone nuclei and >70% of the side-chain nuclei. *In silico* predictions from primary sequence alone suggested that S4T would have a predominantly alpha helical conformation and this agrees with both chemical shift analysis and a preliminary 3D model from CS-ROSETTA [3] that describes the structure of S4T as a bundle of 3 trans-membrane helices. Furthermore, calculated short range (2-6Å) and long range (15-25Å) inter-nuclear distances have been obtained by the acquisition of traditional NOESY experimentation and paramagnetic relaxation enhancement (PRE) experiments (with six S4T cysteine mutants), respectively, allowing us to calculate a structural model for S4T in LMPG micelles. Additionally, utilising the protocol optimisations from subunit 4, triple resonance experiments regarding a full-length construct of subunit g (115 residues) have now also been acquired and the backbone assignment is in progress.

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