NEWSLETTER OF THE FP7-PEOPLE-2012-ITN "PNMR" PROJECT

Pushing the Envelope of Nuclear Magnetic Resonance Spectroscopy for Paramagnetic Systems

pNMR news

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Solid state NMR of paramagnetic metalloproteins

Spinning faster, less blind ?

Andrea Bertarello, CNRS Lyon, ESR10

METALLOPROTEINS PLAY A CRUCIAL ROLE IN BIOLOGY, considering that at least 50 % of all known proteins contain a metal ion as cofactor. In some cases this implies the presence of paramagnetic species at the active site of the protein which represents an interesting but yet challenging world : the interaction between the unpaired electrons and the nuclei can provide information about structure and dynamics of the protein and, more interestingly, a direct probe of the electronic structure at the active site, which intimately correlated to the activity of a metalloprotein. However, the same interactions can cause severe problems NMR spectra of these samples: the presence of unpaired electrons provides very efficient relaxation pathways and induces very large. The result is that NMR lines are usually very broad, sometimes beyond the limit of detection, and the spectroscopist has to deal with very large spectral windows not uniformly excited by conventional experiments. This lead to the common assumption that in paramagnetic proteins there is always a sort of blind sphere around the metal centre which is unaccessible to NMR.

Paramagnetic proteins have a unaccessible blind sphere around the metal centre

Because of all these reasons, solid state NMR has not yet been widely used in the study of paramagnetic metalloproteins. Yet, it would be a very attractive technique for all the systems (like microcrystalline samples, membrane proteins, big aggregates) which cannot be easily inspected by more conventional techniques like X-ray crystallography or liquid-state NMR. Recent developments in the technique allowed a proper "toolkit", with which paramagnetic effects can be alleviated enough to acquire resolved spectra of nuclei surrounding metal center. This toolkit includes : i) echoed acquisition, to obtain; ii) adiabatic pulses, to uniformly and refocus signals over very large spectral windows; iii) rates.

Recent developments alleviated the paramagnetic effects enough to acquire resolved spectra of nuclei surrounding the metal center

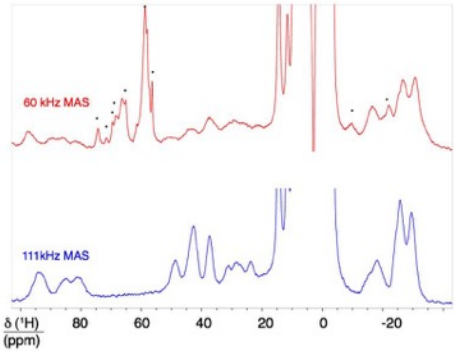


*Figure 1. ^{1}H spectrum of HiPIP 1 from E. halophila acquired at 60 kHz MAS and 111 kHz MAS at 23.5 T. Sidebands are marked with an *.*

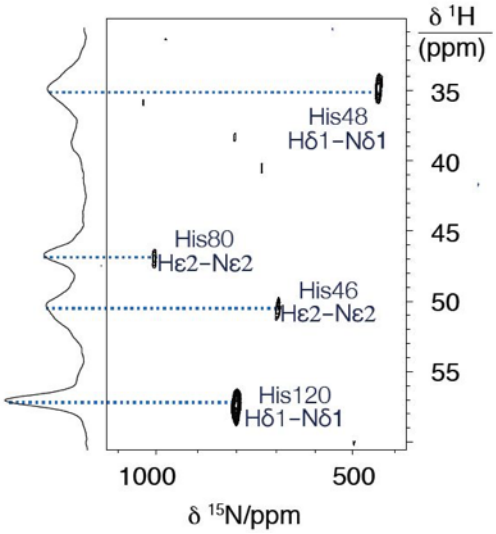


Figure 2. {^{1}H}-^{15}N TEDOR spectrum of Cu(II)-Co(II)SOD acquired at 60 kHz MAS and 111 kHz MAS at 11.7 T.

The effect of high MAS rates can be understood by looking at Fig. 1, showing the ^{1}H spectrum of the protein HiPIP1 from E. halophila acquired: higher MAS rates allow to increase both resolution and sensitivity, due to the fact that is spread over fewer sidebands (therefore the intensity of the central band is enhanced). Coherences lifetimes are longer as the internuclear dipolar coupling is more efficiently averaged.

The toolkit can be further expanded to obtain correlations at the active site in paramagnetic samples. Fig.2 shows the TEDOR sequence can be efficiently used to obtain proton-nitrogen correlations of the histidine moieties in the active site of Cu(II)-Co(II) superoxide dismutase.

We can say that the recent developments in solid state NMR finally open the way to the study of paramagnetic metalloproteins, with the possibility to turn our attention, in the future, to biologically interesting systems… the blind sphere is not blind anymore!

LIVE FROM THE pNMR LABS

University of Oulu Finland

1H chemical shifts in paramagnetic Co(II) pyrazolylborate complexes

SYED AWAIS ROUF, ESR3

Paramagnetic Nuclear Magnetic Resonance (pNMR) is gaining importance in biomolecular and materials science. The NMR research group at University of Oulu worked on further improvement of practical pNMR chemical shift theory presented in 2008 by Pennanen and Vaara. During the application of this theory on Cobalt (CoII) pyrazolylborate complexes, it has been found important to include the so-called magnetic couplings present in the Kurland-McGarvey theory. Further, we found that it is essential to consider both the effects (1) the magnetic couplings between the states belonging to the ground multiplet and (2) the thermal occupation of the states to determine the correct temperature dependence of shielding parameters. Therefore, we can suggest that the combination of this pNMR theory with higher level of computational methods will improve the findings of NMR chemical shielding.

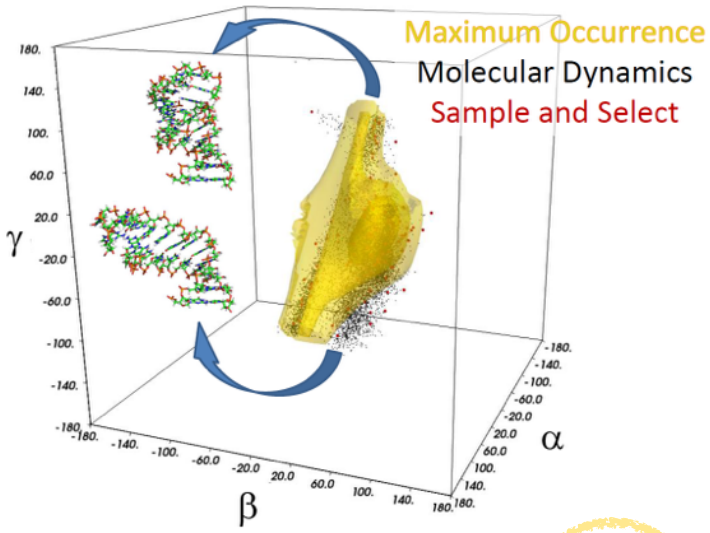


Fig 1 : The most sampled conformations of HIV1TAR (shown in the space of angles between two rigid helical elements)

CERM Italy

Dynamics in biomolecules: an RNA strand from the HIV

WITOLD ANDRALOJC, ESR 6

The transactivation response element (TAR) RNA from the HIV-1 virus is an important drug target that plays essential roles during viral replication. The Florence lab, in collaboration with the al-Hashimi group, has studied the internal motions present in this RNA strand using NMR spectroscopy and the Maximum Occurrence (MaxOcc) computational methodology. The study has revealed the most probable main conformational states sampled by the system. The obtained results are in line with the previous analysis using molecular dynamics and may yield insights into how the HIV1TAR interacts with the drug molecules that target it.

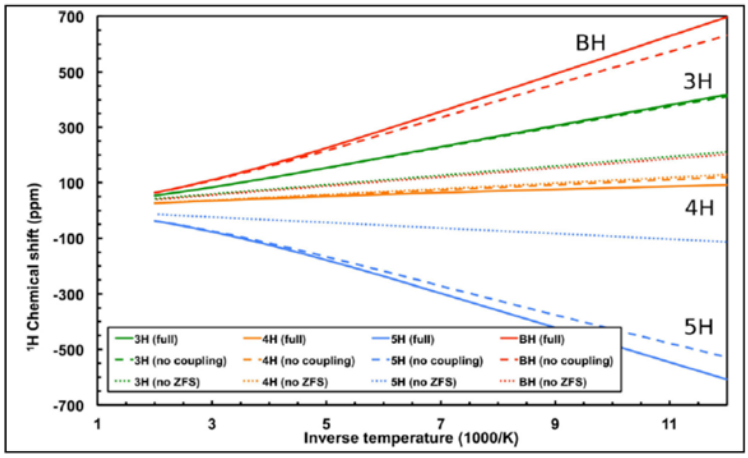


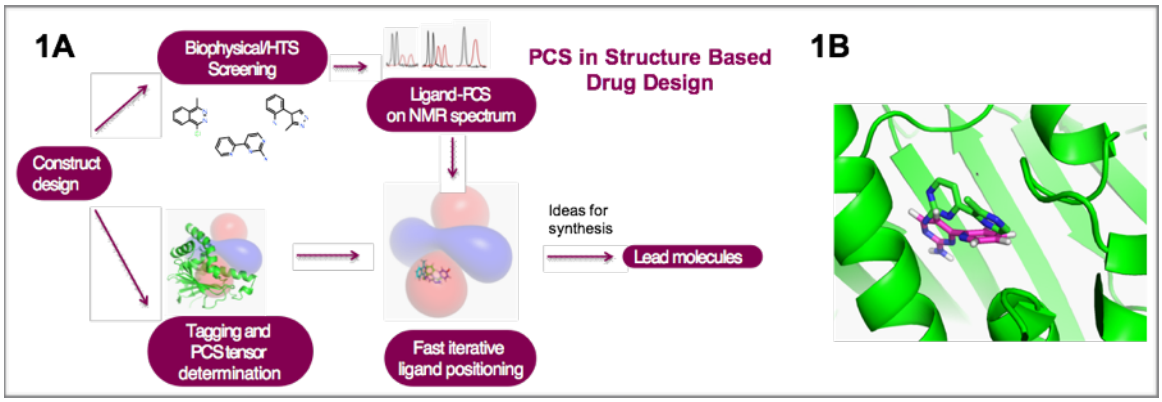
Figure 2 : Inverse temperature dependence of proton chemical shift in CoII pyrazolylborate "HPYBCO" complex. Full stands for full theory with zero-field splitting (ZFS) and magnetic coupling effects. (Fig Credit: Prof. Juha Vaara)

Paramagnetic NMR in Structure Based Drug Design

PUNEET AHUJA, ER 3

In Fragment Based Lead Generation (FBLG), the availability of 3D structural information greatly improves the chances to progress screening hits to viable lead molecules. This information is commonly extracted from X-ray crystallographic structures of targets in complex with the screening hits. However, the low affinities of the hits often make them difficult to crystallize.

Paramagnetic NMR can be used to orient the hits in the binding site. This requires docking the ligands based on the PCS transferred to the binding ligands (Ligand-PCS). In collaboration with Ubbink group from Leiden University and the Florence laboratory, the researchers at AstraZeneca R&D Gothenburg tagged HSP90 with lanthanides and docked various fragment hits based on PCS. Fig 1B shows the PCS based docked binding pose of a fragment to HSP90 that is similar to the crystal structure of a close analogue. To demonstrate the versatility of the methodology, the structures of several ligands ($10\mu M < K_d < 10mM$) bound to HSP90 will be elucidated.



AstraZeneca Sweden

Fig 1 A : Schematic diagram showing the proposed role of PCS based ligand positioning in Structure Based Drug Design (SBDD).
B : Comparison of the PCS based binding pose (Violet) with the crystal structure (Green) for fragments bound to HSP90.

Young Investigator profile
Tobias Schubeis
GIOTTO (Italy)

Please introduce yourself.
My name is Tobias Schubeis and I am from Essen, Germany. I did my PhD at the Helmholtz center for Infection Research in Braunschweig, Germany, but the PhD title was awarded by the local University. I worked in the department of structural biology in the field of biomolecular NMR. My PhD project covered the production of isotope labeled amyloid fibrils, the application of specific labeling schemes, solid state NMR measurements and data analysis.

Where were you enrolled in the last 2 years ?
I worked at Giotto Biotech, close to Florence, Italy, through the pNMR network. The research I performed was conducted in close collaboration with one of the ESRs at the CNRS Lyon. Being part of such a network helped me improving my interpersonal, communication as well as organizational skills, which eventually opened up the opportunity to continue my scientific career at another member of the network's premises.

To what extent being enrolled in a private organization complemented your academic training ?
Since it is a spin off and that labs are shared, the work environment at Giotto Biotech is much like a University's. Beside that, it is interesting to get an insight into a product-oriented way of performing research. I experienced this on an even bigger scale during one of my secondment.

Where did you go on secondments ?
I spent 3 months at the CNRS in Lyon, France, and one month at AstraZeneca in Mölndal, Sweden.

Did you work with ESRs (Early Stage researchers, i.e. PhD Students)?
I worked with Andrea Bertarello during his secondment in Florence. I helped him gaining basic molecular biology skills, a new field for him.

Do you know what was the name of the European Union 'program' and 'sub-program' you were part of ?
The pNMR project is an Initial Training Network funded by the People Programme (Marie Curie Actions) of the European Union's 7th Framework Programme.

What did you prefer about being part of an Initial Training Network project ?
It is very interesting to meet people with different backgrounds, working on the same topic, but with diverse approaches. Another advantage is the integration of private companies, which can help in the always-present question whether one should continue a career in academia or change to industry.

Marie-Curie Actions support research training and career development focused on innovation skills : do you feel you gained skills from this experience ?
The whole field of paramagnetic NMR was new to me, therefore during my time in Florence, and at the pNMR workshops and meetings I learned a lot about the different aspects of the topic. The exchange of ideas also helps to sharpen your own view on the research world and define your own interest better.

Another crucial aspects of Marie-Curie Actions is offering researchers attractive working conditions. What can you say about this ?
The working conditions are indeed attractive. Regular postdoc salaries differ greatly between countries in the EU. In Italy, especially in an expensive city like Florence, the basic postdoc salary might be insufficient and fellowships like those of the Marie Curie Actions greatly improve your possibilities.

Do you think participating to the pNMR project helped you gaining competences useful for your career ?
Of course I did improve my research and presentation skills. This comes naturally by two extra years of experience. Through the pNMR project I had the chance to meet researchers from all over the world. Besides that, leaving your home country to move to a country where you do not know anybody and do not even speak the language is a great challenge. Getting to know new people and cultures is an essential achievement.

Mobility (transnational, intersectoral and interdisciplinary) is encouraged : do you think the pNMR project implemented this?
Yes I think the network covered this well, as partners come from numerous countries with different backgrounds and we had the opportunity to exchange idea during the pNMR meetings. Nevertheless, a fast exchange of thoughts between the students might be desirable. One way of realizing it would be a pNMR blog, were the fellows share their results and ongoing challenges, experiences. Of cause this would require a greater commitment from fellows.
I addition, we were given the chance to acquire a wider range of competences through the secondments scheme by visiting labs doing something entirely different. Unfortunately, a 2 years postdoc is a rather short time. Such a scheme makes it difficult to succeed in your own research project, and means you might learn techniques not necessarily closely related to your own project. However, it gives you an understanding, and more importantly knowledge of whom to consult to solve a given problem.

Did participating to an EU-funded project give you an insight of grant writing and project management ?
Unfortunately I haven't been involved in any grant writing so far. Yet, by writing reports and keeping a career development plan updated, I got an idea of how progress is monitored in an EU funded project.

Do you think being part of such network is an opportunity ? Would you recommend Masters and/or PhD students to apply ?
I would always recommend students to apply. I guess the high salary is a good reason enough, but being part of such a network is also a great opportunity to get to know people and broaden your skills and interests.

In a nutshell, what could you say about the past 2 years ?
I had a great time in Italy, working in a vibrant research environment and living in the beautiful city of Florence. Through the network I gained an insight into many different fields of research and made new friends. I would do it again.

PNMR NETWORK TRAINING

INTEGRAL TO the research-based training programme is the series of workshops, practical training courses, international conferences, and outreach actions, located at the different sites. These will i) train the young researchers of the network in the basics of pNMR and ii) disseminate the results of the network to the larger NMR community and to the general public.

As the two previous years of the pNMR project, a couple of events were organised in ***2015***, lead by the Technische Universität Berlin.

An *'Introduction to practical pNMR shift calculations'* applied training was held in **Berlin** on **29th-30th June**, consisting of seminars on the theoretical background, as well as explicit test calculations. The 6 fellows participating had the opportunity to learn how practical calculations are done, both for molecular systems and for solids, from 2 other fellow ESRs as well as lecturers from TU Berlin and our other partner Oulu University.

On the molecular side the training focused on the 2008 Pennanen/Vaara shift theory and some recent extensions to understand how different terms in the theory can influence the shifts in various systems. This includes contact as well as pseudo-contact shifts together with orbital contributions. Calculations used a combination of molecular codes (Gaussian, ORCA, MAG) combined via shell scripts. Solid-state calculations were limited to the theory without ZFS but touch on contact and non-contact terms as well. The comparison between different possible codes (CRYSTAL, CP2K, Quantum Espresso) and the role of bulk susceptibility corrections were in focus.

This training was immediately followed by a **Complementary Skills training** in... **Prague**, on **2nd-3rd July,** dedicated to *'Personal skills, conference presentation and communication'*, given by an external trainer from the UK. The course focused on two main themes: 'Personal effectiveness in the final stages of one's project' (career and time management, completion of one's project, making the transition to the next career stage), and 'Presenting one's research with impact' (making the most of conferences, confident communication to a range of audiences, presenting one's work as a thesis and in the viva)'. 10 out of the 13 pNMR funded fellows

participated.

The day after (**4th-5th July**), our **3rd Annual Workshop** opened at **Prague**'s Conference Center, and intended to discuss the ***'challenges and potentials of NMR on paramagnetic molecules'.*** Leading specialists as well as 12 pNMR fellows described their recent work on experimental and theoretical techniques along with its challenges and applications to a wide range of paramagnetic systems such as spin relaxation, paramagnetic solids, battery materials and supported catalysts, drug discovery, paramagnetic proteins in solution and in crystals. This event was organised as a satellite event to the EUROMAR 2015 Conference.

2016 EVENTS

For more details and registration visit www.pnmr.eu

11-13 MAY 2016 | APPLIED TRAINING COURSE
Expression and purification of metalloproteins for structural studies
Paramagnetic restraints in hybrid methods for protein structural analysis
Sesto Fiorentino, Florence, Italy

5 JUNE 2016 | TUTORIAL COURSE
Structure and dynamics of biomolecules by MAS NMR
CHIANTI WORKSHOP 2016 SATELLITE event | Principina Terra (Grosseto),Italy

8-9 JULY 2016 | WORKSHOP
pNMR: The roots of the matter. Foundations and future challenges for theory and experiments of paramagnetic NMR
EUROMAR 2016 SATELLITE event | Aarhus, Denmark

27-30 Sept 2016 | FINAL WORKSOP | Venice, Italy
Registration deadline : 15th August 2016