XXIII Swiss NMR Symposium

Tuesday 14 January 2020 - Tuesday 14 January 2020

CERN

Book of Abstracts

Contents

NMR session 2 / 3

NMR tools for studying large (>100 kDa) protonated proteins produced in human cells

Author: Alvar Gossert[1]

Co-authors: Philip Rössler [1]; Simon Rüdisser [1]

[1] *ETH Zürich*

Our aim is to study proteins that are relevant for human diseases at atomic resolution. Many of the important drug target proteins –like G-protein coupled receptors –are difficult to study by NMR in solution, as they can often only be produced in eukaryotic expression hosts and are larger than 100160;kDa.
We have therefore developed protocols for isotope labeling in eukaryotic cell lines, including human HEK293 cells. However, to date we are not able to produce highly deuterated samples in these cell lines, that's why we developed an alternative approach to study large proteins based on ¹³C-methyl group labeling and novel NMR experiments. Using a T₁- and T₂-relaxation optimized HMQC pulse sequence and introducing the concept of delayed decoupling, we are able to record spectra with more than 3-fold higher sensitivity. This enables studies of protonated membrane protein complexes of limited stability and characterizing clinically relevant antibodies (150 kDa) at natural ¹³C abundance.

NMR session 1 / 4

ShiftML. A machine-learning model for chemical shifts in molecular crystals with uncertainty quantification

Authors: Michele Ceriotti[1]; Andrea Anelli[2]; Lyndon Emsley[2]; Edgar Engel[3]; Albert Hofstatter[2]; Felix Musil[2]; Federico Paruzzo[2]

[1] *EPFL - EPF Lausanne*

[2] *EPFL*

[3] *Cambridge*

Corresponding Author: michele.ceriotti@epfl.ch

NMR cristallography is gaining traction as a tool to achieve structure determination for molecular materials, but it relies on computationally-demanding electronic structure calculations to estimate the chemical shieldings of candidate structures.
Here I discuss the construction of a machine-learning model trained on electronic-structure data, that can predict accurately chemical shieldings in molecular materials containing C, H, N, O, S, and that incorporates an uncertainty estimation model that makes it possible to assess quantitatively its reliability for different compounds.
I will demonstrate that it can substitute for electronic-structure calculations to support NMR crystallography, and discuss how it can be combined with a Bayesian framework to determine the confidence of NMR-based structural determination.

NMR session 3 / 5

Should we care about imperfect pulses?

Author: Matthias Ernst[1]

[1] *ETH Zurich*

Corresponding Author: maer@ethz.ch

Pulse sequences in solid-state NMR are usually designed and simulated using the assumption of perfect rectangular or shaped pulses. Since the early 50s it had been recognized that the limited bandwidth of the resonance circuit leads to pulse transients (amplitude and phase) at discontinuities of the phase or amplitude. Therefore, the rotations that the spins experience are not the same as the programmed ones. Transient compensation based on linear response theory allow the generation of pulses with a well defined rotation axis over the complete pulse. We have investigated which pulse sequences benefit from transient-compensated pulses and which sequences are insensitive to pulse errors generated by transients. Experimental results can be understood using theoretical models based on Floquet theory.

NMR session 3 / **6**

Single-spin magnetic resonance using Nitrogen-Vacancy centers in diamond

Author: Takuya Segawa[1]

[1] *ETH Zurich*

Corresponding Author: takuya.segawa@phys.chem.ethz.ch

Nitrogen-Vacancy centers in diamond are fluorescent crystal defects with a unique property: Their electron spin (S = 1) can be polarized and read out optically in a fluorescence microscope. This optically-detected magnetic resonance (ODMR) effect is so sensitive that a single defect can be recorded at ambient temperature and pressure.

We will discuss the potential, recent achievements and current challenges of this single-spin magnetic resonance technology giving an insight into the areas of NMR/EPR spectroscopy and nanoscale sensing.

NMR session 4 / **7**

Diffusion-Weighting for in vivo Spectroscopy

Authors: Roland Kreis[1]; André Döring[1]; Kadir Simsek[1]; André Pampel[2]; Harald E. Möller[3]

[1] *University Bern*

[2] *MPI, Leipzig*

[3] *MPI Leipzig*

Corresponding Author: roland.kreis@insel.ch

University Bern, MR Methodology, Departments of Radiology and Biomedical Research, Bern, CH
Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, D

Diffusion-weighting is well established as clinical tool in MRI and as a valuable research tool for the investigation of fiber and micro-structure. Diffusion weighted MR spectroscopy (DW MRS) [1,2] is also becoming more and more of interest to investigate brain microstructure in vivo. The paramount advantage compared to DW water imaging lies with the fact that diffusing metabolites exclusively and selectively probe intracellular space, while DW MRI is complicated by fast intra/extracellular exchange.
In this contribution, we report on two quite distinct aims in the context of DW MRS:

1st we developed DW MR methods to gain microstructural information, where we have extended the range of exploitable diffusion times on clinical systems to a much shorter temporal regime using oscillating gradients [3,4].
2nd we currently use ultra-strong diffusion gradients and interrelated data modeling to help define the macromolecular (MM) signals [5] encountered in clinical short echo-time spectroscopy, where they overlap with the spectral components of small mobile metabolites and hinder the precision of their evaluation if the MM signals are not well defined and have to be represented with flexible or inaccurate models.

References
1. Ronen I, Valette J. Diffusion-Weighted Magnetic Resonance Spectroscopy. eMagRes: John Wiley & Sons, Ltd; 2015:733-750.
2. Palombo M, Shemesh N, Ronen I, Valette J. Insights into brain microstructure from in vivo DW-MRS. NeuroImage. 2018;182:97-116.
3. Doring A, Adalid V, Boesch C, Kreis R. Diffusion-weighted magnetic resonance spectroscopy boosted by simultaneously acquired water reference signals. Magn Reson Med. 2018;80(6):2326-2338.
4. Doring A, Kreis R. Magnetic resonance spectroscopy extended by oscillating diffusion gradients: Cell-specific anomalous diffusion as a probe for tissue microstructure in human brain. Neuroimage. 2019;202:116075.
5. Kunz N, Cudalbu C, Mlynarik V, Huppi PS, Sizonenko SV, Gruetter R. Diffusion-weighted spectroscopy: A novel approach to determine macromolecule resonances in short-echo time (1)H-MRS. Magn Reson Med. 2010;64:939-946.

NMR session 2 / 8

Real-time monitoring of metabolism and mitochondrial respiration in 3D cell culture systems by NMR

Authors: Andrea Felser[1]; Damian Hertig[1]; Jean-Marc Nuoffer[1]; Peter Vermathen[2]; Sally Maddah[2]

[1] *Institute of Clinical Chemistry, University Hospital Bern*

[2] *DRNN & DBMR, University Bern*

Corresponding Authors: jean-marc.nuoffer@insel.ch, damian.hertig@insel.ch, peter.vermathen@dbmr.unibe.ch, sally.maddah@students.unibe.ch, andrea.felser@insel.ch

The feasibility to determine metabolism and oxygen consumption of living 3D cell culture systems by NMR in standard 5mm tubes is demonstrated. High cell viability was obtained and the measurements were stable and reproducible over >12 hours. We describe the effect of the flow rate on metabolic activity. The sensitivity to detect substrate degradation rates of major mitochondrial fuel pathways is demonstrated and the ability to measure rapid O2 and lactate changes as surrogate markers of oxidative phosphorylation and anaerobic glycolysis.

NMR session 3 / 9

Drug Delivery Systems examined by NMR

Authors: Martina Vermathen[1]; Ilche Gjuroski[1]; Max Meier[1]; Sara Pfister[2]; Luca Sauser[3]; Julien Furrer[1]

[1] *University of Bern*

[2] *ETH Zürich*

[3] *University of Zürich*

Corresponding Authors: sara.pfister@phys.chem.ethz.ch, julien.furrer@dcb.unibe.ch, martina.vermathen@dcb.unibe.ch, ilche.gjuroski@dcb.unibe.ch, max.meier@students.unibe.ch, luca-sauser@hotmail.com

Various polymeric drug delivery systems suitable for physical entrapment of porphyrinic photosensitizers are a focus of our research. In this presentation, it will be shown how NMR can provide useful information on many different aspects such as drug loading and stability of the porphyrin-carrier systems. Classical NMR spectroscopic methods such as NOESY and DOSY are used to analyze drug localization at the polymer interface as well as exchange processes and release from the carrier in physiologic solution. The data contribute to the assessment of simple, biocompatible and cost-effective drug delivery systems that can be used for porphyrinic photosensitizers with a prospect of enhanced in vivo efficacy.

NMR session 2 / **10**

Biomolecular mechanisms unraveled by solution NMR spectroscopy

Author: Sebastian Hiller[1]

[1] *Universität Basel*

Corresponding Author: sebastian.hiller@unibas.ch

Solution NMR spectroscopy is a matured technique to study structure, function, and dynamics of biomacromolecules at atomic resolution. Of particular value in the current era of structural biology are integrative approaches to combine data from multiple structure biology techniques to integrated molecular models. I will give an overview of ongoing projects in my laboratory at Biozentrum Basel with selected examples where solution NMR spectroscopy is fruitfully applied to unravel highly relevant molecular mechanism. These examples include the characterization and development of novel antibiotics, the regulation of a cellular IDPs by molecular chaperones, as well as to substrate recognition by a ˜1 MDa kinase complex. These and related projects will particularly benefit from the sensitivity and resolution gains provided by the upcoming 1.2 GHz magnet.

NMR session 1 / **11**

Opening Remarks

NMR session 1 / **12**

New Methods in Solid-State NMR

A short overview of our recent results developing methods for determining structure and dynamics in materials and molecular solids will be provided. These include machine learning approaches to NMR crystallography, new methods for obtaining high resolution 1H spectra in solids, and concepts for hyperpolarisation of inorganic materials.

NMR session 1 / **14**

Proton-detected Solid-state NMR of Proteins

Co-authors: Alexander Malär [1]; Maarten Schledorn [1]; Anahit Torosyan [1]; Susanne Penzel [1]; Thomas Wiegand [1]; Nils-Alexander Lakomek [1]; Alons Lends [1]; Vlastimil Jirasko [1]; Lauriane Lecoq [2]; Matthias Ernst [1]; Anja Böckmann

[2]

[1] *Laboratory of Physical Chemistry, ETH Zürich*

[2] *Institut de Biologie et Chimie des Protéines, Bases Moléculaires et Structurales des Systèmes Infectieux, Labex Ecofect*

An overview over the research activities in proton-detected fast-MAS will be provided

NMR session 1 / **16**

DNP and Electron Decoupling with Chirped Microwave Pulses and Spinning Spheres

NMR session 2 / **18**

New tools to study protein phase separation by solution NMR

NMR session 2 / **22**

NMR spectroscopy of RNA G-quadruplexes

Author: Silke Johannsen[None]

Co-authors: Simon Jurt [1]; Zenghui Wang [1]; Roland K.O. Sigel [1]

[1] *Department of Chemistry, University of Zurich*

As one kind of non-canonical structures of nucleic acid, G-quadruplexes (G4) exist universally in the genome of different organisms and in humans. G4 are supposed to play crucial regulatory roles in gene
replication, translation and expression. Therefore, G4s are handled as a promising target for chemotherapy. To date, most studies concentrated on DNA G4s, while the understanding of RNA G4s is far from comprehensive. Here I will present our recent results on investigating structural details of RNA G4s
by NMR spectroscopy.

NMR session 2 / **23**

Intercepting c-di-GMP signaling by a c-di-GMP sequestering peptide

Co-authors: Christoph Schmutz [1]; Tilman Schirmer [2]; Urs Jenal [1]; Chee-Seng Hee [3]; Stephan Grzesiek [2]

[1] *Focal Area of Infection Biology, Biozentrum, University of Basel*

[2] *Focal Area of Structural Biology and Biophysics, Biozentrum, University of Basel*

[3] *Focal Area of Structural Biology and Biophysics, Biozentrum, University of Basel; Focal Area of Infection Biology, Biozentrum, University of Basel*

NMR session 4 / **24**

Beta-detected NMR: from nuclear physics to biology

Corresponding Author: kowalska@cern.ch

NMR session 3 / **26**

Intrinsic magnetic anisotropy of lanthanide chelates and new lanthanide chelating tags for protein NMR spectroscopy

NMR session 4 / **31**

Hyperpolarized Imaging - from experimental towards clinical application

Dissolution dynamic nuclear polarization allows to temporarily enhance polarization of

carbon-13 and other nuclei by up to four orders of magnitude and yields injectable tracers in solution for in-vivo

applications. In recent years, various substrates have been proposed to probe different metabolic pathways or to

act as inert contrast agents. To this end, we show work on long-lived polarization in silicon-29 and its

potential for in-vivo imaging. We demonstrate advanced imaging approaches of pyruvate and urea along with

modelling to assess relevant biological information in both the experimental and human setting and provide an outlook towards optimal experimental design.

NMR session 4 / **34**

The strange Xenon: an inert gas, hyperpolarization and theranostics

Corresponding Author: jean-noel.hyacinthe@hesge.ch

NMR session 4 / **36**

Do We Still Need NMR to Identify Natural Products

Author: Jean-Luc Wolfender[1]

[1] *Université de Genève*

Corresponding Author: jean-luc.wolfender@unige.ch

With the recent progresses made in metabolite profiling methods especially based on Liquid chromatography high resolution mass spectrometry methods (LC-HRMS), a question that arises is: Do we still need to fully identify natural products (NPs) using tedious isolation protocols from complex biological matrices and subsequent 1D and 2NMR characterisation for their unambiguous identification?
High resolution mass spectrometry (HRMS) and data dependent MS/MS analyses provide very valuable information on secondary metabolites for in-depth metabolome annotation studies [1]. The recent development of molecular network (MN) approaches for the mining of such data in combination with spectral database generated in silico [2] gives the possibility to establish relationships between metabolites thus significantly improving the efficiency of dereplication when combined with high quality chemotaxonomic data [3]. Such types of information can be generated with a few mg of extract only and are readily applicable to herbarium scale samples. These data massively acquired on many samples provides a new and efficient way to obtain detailed structural information on many metabolites at once and this considerably improves the dereplication process.
NMR is still required for complete de novo identification of new compounds and, in this case, MS-targeted micro-isolation of given NPs can be performed and sensitive 1D and 2D microNMR with microgram amounts of purified metabolites can be acquired. For bioactivity determination, many bioassays fit also to this scale. Using an ideal combination of methods it is this virtually possible to fully identify any bioactive principles in this way. Integration of other filters to this approach such as permeation studies on extracts additionally provide key information on the possible bioavailability of NPs prior to their isolation. Furthermore the link of a given bioactivity result to those previously reported for compounds similar to those identified can be rationalised through in silico chemical space approaches.
Ideally a combination all these state-of-the-art methods should enable to identify and localise valuable NP efficiently at the analytical scale. In such a way large scale MS-targeted isolation of valuable NPs only can become a very rational way to conduct investigations. Different recent applications of our metabolomics and phytochemical investigations will illustrated these aspects.
References
[1] J. L. Wolfender, J. M.Nuzillard, J.J.J. van der Hooft, J.H. Renault, S. Bertrand 2019. Anal. Chem. 91: 704-742.
[2] P. M. Allard; Peresse, T.; Bisson, J.; Gindro, K.; Marcourt, L.; Pham, V. C.; Roussi, F.; Litaudon, M.; Wolfender, J. L. Anal. Chem. 2016, 88, 3317-3323
[3] P.-M. Allard; Genta-Jouve, G.; Wolfender, J.-L. Curr. Opin. Chem. Biol. 2017, 36, 40-49.

NMR session 3 / **37**

NMR in the web-browser: applications to metabolomics and teaching

NMR session 1 / **38**

Elucidating the structure of surface sites using sensitivity enhanced NMR methods

Authors: Deni Mance[1]; Christophe Copéret[1]

[1] *ETH Zurich*

Corresponding Author: mance@inorg.chem.ethz.ch

Catalytic processes in industry predominantly rely on heterogeneous-catalysts, a prominent example are supported vanadium oxide oxidation catalysts, VOx/Support. Due to the small amount and ill-defined nature of their active sites, little is known about their structure.

While NMR would be a method of choice to probe the structure of surface sites, it particularly suffers from its low. Dynamic Nuclear Polarization (DNP) can be a method of choice to alleviate the sensitivity problem, but it is currently mostly limited to spin ½ nuclei, with limited success with quadrupolar nuclei. Another issue that arises with this method is incompatibility of the radical or solvent required for DNP measurements with the catalyst.

An alternative method to study such systems would be the use of 1H-detection under fast MAS conditions, which has been especially fruitful in the case of studying biomolecules. Here we will present the use of this approach to identify surface sites in Vanadium oxide supported catalyst, we were able to detect low-abundant (<1%) surface species and characterize them with various correlation spectra in a time effective manner. Thereby showcasing how 1H-detection can be used for this case, which can be further expanded to other catalysts.

NMR session 3 / **39**

Targeting FPPS by fragment-based lead discovery

Author: Joy Kristin Petrick[1]

Co-authors: Lena Muenzker [1]; Andreas Marzinzik [1]; Wolfgang Jahnke [1]

[1] *Novartis*

Trypanosoma cruzi (*T. cruzi*) is the causative agent of Chagas disease, which is considered a neglected disease. Medication for this disease is based on empirically discovered drugs with low efficacy, difficulties in administration and severe side effects. The development of a safe and efficient drug is therefore urgently needed.

Farnesyl pyrophosphate synthase (FPPS) is a key enzyme in isoprenoid biosynthesis. The parasite is dependent on isoprenoids, such as ergosterol, as they cannot be acquired by other mechanisms. Bisphosphonates (BPs) are active site directed FPPS inhibitors. They are used in the clinic as drugs for bone diseases due to their ideal pharmacokinetics in targeting bone tissue. They can also combat *T. cruzi* flagellates but are not ideal to treat Chagas disease. Several non BP inhibitors that bind to an allosteric pocket were found for human FPPS by fragment-based screening (FBS). More recently it was shown that the product of FPPS, farnesyl pyrophosphate (FPP) can bind to this pocket and locks the enzyme in an open and inactive state.

Encouraged by these findings, we started our investigations by FBS against *T. cruzi* FPPS. Screening and validation of 1806 fragments by NMR spectroscopy revealed 118 diverse fragment hits. Counter screening against human FPPS and *T. brucei* FPPS, the causative agent of African sleeping sickness, showed selectivity of the fragments at this early stage of screening. To enable follow up by X-ray crystallography, a crystallization system was set up that yielded apo-crystals of *T. cruzi* FPPS with a diffraction limit of around 1.6 A. 72 fragments were employed to soaking experiments that resulted in two structures. One ligand was active site directed and the other binding to the homodimer interface. The major break trough was achieved by FBS by X-ray crystallography at the XChem facility in Harwell, UK, and the HTXlab in Grenoble, France. In total 1113 data sets were collected and analyzed using the statistical analysis tool Pan-Dataset Density Analysis (PanDDA). More than 50 hits with non-bisphosphonate scaffolds were obtained. Binding sites were distributed over the entire protein, including the active site, the allosteric site, the homodimer interface, sites on the surface and a new site in close proximity to the active site.
In summary, we found active site binders of a novel scaffold and discovered the first allosteric site binders for *T. cruzi* FPPS. Both will deliver starting points for medicinal chemistry. Thus, the herein reported findings will give new impulses in drug discovery for Chagas disease.

NMR session 1 / **40**

Investigating Seven-Transmembrane Proteins by Solution NMR

Methods

Author: Oliver Zerbe[None]

I will report on our developments of methodology for assigning 7-TM membrane proteins by solution NMR methods. To this end, information from triple-resonance NMR spectra, from 3D and 4D NOESY spectra and from biochemical and topological information is fed as restraints into the FLYA module of CYANA. Thereby we could assignment more than 60 % of backbone and methyl groups of bacteriorhodopsin incorporated into lipid nanodiscs without mutagenesis. In addition, we probed dynamics of methyl groups.

Furthermore, I present first results of our NMR studies conducted on the a1b adrenergic receptor. I will in particular highlight our efforts to produce receptor constructs stable for NMR measurements at elevated temperature.

NMR session 2 / **41**

Multiple states of proteins in vitro and in cells

Author: Roland Riek[1]

[1] *ETHZ - ETH Zurich*

Corresponding Author: roland.riek@phys.chem.ethz.ch

The 3D structure and dynamics of proteins are key of their functions. Proteins undergo thereby structural changes from one state to another one.
With the advent of exact NOEs structural ensembles of proteins are determined at atomic resolution highlighting this structure-dynamic mechanism in the case of enzymes and protein allostery in vitro. In parallel, in cell NMR shows the presence of multiple structural states inside a cell that interchange both in the slow and fast exchange dynamics regime indicating that a protein exists in vivo on a structural landscape rather than a single state.

NMR session 3 / **42**

Spin thermometry: deuterium NMR spectra give a straightforward measure of very low spin temperatures resulting from dynamic nuclear polarization

Co-authors: Behdad Aghelnejad [1]; Diego Carnevale [1]; Mathieu Baudin [1]; Sina Marhabaie [1]

[1] *Ecole normale supérieure, Paris*

Dynamic nuclear polarization of samples at low temperatures, typically between 1.2 and 4.2 K, allows one to achieve spin temperatures as low as 2 mK, so that for many nuclear isotopes the high-temperature approximation is violated. This leads to characteristic asymmetries in powder spectra. We show the lineshapes due to the quadrupolar couplings of deuterium spins that are present in virtually all solvents used for such experiments allows the quick yet accurate determination of the spin temperature, or, equivalently, of the polarization. The observation of quadrupolar echoes excited by short pulses allows one to monitor the build-up and decay of positive or negative hyperpolarization when switching the frequency of the microwave irradiation.

NMR session 4 / **43**

Closing Remarks