SPEAKERS ABSTRACTS

EUROMAR2026

GÖTEBORG

June 28 - July 02

Eriksbergshallen

Table of Contents

Table of Contents (continued)

Hyperpolarization 1

Liquid Methods 1

Low field / Benchtop

Solids Methods 1

Table of Contents (continued)

Table of Contents (continued)

Table of Contents (continued)

Hardware

Liquid methods 2

AI/Theory/Computation 2

EPR & paramagnetic 2

Speaker Registry

Speaker Registry (cont.)

Monday I Hall A I 08:30-9:10

Mei Hong

Structures and Dynamics of Tau Assemblies From Solid-State NMR

The intrinsically disordered and highly charged tau protein aggregates into β-sheet amyloid fibrils that spread in the brain of many neurodegenerative diseases, including Alzheimer's disease (AD). In these pathological tau aggregates, a small rigid β-sheet core is surrounded by large intrinsically disordered segments. Different tauopathies have different rigid-core structures while each tauopathy exhibits a single structure. To understand how this intrinsically disordered protein converts to well-defined and disease-specific β-sheet structures, we use solid-state NMR coupled with cryoelectron microscopy to investigate the structures and dynamics of *in vitro* assembled tau fibrils. Our results show that tau fibrils assembled in the presence of the anionic cofactor heparin adopt homogeneous structures, but these structures deviate from brain tau structures. Shortening the intrinsically disordered regions facilitated fibril formation, and some constructs adopt the AD fold, but only under specific pH and salt conditions. Thus, tau aggregation is sensitive to environmental conditions. Incorporating charge-reducing phospho-mimetic mutations allowed cofactor-free aggregation of full-length tau, with some structures mimicking the three-dimensional fold of tau in certain tauopathies. Seeding recombinant tau with AD brain tau led to the accurate reproduction of AD tau structure. However, these gen-1 fibrils are unable to template further generations, indicating that they lack certain properties of AD tau. These results suggest that the intrinsically disordered fuzzy coat of tau fibrils, which contain the majority of posttranslational modifications in pathological tau, may regulate the conversion of monomers to β-sheet structures to elongate existing fibrils. We are now characterizing the dynamics of the tau fuzzy coat and its interaction with the rigid core using solid-state and solution NMR. These solid-state NMR data give insights into the aggregation mechanism of tau, and suggest therapeutic targets for intervention of tauopathies.

Operando NMR and MRI characterisation of the transport and transformation of ions and molecules in porous materials.

Britton, Melanie

The transport and transformation of molecules and ions through porous materials are critical processes across a diverse range of energy storage and separation technologies. Understanding the structural and chemical factors controlling these processes, under working conditions, is essential for their development and application. We have employed multi-nuclear nuclear magnetic resonance (NMR) spectroscopy and imaging (MRI) to observe and quantify these processes, in operando, within porous materials employed across a range of energy storage and separation applications. Our experiments apply both direct and indirect detection of the transport, distribution and chemical evolution of species.

In this talk, I will present examples exploring the insight achieved using operando NMR and MRI to understand the transport and transformation of molecules and ions in three classes of porous material. In the first example, we investigate the competitive adsorption of water and CO_2 in the CALF-20 metal-organic framework (MOF). We employ ^{1}H NMR to observe water adsorption, ^{13}C NMR to track structural changes in the framework, and ^{17}O and ^{2}H NMR to quantify the dynamics of pore-confined water as a function of relative humidity. We observe competitive CO_2 adsorption, indirectly, using ^{1}H NMR. In the second example, we employ ^{1}H and ^{7}Li NMR relaxation and diffusion studies to observe the transport and separation of ions and molecules in ion-conducting and ion-selective polymer membranes [1,2]. We explore the link between polymer membrane pore size, water diffusivity and permeability. In the third example, we employ ^{1}H and ^{7}Li diffusion NMR and MRI, revealing electrolyte transport and distribution in battery separators during formation and charge cycling in lithium-ion batteries, as well as ^{1}H and ^{23}Na NMR and MRI revealing the development of metallic sodium during formation and fast charging in sodium-ion batteries [3].

[1] A. Wang et al. *Nature* 635 (2024) 353

[2] D. Yang et al. Nature Water 3 (2025) 319.

[3] J. M. Bray et al. *Nat. Commun.* 11 (2020) 2083.

Experimental methods and computational tools for battery research and membrane science

Clément, **Raphaële**,[1,2] Nguyen, Howie,[1,2] Bassey, Euan,[1,2] Garcia Ponte, Elizabeth,[1,2] Gordon, Leo,[1,2] Ouimet, Jonathan,[3] Sujanani, Rahul,[3] Sebti, Elias,[1,2] Kitchaev, Daniil,[1,2] Behara, Sesha Sai,[1] Latchem, Emma,[1,2] Freeman, Benny,[4] Christopher, Phillip,[3] Segalman, Rachel,[1,2,3] Van der Ven, Anton[1]

[1]Materials Department, University of California, Santa Barbara, CA 93106, USA.
[2]Materials Research Laboratory, University of California, Santa Barbara, CA 93106, USA.
[3]Chemical Engineering Department, University of California, Santa Barbara, CA 93106, USA.
[4]McKetta Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712, USA.

Two major challenges facing society today are ensuring reliable energy and clean water supplies for all. In this context, the development of high-performance secondary batteries is critical for enabling the widespread use of renewable energy sources. At the same time, ion-selective polymer membranes play a key role in critical mineral recovery—for example, for battery materials—and in water purification.

Designing next-generation batteries and ion-selective membranes requires an atomic-level understanding of composition–structure–property relationships. Magnetic resonance methods provide unique insight into the local structure and ion transport processes in battery and membrane materials. Our group has focused on developing new equilibrium and time-resolved measurements for battery devices and membrane systems, enabling the separation of thermodynamic and kinetic parameters that govern their performance. In parallel, we have developed computational tools to facilitate the interpretation of complex experimental data.

In this talk, I will present our work on *operando* EPR and magnetometry of battery cathode materials, along with *ab initio* cluster expansion Monte Carlo simulations used to predict the room-temperature NMR and magnetic properties of transition metal-containing compounds. I will also discuss our use of spatially- and temporally-resolved NMR to determine partitioning and diffusion coefficients of solvents and solutes in polymeric membranes.

Understanding complex systems with (mostly) simple tools: from functional protein dynamics to metabolomics

Rafael Brüschweiler

Department of Chemistry and Biochemistry and Department of Biological Chemistry and Pharmacology, The Ohio State University, Columbus, OH 43210

Despite the central role of K-Ras protein in many different types of human cancer, major gaps in atomic-level information by traditional structural biology methods (X-ray, cryo-EM, NMR, modeling) severely limit our understanding of K-Ras function in health and disease. This is primarily due to the challenge to work with K-Ras when bound to its active, hydrolyzable guanosine triphosphate (GTP) ligand and the difficulty to observe the functionally critical Switch I and Switch II regions of K-Ras. I will describe recent advances to address these challenges by state-of-the-art solution NMR spectroscopy revealing the functional dynamics of K-Ras at atomic resolution on nanosecond-to-millisecond timescales using relaxation dispersion, residual dipolar couplings, and heteronuclear spin relaxation. The experimental data reveal cooperative transitions of active K-Ras·GTP to a dynamic excited state that resembles the partially disordered "inactive" K-Ras·GDP state. Further insights can be gained by molecular dynamics computer simulations that were carefully validated against our large body of experimental NMR data. The results help understand differential GTPase activities and signaling properties of WT versus oncogenic mutants guiding new strategies for the development of therapeutics. I will also discuss methodological advances that enabled this research including nanoparticle-assisted spin relaxation (NASR) and machine-learning based algorithms for data analysis (DEEP Picker) that can be broadly used also in other NMR fields, such as metabolomics.

Breaking the limits in understanding glycan recognition by NMR

J. Jimenez-Barbero,[a,b,c]

[a] *CIC bioGUNE, Bizkaia Technology Park, Building 801, 48162 Derio, Spain*
[b] *Ikerbasque, Basque Foundation for Science, Bilbao, Spain*
[c] *Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, Madrid, Spain*

jjbarbero@cicbiogune.es

Molecular recognition by specific targets is at the heart of the life processes. Carbohydrates (glycans, saccharides, sugars), prevalent on mammalian cells and in the extracellular matrix, collectively form the glycocalyx, impacting physical properties, regulating protein function, and acting as ligands for lectins (glycan-binding proteins). Indeed, dysregulation of glycosyltransferases results in tumor-associated carbohydrate antigens (TACAs), unique to tumor cells. TACAs play a key role in modulating immune responses within the tumor microenvironment through interactions with lectins. Similar interactions are observed in bacterial and viral-mediated infectious events. Therefore, the interactions between lectins, enzymes, antibodies and glycans mediate a broad range of biological activities, from fertilization and tissue maturation to pathological processes. In this context, the elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, unravelling the structural, dynamic, and conformational factors that rule the interactions of these molecules is of paramount interest.

Solution NMR is unique in providing stereochemical, conformational, and dynamic information. Given the inherent flexibility and dynamic properties of glycans, we use NMR as key tool for deducing, at atomic resolution, molecular recognition events in which glycans are involved. Together with state-of-the-art NMR, we employ a combination of diverse and synergic methodologies, including chemical synthesis, protein biochemistry and molecular biology, biophysics (ITC, BLI), molecular modelling, and structural biology techniques (X-Ray crystallography and CryoEM). In this way, we investigate the interactions of glycans related to immune responses, from cancer to bacterial and viral infections.

This presentation focuses on the application of our NMR methodology, both from the ligand and receptor's perspective, to study conformation and dynamics of glycans and molecular recognition events with their receptors of biomedical interest. Recent examples will be shown, including new NMR avenues to unravel the interactions of glycans on intact glycoproteins and on cell surfaces.[1-7]

References

1. Unione et al., *ACS Cent Sci* **2019**, *5,* 1554; *Curr Opin Struct Biol* **2021**, *68,* 9; *Angew Chem Int Ed,* **2022,** *61: e202201432;* Østerlid et al., *Angew Chem Int Ed*. **2025**, *64*, e202511378.
2. Gimeno et al., *Curr. Opin. Struct. Biol.* **2020**, *62*, 22; Angulo, Jiménez-Barbero et al., *Prog NMR Spectrosc.* **2024**, *144,* 97. Quintana et al., *J Biol Chem*. **2024**, *300*, 107577; *Glycobiology*. **2024**, *34*:cwae002.
3. Lenza et al., *Angew Chem Int Ed.* **2020**, *59*, 23763; *JACS Au.* **2023**, *3,* 204; *Nat Comm*. **2023**, *14,* 3496
4. Moure et al., *Angew Chem Int Ed* **2021**, *60*, 18777–82. Atxabal et al., *Chem Sci* **2024**, *15*, 10612
5. Bertuzzi et al., *ACS Chem Sci.* **2022**, *8,* 1415; *Chem Eur J.* **2025**, *31*:e202403102.
6. Wang et al., *J Am Chem Soc* **2023**, 145, 14052; *Angew Chem Int Ed*. **2023**, *62*: e202211940.
7. Fittolani et al., *Nat Chem*. **2023**, 15, 1461; Yadav et al *J Am Chem Soc* **2024**, 146, 6369.

Reawakening a sleeping giant? Steady-state high resolution NMR in chemistry, biology and beyond.

Lucio Frydman
Department of Chemical and Biological Physics, Weizmann Institute, Rehovot, Israel

The steady-state free-precession (SSFP) NMR experiment proposed by Carr nearly 70 years ago applies a rapid train of pulses at repetition times TR<<T2,T1, while observing the emitted signal. As shown by Carr this can provide a steady transverse emission of up to 50% of the thermal equilibrium magnetization, and thereby a superior sensitivity/time than FT-based experiments. This was explored in the early days by NMR's pioneers, but eventually abandoned for FT-based acquisitions: SSFP could not deliver a comparably high spectral resolution, its rapid pulsing led to "blind" spectral regions, and it was incompatible with sophisticated multiple-resonance polarization transfer techniques. Hence, although widely used in MRI –with ≈100% of cardiac and angiography studies relying on SSFP and over 23,000 papers quoting its use– SSFP is virtually unknown in the realm of spectroscopy. The present study tries to reawaken this sleeping giant, by introducing ways to overcome SSFP's handicaps, leading to a new approach to high resolution NMR that matches or exceeds the sensitivity of FT-NMR methods. It is also shown that coherent polarization transfers can be combined with these SSFP schemes, to enhance even further the sensitivity gains. This can in turn be used to endow 13C spectroscopic imaging with sufficient sensitivity, to image metabolic aberrations associated with the presence of tumors without hyperpolarized approaches. With the introduction of high-resolution into SSFP it was realized that numerous basic physical features that are "bread and butter" in spectroscopic NMR –topics such as the effects of J-couplings, chemical exchange, magic-angle spinning and radiation damping– have never been explored within the framework of this decades-old experiment. These and other unexplored but exciting phenomena arising in liquids and solids applications of SSFP NMR will be briefly surveyed.

Heavy mice and lighter things: solid-state NMR investigations of chemistry in tissues

Melinda J. Duer

Yusuf Hamied Department of Chemistry, University of Cambridge, UK
e-mail: mjd13@cam.ac.uk
web-page: http://www.ch.cam.ac.uk/group/duer/

The extracellular matrix (ECM) forms the bulk of our structural tissues and provides them with their mechanical properties. Equally importantly, cell fate and function is determined by the molecular structures of the ECM and their interface with cells. Thus, the ECM manages very different roles on different lengthscales, all ultimately underlined by the ECM molecular structures, dynamics and organization. If we can understand how the ECM molecular structure and dynamics dictates the behaviour of cells and the tissue mechanical properties, then we can develop ways to combat the effects of ageing, reprogram cancer cells and effectively treat many degenerative diseases.

Arguably, solid-state NMR spectroscopy is one of the few techniques that can deliver molecular structural and dynamics information in such heterogenous materials as the ECM. In this talk, I will discuss some of the solid-state NMR approaches that give access to important structure and dynamics information on ECMs from bone to brain.

Tissue mechanics are as important as the ECM as a regulator of cell behaviour and I will describe new work that allows us to understand how stretching a tissue changes ECM molecular structure and dynamics.

Finally, I will address whether we can use NMR to understand how to therapeutically treat ECM to improve disease outcomes, particularly in cancer.

Single-spin magnetic resonance using superconducting quantum circuits

P. Bertet, CEA Saclay

Pushing the sensitivity of magnetic resonance spectroscopy to the single-spin limit promises structural information at the scale of individual atoms or molecules. We developed a new method to achieve single-electron-spin sensitivity by using superconducting circuits at milliKelvin temperatures. It consists in enhancing the rate at which an electron spin spontaneously relaxes by emitting a microwave photon into a low-mode-volume, high-quality-factor microwave superconducting resonator [1]. The emitted photon is then detected by a microwave photon counter, based on a superconducting qubit [2]. The electron spin can be used as a nano-antenna to polarize and probe nearby nuclear spins, allowing single-spin NMR spectroscopy [3]. I will present application of the method to the spectroscopy of individual quadrupolar nuclei (^{43}Ca and ^{93}Nb) in a $CaWO_4$ crystalline matrix, detected by proximal lanthanide paramagnetic impurities. Owing to the low nuclear spin background in this matrix and to the absence of inhomogeneous broadening, high spectral resolution is reached ; in particular, fitting the ^{93}Nb data requires including a hexadecapolar term in the nuclear spin Hamiltonian which may constitute a first evidence for a nuclear hexadecapolar moment [4]. I will discuss perspectives to develop the method into a general-purpose EPR and NMR single-molecule spectrometer.

[1] Z. Wang et al., Nature 619, 276 (2023)

[2] L. Balembois et al., Phys. Rev. Applied 21, 014043 (2024)

[3] J. Travesedo et al., Sci. Adv. 11, eadu0581 (2025)

[4] J. Travesedo et al., arxiv:2605.02857

Very Attractive Instruments: A Love Story About Permanent Magnets

Blümler, Peter

As magnetic resonance moves toward more compact, accessible, and application-specific systems, permanent magnet design has re-emerged as a key enabling technology - and there is more to it.

This talk offers a tour from the fundamental properties of permanent magnets, through idealized continuous magnetization distributions in one to three dimensions, to analytical solutions for realistic configurations composed of a fixed number of finite-size magnets. Inspired by Halbach designs, key topics include methods for field homogenization and controlled field variation, as well as strategies for opening magnet structures while minimizing the required forces.

These fundamental design principles are illustrated through applications in NMR, MRI, DNP, EPR, magnetic guiding, and *n*MR (magnetic resonance on free neutrons from a nuclear reactor inside a magnetic trap).

Mathematics and computer science of steady state DNP simulations

Maximilian Keitel[1], Callum Musselwhite[1], Shebha Anandhi Jegadeesan[2], Guinevere Mathies[2], Ilya Kuprov[3]

[1]School of Chemistry and Chemical Engineering, University of Southampton, United Kingdom; [1]Department of Chemistry, University of Konstanz, Germany; [3]Chemical and Biological Physics Department, Weizmann Institute of Science, Rehovot, Israel.

Solid state dynamic nuclear polarisation (DNP) experiments pump nuclear spin transitions by transferring magnetisation from unpaired electrons through repeated application of a microwave pulse sequence [1,2]. This is an active research area and a competition; the pulse sequence that produces stronger nuclear magnetisation in shorter time with lower average microwave power is a better one. Theoretical simulations [3,4], including optimal control theories [5], are often used to assist DNP sequence design and optimisation efforts. However, DNP is a difficult simulation target: the nuclear magnetisation pertains to the asymptotic steady state, and sometimes a steady orbit, of driven dissipative dynamics generated in a spin system ensemble by imperfect control hardware, including distributions in distances, orientations, concentrations, conformations, microwave amplitudes, and spin relaxation times [6,7].

In this presentation, we report several new methods for efficient simulation and optimal control of asymptotic steady states of heterogeneous dissipative ensembles that we have recently implemented in *Spinach* library (https://github.com/IlyaKuprov/Spinach):

(a) a Newton-Raphson steady state solver that efficiently (a few matrix-vector operations) calculates the asymptotic steady state of a dynamical system produced by repeated action by a user-specified propagator on any initial spin state [7].

(b) a modification that allows gradient ascent pulse engineering (GRAPE) method to target stroboscopic steady states and limit cycles that are approached asymptotically as a particular control sequence is repeated infinitely many times.

(c) improvements in sparse matrix logistics of spin dynamics simulations that reflect different additive and multiplicative operation efficiency of compressed sparse column (CSC) and coordinate (COO) sparse matrix storage formats.

(d) improvements in GPU and parallelisation logistics that reflect the balance between communication and computation efficiency of the linear algebra of spin dynamics simulations on different types of computing devices.

Due to the complexity and heterogeneity of solid state DNP samples, most simulations in the current literature only achieve qualitative agreement with experimental data. We demonstrate that steady state simulations with distributions in electron-nuclear distances, B_1 fields, and relaxation rates are in close quantitative agreement with experimental data.

[1] A.W. Overhauser, Physical Review (1953) 92(2) 411; **[2]** T.R. Carver, C.P. Slichter, Physical Review (1953) 92(1) 212; **[3]** F. Mentink-Vigier, S. Vega, G. De Paëpe, Physical Chemistry Chemical Physics (2017) 19(5) 3506; **[4]** F.A. Perras, M. Pruski, Journal of Chemical Physics (2018) 149(15) 154202; **[5]** J.P. Carvalho, D.L Goodwin, N. Wili, A.B. Nielsen, N.C. Nielsen, Journal of Chemical Physics (2025) 162(5) 054111; **[6]** F. Mentink-Vigier, Journal of Magnetic Resonance (2021) 333 107106; **[7]** S.A. Jegadeesan, Y. Zhao, G.M. Smith, I. Kuprov, G. Mathies, Journal of Chemical Physics (2025) 163 034111.

A Journey Through the World of Spin Polarisation

Kevin B. Henbest (1), Claudia Tait (1), Sebastian Kopp (1), Patrick Murton (1), Jamie Gravell (1), Ana Stuhec (1), Daniel Cubbin (1), Victoire Dejean (1), Tommy L. Pitcher (1), Gabriel Moise (1), Sabine Richert (1), Jingjing Xu (2), Henrik Mouritsen (2), P. J. Hore (1), Harry Anderson (1), Stuart R. Mackenzie (1), Christiane R. Timmel (1)

(1) Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK, (2) AG Neurosensory Sciences/Animal Navigation, Institut für Biologie und Umweltwissenschaften, Carl-von Ossietzky Universität Oldenburg, 26111 Oldenburg, Germany.

christiane.timmel@chem.ox.ac.uk

Over the last decade, interest in photogenerated spin systems far from equilibrium has grown dramatically. While the fundamental spin physics and chemistry of these systems have been established for many years, emerging applications in quantum information science and molecular electronics have brought renewed focus to these phenomena and accelerated progress across the field.

Three representative classes of photogenerated spin systems are highlighted: (1) radical pairs as quantum sensors, including their proposed role in animal magnetoreception; (2) triplet pairs generated by singlet fission in polyacenes and their remarkable performance as quantum compasses; and (3) photogenerated triplet states as probes of supramolecular architectures and molecular wire assemblies.

A combination of optical spectroscopy, magnetic field effect studies, and electron paramagnetic resonance, supported by theoretical modelling and density functional theory calculations, is employed to investigate these systems. The integration of complementary experimental and computational techniques provides unique insight into spin dynamics, electronic structure, and function in molecular systems far from equilibrium.

 Songi Han

Water as essential building blocks to direct protein structure and function

Active Role of Water in Protein Assembly to Qubits

Water is central to how biological molecules interact, assemble, and perform chemical and biological work, yet it remains exceptionally difficult to characterize. It behaves as both a liquid and a structured material, adopting multiple distinct configurations around dissolved solutes. A combination of ^{1}H and ^{17}O nuclear magnetic resonance and electron-nuclear double resonance spectroscopy can resolve the dynamical, structural, and thermodynamic properties of water as they vary with the surface chemistry and topology of small molecules and biological macromolecules. I will outline the measurement principles and why they are not yet routinely applied and discuss the conditions under which the shaping of water occurs and why it matters for biomolecular function. Control experiments with DMSO, glycerol, and phosphate reveal that even these "simple" mixtures carry rich and unexpected structural signatures of their own.

A central worked example will be the LOV2 photoreceptor domain of Avena sativa (AsLOV2), where we find that blue light triggers the concerted, reversible eviction of low-entropy, tetrahedrally structured wrap water from the protein surface, and that this hydration change precedes — and appears to drive — the long-range conformational extension that releases the Jα helix from the LOV core. Strikingly, hydrostatic pressure recapitulates this activation in the absence of light, shifting the same conformational equilibrium with a comparable free-energy contribution. Water in this system is not a passive solvent, but a hydraulic actuator that performs mechanical work on the protein. I will also show that the same lens — water shaped by an active surface modulating the thermodynamic of protein motion and function — extends to ongoing work on tau protein aggregation to the design of antifreeze molecules. In each case, our working hypothesis runs counter to several established design criteria, and connects the role of water to the function and activity of proteins.

Deep Learning as a New Dimension in NMR: From small molecules to large biomolecular complexes

Hansen, D. Flemming

Deep learning is rapidly changing how MR spectra are recorded, processed and interpreted. In the tutorial, it will initially be discussed why NMR is well suited for artificial intelligence, including the fact that training data can be generated synthetically, and pulse sequences can encode information in forms that neural networks can decode. The tutorial will emphasise that deep learning cannot create information absent from the experiments; its power lies solely in extracting latent information from carefully designed and information-rich data. As spectroscopists our task is thus to develop experiments and pulse sequences that generate information-rich data, albeit these might look complex in nature to us.

The tutorial will cover both biomolecular and small-molecule NMR. For proteins, I will show how deep learning can enhance the resolution and virtually decouple aromatic and methyl ^{13}C-^{1}H spectra. Longitudinal exchange and titration experiments show how quantitative information on dynamics and interactions can be extracted from deep learning-transformed data. The recently developed single-pulse methods that reconstruct 2D spectra of very large biomolecular complexes will also be discussed.

For small molecules and mixtures focus will be on deep-learning assisted pure-shift methods that preserve quantitative information. This will include new lightweight architectures that generate high-sensitivity pure-shift 1D and 2D spectra from conventional and spin-echo data, with applications to complex mixtures. Finally, I will discuss limitations, artefacts, out-of-distribution behaviour and implementation, including deployment in TopSpin. Focus will generally be on providing a practical framework for using deep learning as an integrated component of modern NMR spectroscopy.

Principles and Applications of EPR in Chemistry and Biology

Goldfarb, Daniella

This tutorial lecture will introduce the principles and applications of Electron Paramagnetic Resonance (EPR) spectroscopy in chemistry and biology, with emphasis on modern pulse EPR methods for studying molecular structure and dynamics. The presentation will begin with an overview of the types of paramagnetic systems accessible by EPR and the interactions that determine EPR spectra, while highlighting the similarities and key differences between EPR and NMR spectroscopy. Continuous-wave (CW) EPR will be briefly discussed as a method for recording EPR spectra and probing molecular motion through spectral line-shape analysis. The development of rapid-scan EPR methods will also be briefly introduced.

The tutorial will then focus on pulse EPR techniques that provide structural information on the nanometer scale. Particular attention will be given to pulse dipolar spectroscopy, especially DEER/PELDOR, which enables distance measurements between paramagnetic centers. Examples will demonstrate how these methods reveal conformational changes, ligand binding, and biomolecular dynamics. Different spin labels, including nitroxides and metal-based probes such as Gd(III), Cu(II), and Mn(II), will be introduced together with approaches for distance-distribution analysis.

In addition, hyperfine spectroscopic techniques such as ENDOR, ESEEM, HYSCORE, and ELDOR-detected NMR will be presented as methods for probing electron–nuclear interactions and local structure, accompanied by selected examples. Recent developments including high-field EPR, , and advanced pulse schemes will also be presented. Through selected examples, the lecture will illustrate the broad applicability of EPR spectroscopy to contemporary problems in chemistry, structural biology, and biophysics and will and will conclude with an outlook on future developments in the field.

Symmetry-Based Pulse Sequences in Solids and Liquids

Malcolm H. Levitt

University of Southampton, UK

Symmetry-based pulse sequences are trains of resonant radiofrequency pulses which form a symmetrical pattern in space and time. The repeating pattern is synchronised with a periodic modulation applied to the spin system, for example the magic-angle rotation of a solid sample. The symmetrical structure of the pulse sequence generates selection rules which constrain the spin dynamics. This design principle allows the spectroscopist to steer the spin evolution, and hence to construct pulse sequences which have a desired effect, within some approximation. The selection rules may be described by simple inequalities, which may be represented in a diagrammatic form.

The symmetrical construction procedures are defined by their class (either C or R), and by the values of three integers, which are called symmetry numbers. These symmetry numbers define selection rules on the average Hamiltonian, which govern the nuclear spin dynamics. For example, sequences of the C class, and with symmetry numbers 7, 2 and 1, generate double-quantum recoupling of dipole-dipole interactions in solids, and may be used for defining structural connectivities and for estimating internuclear distances.

Recently it has been shown that symmetry-based sequences may also be used in solution NMR. We have used symmetry-based pulse sequences to selectively drive singlet-triplet transitions, and to excite double-quantum coherences, in strongly coupled systems in solution NMR.

Some methods used in the spectroscopy of single-electron sites, such as nitrogen-vacancy centres in diamond, may also be interpreted as symmetry-based pulse sequences.

In this tutorial I will sketch out the principles and theory of symmetry-based pulse sequences, and give examples from both solid-state and solution-state NMR.

abstract missing

Combining CPMAS CryoProbe-detected SSNMR and cryoEM for the structural elucidation of a functional amyloid

Polonio, Paula, López-Alonso, Jorge Pedro, Jiang, Hanxing, Andrés-Campos, Sara, Escobedo, Fátima, Titaux, Gustavo, Ubarretxena-Belandia, Iván, Mompeán, Miguel.

In nature, the conformation of some proteins has evolved to assemble into functional amyloids to optimize essential biological processes.

An example of functional amyloid involvement can be found in necroptotic signaling pathways, where distinct homomeric and heteromeric assemblies mediate signal transduction. Upon activation of canonical necroptosis, receptor-interacting protein kinase 1 (RIPK1) self-associates and becomes activated, subsequently recruiting RIPK3 monomers to form a heteromeric complex. This assembly serves as a nucleation seed for further homomeric RIPK3 amyloid, ultimately leading to necroptotic execution.

All amyloidogenic interactions within these pathways rely on a short amyloid-forming sequence known as the RIP homotypic interaction motif (RHIM). Accessing the structural features of this region in human RIPK1 will clarify the molecular basis of RHIM-mediated specificity in these signaling complexes.

To address this question, we employed an integrative approach combining CryoProbe-detected solid-state nuclear magnetic resonance (SSNMR) and cryo-electron microscopy (cryo-EM). SSNMR measurements on isotopically diluted fibrils revealed the local fold of monomeric layers within the fibril, identifying hydrogen interactions that appear critical for monomer incorporation and fibril stabilization. In parallel, cryo-EM imaging enabled the resolution of the macromolecular architecture, including the symmetry, helical twist, and axial rise of the stacked protomers. Finally, the introduction of single-amino-acid mutations demonstrated how individual residues can modulate both homomeric and heteromeric RHIM assemblies, providing insight into the specificity of these interactions.

Acknowledgment. This work is funded by the European Union (ERC, 101042403 – BiFOLDOME, to M.M). NMR experiments were performed in the “Manuel Rico” NMR Laboratory (LMR) of the Spanish National Research Council (CSIC), a node of the Spanish Large-Scale National Facility (ICTS R-LRB).

Fast MAS NMR of a CCHFV glycoprotein n domain in membrane lipids

Morgane Callon, Maëlys Laux, Laura Troussicot, Susanne Penzel, Caroline Marteau, Edward P. Saliba, Lauriane Lecoq, François-Loïc Cosset, Beat H. Meier, Alexander Barnes, Marie-Laure Fogeron, and Anja Böckmann

We here report ^{1}H-detected fast magic-angle spinning (MAS) solid-state NMR studies of the membrane-bound cytosolic domain of the envelope glycoprotein Gn of the Crimean-Congo hemorrhagic fever virus. This highly deadly virus is currently emerging in Europe, and there is no approved vaccine or treatment. The cytosolic domain of interest is likely active in ribonucleoprotein packaging during viral particle assembly. Although its structure in isolation is known, its membrane-bound state remained uncharacterized.

While divide-and-conquer strategies for soluble membrane proteins domains are commonly used to bypass the complexity of membrane protein expression, the membrane environment can significantly influence protein structure and dynamics. We combined ^{1}H-detected fast MAS solid-state NMR with cell-free protein synthesis (CFPS) to study the cytosolic domain with its transmembrane regions.

While the protein shows broader lines than typical model proteins, CFPS-enabled selective [^{13}C,^{15}N] labeling of Cys, Gly and Phe residues provided spectral resolution. Our results show that the protein's zinc-finger motif is retained in membrane-bound form of the Gn cytosolic domain. Interestingly, however, we observed peak doubling, suggesting two conformational states in presence of the membrane. This could indicate an at least partially asymmetric multimer or a second distinct conformation.

To further investigate the origins of line broadening, we measured site-specific relaxation rates of the labeled residues and compared them to reference data we established for Ubiquitin, ASC and HET-s proteins. This revealed that the total line broadening is partly due to heterogeneity but also includes dynamic components.

The *in-situ* structure of outer membrane protein A and its role in bacterial envelope stability

Johannes Thoma[1,2], Tanguy LeMarchand[3], James Tolchard[3], Jackson Crowley[4], Tobias Sparrman[5], Luca Monticelli[4,6], Guido Pintacuda[3], Björn M. Burmann[1,2,7,8]

[1] Department of Chemistry and Molecular Biology, University of Gothenburg, 405 30 Göteborg, Sweden
[2] Wallenberg Centre for Molecular and Translational Medicine, 405 30 Göteborg, Sweden
[3] Centre de RMN à Très Haute Champs, UMR 5082 (CNRS/Université Claude Bernard Lyon 1/Ecole Normale Supérieure de Lyon), University of Lyon, 69100 Villeurbanne, France
[4] Molecular Microbiology and Structural Biochemistry, UMR 5086 CNRS, University of Lyon, 69367 Lyon, France.
[5] Department of Chemistry, Umeå University, 901 87 Umeå, Sweden
[6] Institut National de la Santé et de la Recherche Médicale, 69367 Lyon, France
[7] Swedish NMR Centre, University of Gothenburg, 405 30 Göteborg, Sweden
[8] Science for Life Laboratory, Integrated Structural Biology Platform, University of Gothenburg, 405 30 Göteborg, Sweden

The structure and function of membrane proteins strongly depend on their surrounding lipid environment. This is particularly relevant for the outer membrane of Gram-negative bacteria, which has a unique asymmetric architecture with phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet. Because this complex environment cannot be experimentally reproduced, structural studies of outer membrane proteins typically rely on membrane-mimetic systems. Here, we present the first *in-situ* structure of an outer membrane protein in its native membrane. Using engineered outer membrane vesicles (OMVs), we determine the structure of outer membrane protein A (OmpA) from *Klebsiella pneumoniae* embedded in the native asymmetric membrane by ^{1}H-detected ultra-fast magic-angle spinning solid-state NMR spectroscopy. We find that OmpA adopts an extended β-barrel fold in the native membrane that differs from structures observed in membrane-mimetic systems. Supported by molecular dynamics simulations and atomic-force microscopy, our data indicate that this conformation is stabilized through direct interactions with LPS, which in turn contribute to membrane integrity and reinforce the structural role of OmpA in the Gram-negative cell envelope. Together, our work provides a blueprint for *in-situ* structural studies of membrane proteins in their native lipid environments

Capturing the interactions of small heat shock proteins and their clients with advanced NMR spectroscopic tools

Debelouchina, Galia

Cells regulate condensate formation and aberrant protein aggregation through the action of chaperones, including the ATP-independent small heat shock protein HSPB1/Hsp27. The HSPB1 monomer consists of a central α-crystallin domain (ACD) flanked by an N-terminal domain (NTD) and a C-terminal domain (CTD). In the absence of client proteins, HSPB1 assembles into polydisperse oligomers that have presented a significant challenge for structural characterization due to their heterogenous and dynamic nature. While the interactions of several client proteins and HSPB1 dimers stabilized by mutations have been described by solution NMR spectroscopy, how the wild-type HSPB1 oligomers regulate proteins in the context of biological condensates and protein aggregation is still unknown. Here, we use magic angle spinning (MAS) NMR spectroscopy to capture the structure and dynamics of HSPB1 oligomers in the presence and absence of the low complexity domain (LC) of the fused in sarcoma (FUS) protein. Our work has significantly benefited from recent advances in the field that have allowed us to record high resolution ^{1}H-based correlations of just 50 µg of fully protonated 250 – 500 kDa HSPB1 oligomers. We have combined this methodology with intein-mediated segmental labeling and coarse-grained molecular dynamics simulations to paint a comprehensive picture of the interactions of each HSPB1 domain in the context of oligomers and in the presence of FUS LC. Our results point to the importance of the NTD domain in mediating interactions with aggregating client proteins and condensate regulation.

The Power of Ultralow-Field Zeeman-Perturbed NQR to Study Quadrupolar Nuclei in Materials

Bryce, David L.

In NQR spectroscopy, the quadrupolar interaction is dominant and one may introduce a small perturbative Zeeman field to access additional information – an experiment described in some of the very earliest literature. Here, we describe advances resulting from a holistic view of the complete gamut of spectroscopies arising from the complete range of ratios of the Larmor frequency to the quadrupolar frequency, i.e., Zeeman-perturbed NQR (Zp-NQR) to ultrahigh-field quadrupolar-perturbed NMR. For the ultralow-field work, we describe the repurposing of an adjustable EPR electromagnet as well as various practical aspects of recording, analysing, and interpreting Zp-NQR spectra. Applications to a range of solid halogen-bonded supramolecular architectures are presented, where ^{127}I and $^{79/81}$Br spectra can be recorded in a matter of minutes including for covalently-bonded iodine sites with quadrupolar coupling constants in excess of 2 GHz. These data are interpreted in concert with DFT calculations to provide information on the strength of the halogen bond. The concept of multisite spectral fitting in Zp-NQR is presented and, given the lack of central Larmor frequency, demonstrated to be valuable in differentiating between crystallographic sites not only for the same isotope, but also for different isotopes with similar quadrupolar frequencies. This methodology is successfully applied to a series of mechanochemically-prepared cocrystals. Overall, these results suggest that a modern, sustainable, cryogen-free implementation of Zp-NQR spectroscopy could open doors to studying a range of quadrupolar nuclei in advanced materials, the quadrupolar couplings of which may be too large to study practically even in the highest available applied magnetic fields.

Probing Confinement and Interactions of Biomass Mimic Guaiacol in ZSM-5 Zeolite via Advanced NMR Spectroscopy.

Kyle A. Watson, Ming-Feng Hsieh, Stephen Day, Luke Tuxworth, and Frédéric Blanc.

High-resolution solid-state NMR spectroscopy provides a powerful approach for probing host-guest interactions in heterogeneous catalysts. Here, we showcase a suite of multinuclear NMR methods used to investigate the adsorption, confinement and reactivity of oxygenated biomass-derived molecules within aluminosilicate zeolites relevant to catalytic fast pyrolysis and bio-oil upgrading. Using guaiacol and others as bio-oil model compounds, ^{1}H and ^{13}C MAS NMR experiments reveal distinct in-pore and ex-pore species based on signal linewidth, chemical-shift dispersion, and mobility-dependent relaxation behaviour in both adsorbed and activated ZSM-5 zeolite *via* quantitative 1D and correlation measurements. Crucially, the ^{17}O isotopic enrichment of ZSM-5 has transformed zeolite framework oxygens into active probes of host-guest interactions. Two-dimensional ^{1}H-^{17}O dipolar heteronuclear multiple-quantum coherence (D-HMQC) NMR experiments were performed to selectively detect short-range heteronuclear dipolar couplings, providing sensitivity to the spatial proximity and confinement geometries of reactive species trapped within the channel intersections and sinusoidal/straight pores. Numerical simulations of the ^{1}H-^{17}O signal intensities and recoupling efficiencies provide estimates of dipolar coupling constants and hence insight into the spatial arrangement, restricted motion, and local binding environments of trapped guaiacol. Together, these results reveal distinct confinement regimes and differences in guest–framework hydrogen-bonding motifs, providing molecular level insight which can inform the rational design of next generation catalysts with faster activity, better selectivity and improved stability for catalytic upgrading.

Fluid Flow Regimes in Triply Periodic Minimal Surface Structures Studied by Dynamic NMR Microscopy and CFD

Daniel A. Clarke[1,2], Petrik Galvosas[1], and Daniel J. Holland[3]

[1] School of Chemical and Physical Sciences, Victoria University of Wellington, Wellington 6140, New Zealand
[2] Division of Physical Chemistry, Lund University, 221 00 Lund, Sweden
[3] Department of Chemical and Process Engineering, University of Canterbury, Christchurch 8140, New Zealand

Magnetic Resonance Imaging (MRI) in combination with pulsed field gradients allows the spatially resolved detection of flow and random motion using Dynamic NMR Microscopy [1] . This technique measures coherent motion (flow) via phase shifts in the NMR signal, while random motion (diffusion and dispersion) is extracted from signal attenuations. Dynamic NMR Microscopy can be tuned to measure fully resolved averaged propagators or only its lower order moments, i.e., focussing on mean velocities and dispersion. It may also be optimised for high spatial or temporal resolution.

Triply Periodic Minimal Surface structures (TPMS) are ordered periodic porous materials defined by mathematical expressions to form internal surfaces with minimal local energies. These structures may offer improved performance for instance for heat exchangers or chromatographic columns. We studied the time averaged mean velocities and velocity fluctuations in TPMS systematically, covering creeping flow at Reynolds numbers as low as Re = 1.25 [2], inertial flow at Re = 17.6 and 32 [2], the transition into unsteady laminar flows between Re = 101 and Re = 152 [3], and turbulent flows of up to Re = 1470 [4]. MRI velocity data were cross-validated by Computational Fluid Dynamics (CFD) simulations, where concordant results validated the simulation methods and the MRI experiment design.

In addition to time averaged data we recently recorded velocity vector images with a temporal resolution of 12 ms (at a spatial resolution of 32x32) and 21 ms (at a spatial resolution of 64x64) for flows in TPMS with Reynolds numbers of up to 2500, revealing time resolved details of the flow under unsteady and turbulent regimes [5].

[1] Callaghan, P. T.; Eccles, C. D. and Xia, Y., *J. Phys. E* **21** 820-822 (1988)
[2] Clarke, D. A.; Dolamore, F.; Fee, C. J.; Galvosas, P. and Holland, D. J., *Chem. Eng. Sci.* **231** 116264 (2021)
[3] Clarke, D. A.; Galvosas, P. and Holland, D. J., *AIChE J.* **69** 18097 (2023)
[4] Clarke, D. A.; Coe, M. J.; Galvosas, P. and Holland, D, J., submitted to *Chem. Eng. J.*
[5] Clarke, D. A.; Galvosas, P. and Holland, D, J., in preparation

Probing Platinum Surface Sites *via* DNP-Enhanced Indirect ^{195}Pt NMR Detection

Insinna, Teresa,[1] Gioffré, Domenico,[2] Koppe, Jonas,[2] Copéret, Christophe,[2] Lesage, Anne[1]

[1]Centre de RMN à Très Hauts Champs de Lyon, CNRS, 5 Rue de la Doua, 69100 Villeurbanne, Rhône-Alpes, France

[2]Department of Inorganic Chemistry, ETH Zurich, Vladimir-Prelog-Weg 1-5/10, 8093 Zürich, Switzerland

Solid-state NMR spectroscopy is a powerful technique for elucidating the atomic-scale structure of active sites in heterogeneous catalysts. In particular, when the metal center (*e.g.* Pt) is NMR-active, its spectral features provide unique insights into the local structure and reactivity of the active sites. Direct detection of ^{195}Pt NMR offers rich information on the local chemical and electronic environment, but its application is often limited by low sensitivity due to the ^{195}Pt large chemical shift anisotropy (CSA) and the low concentration of Pt sites (≤ 5 wt%). To overcome these limitations, Dynamic Nuclear Polarization (DNP) NMR spectroscopy can be applied. While DNP-enhanced ^{195}Pt spectra have been demonstrated for a few organometallic complexes, indirect detection through more sensitive nuclei (^{1}H, ^{31}P) offers an attractive alternative. In this study, we combine DNP with ^{195}Pt indirect detection *via* ^{31}P to investigate the local environment of a Pt-phosphine complex grafted onto diverse oxide supports, including silica and various aluminas, at loadings < 1 wt%. Exogenous DNP is used to enhance the ^{31}P signal from surface-bound Pt complexes. The enhanced ^{31}P signal is then exploited to indirectly detect the ^{195}Pt CSA pattern by implementing *J*-based Heteronuclear Multiple Quantum Correlation (HMQC) experiments and Variable Offset Cumulative Spectroscopy (VOCS). This approach not only circumvents sensitivity challenges of direct ^{195}Pt detection, delivering substantial overall sensitivity gains, but also enables site-selective characterization of ^{195}Pt CSA patterns *via* their resolved ^{31}P signatures. Interestingly, structural differences in the supported species are revealed depending on the support, uncovering unexpected surface structures.

Molecules Under Pressure: How Nanoconfinement Dictates CO_2 Behaviour

Sardo, Mariana; Pereira, Daniel; Marin-Montesinos, Ildefonso; Lourenço, Mirtha; Vieira, Ricardo; Mafra, Luís

While porous CO_2-chemisorbents are crucial to the success of direct air capture and industrial decarbonization, the elusive atomic-level nature of the gas–solid interface remains a barrier to rational materials design. Unravelling the intricate reactivity and atomic-level nuances of adsorption in these systems requires moving beyond bulk observations. This presentation explores our recent breakthroughs in decoding speciation within the confinement of nanopores through the combination of solid-state NMR and computational modelling, providing a dual-lens view of molecular behaviour under both dry and humid conditions.

Our methodology quantitatively resolves multiple co-existing species - from physisorbed CO_2 to chemisorbed carbamates and moisture-induced CO_2 species (bicarbonates) - enabling the first-ever generation of "species-specific" adsorption isotherms. Unlike conventional volumetric or gravimetric techniques, NMR uniquely resolves these species, allowing the generation of individual CO_2 isotherms for each adsorbed component. The versatility of our methodology is showcased across different porous systems, including amine-modified silicas, covalent organic frameworks (COFs), metal-organic frameworks (MOFs) and polysaccharide-based biopolymers, thus providing a powerful framework for rationalizing adsorption mechanisms at confined material's surfaces.

Acknowledgements
This work has received funding from ERC under the European Union's Horizon 2020 research and innovation program (GA 865974) and CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025) & UID/PRR2/50011/2025 (DOI 10.54499/UID/PRR2/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC).

MRI goes vertical: A large-bore 3 T system for chemical engineering and biotechnology

Penn, Alexander

Magnetic resonance imaging is mature, but its hardware and methods have been optimised almost entirely for one kind of sample: the human body. This talk asks what changes when MRI is instead built for an unfamiliar class of samples, the multiphase reactors of chemical and bioprocess engineering, which are large, heterogeneous, often fast-moving, and combine phases with very different spin density and relaxation behaviour. At its centre is a large-bore vertical MRI system at Hamburg University of Technology, built around a 3 T conduction-cooled magnet and developed for process imaging together with Tesla Engineering and Philips Healthcare. Its vertical geometry lets samples several metres tall and up to 40 cm in diameter pass through the magnet, its gradient and radiofrequency systems go beyond clinical specifications, and radiofrequency coils and coil arrays are built in house to fit each reactor. On this platform we bring familiar MR methods, including phase-contrast velocimetry, accelerated by compressed sensing and parallel imaging, to gas-solid and gas-liquid systems, mapping velocity fields, phase distributions, and transport in equipment that is otherwise hard to probe. The talk reflects on the measurement challenges these samples raise and on how the magnetic resonance community might help take process MRI further.

Lithium deposition in anode-free batteries studied by conduction electron paramagnetic resonance imaging

Wolff, **Beatrice**, Jakes, Peter, Eichel, Rüdiger-A., Hausen, Florian, Granwehr, Josef

Lithium deposition is a crucial process in anode-free lithium batteries. The homogeneity of the deposition process governs the formation of dendrites and thereby potential failure of the battery. Several factors influence the deposition morphology, including current density and electrolyte composition. Conduction EPR differentiates between microstructure and bulk deposition and is therefore a valuable tool for the study of lithium deposition. Moreover, a spatially resolved view of lithium deposition can be provided by conduction EPR imaging (CEPRI).

In this contribution, we present a study of lithium deposition via 2D CEPRI, studying different deposition current densities as well as the influence of electrolyte additive vinylene carbonate. The CEPRI signal selectively probes lithium metal and exhibits different intensities. To assess the relationship between CEPRI signal intensity and deposition morphology, CEPRI maps are correlated to atomic force microscopy (AFM). AFM probes the surface morphology as well as local stiffness and enhances the spectroscopic information gained by CEPRI. Furthermore, a significantly smaller length scale in the sub-micrometre regime is probed by AFM, complementing the global view of the sample obtained by CEPRI. For future applications, 2D spectral-spatial imaging (SSI) will provide deeper insights into the deposition process by differentiating deposited microstructure and bulk lithium from the EPR signal shape in the CEPRI map.

Multidimensional MRI Reveals Signatures of Neuroinflammation in Brain Organoids

Willett, Matthew, Gross, Phillip, Fishbein, Kenneth, Rather, Kara, Marsan, Elise, Benjamini, Dan

Glial reactivity is strongly linked to TDP-43 pathology and cognitive decline. Novel MRI biomarkers for neuroinflammation have been identified primarily through *ex vivo* imaging, highlighting the need for model systems to better understand these signatures *in vivo*. Brain organoids are created by reprogramming adult skin cells into stem cells, which are then differentiated into neural cell types such as neurons and glia. Cortical organoids carrying progranulin (GRN) mutations are associated with increased neuroinflammation, including astrogliosis, and serve as useful models for Alzheimer's disease and related dementias (AD/ADRD). In 29-week-old mutant organoids (GRN/GRN), histological and molecular analyses revealed increased levels of reactive astrocytes compared to revertant controls (REV/REV). To study *in vivo* biomarkers of astrogliosis in these organoids, a perfusion system was integrated into a 14 T Bruker NMR magnet. We hypothesized that multidimensional MRI (MD-MRI), an imaging modality which spatially encodes diffusion and relaxation domains, would show a unique signature due to increased astrocyte content. Projecting full distributions into 3D parameter space ($D_{iso} - D_{\Delta}^{2} - R_2$) revealed distinct redistributions between control and mutant organoids across different microenvironments. Voxels mapped in anisotropic (*i.e.*, high D_{Δ}^{2}) spectral subdomains were statistically more prevalent in mutant organoids, likely due to increased astrocytic process hypertrophy, while voxels in isotropic and higher R_2 regions were dominated by the control organoids. This study demonstrates that MD-MRI can be successfully applied to find signatures for neuroinflammation in brain organoids and may help translate these biomarkers to clinical MRI studies.

Advanced NMR Approaches for *Operando* Studies of Heterogeneous and Multiphase Catalytic Reactors

Kononenko, **Elizaveta S.**[a,b]; Skovpin, Ivan V.[a]; Burueva, Dudari B.[a]; Kovtunova, Larisa M.[a,c]; Rogozhnikov, Vladimir N.[c]; Gökpek, Yenal[d]; Pravdivtsev, Andrey N.[d]; Hövener, Jan-Bernd[d]; Eid, Mahmoud E. A.[e,f]; Guda, Alexander A.[e]; Koptyug, Igor V.[a]

a) International Tomography Center SB RAS, 3A Institutskaya St., 630090 Novosibirsk, Russia

b) Novosibirsk State University, 2 Pirogova St., 630090 Novosibirsk, Russia

c) Boreskov Institute of Catalysis SB RAS, 5 Acad. Lavrentiev Pr., Novosibirsk 630090, Russia

d) Section Biomedical Imaging (SBMI), Molecular Imaging North Competence Center (MOINCC), Department of Radiology and Neuroradiology, University Hospital Schleswig-Holstein, Kiel University, Am Botanischen Garten 14/18, 24118 Kiel, Germany.

e) The Smart Materials Research Institute, Sladkova 178/24, 344090 Rostov-on-Don, Russia

f) Physics Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

Nuclear magnetic resonance (NMR) enables operando investigation of catalytic reactions but is often limited by low sensitivity and magnetic field inhomogeneities. Gas-phase reactions are limited by low spin density, while susceptibility mismatches in heterogeneous systems degrade both spectral resolution and sensitivity.

To overcome these sensitivity limitations, we employed parahydrogen-induced polarization (PHIP) in gas-phase hydrogenation. PHIP enhances NMR sensitivity and enables visualization of gas. Using this approach, we detected hyperpolarized propane in segmented-flow microfluidic reactors despite the extremely small sample volumes.

However, under PASADENA conditions PHIP produces antiphase spin order highly sensitive to magnetic field inhomogeneities typical for heterogeneous catalytic systems such as packed-bed reactors. To obtain high-resolution spectra, we applied a COSY pulse sequence that converts antiphase spin order into double-quantum coherences. In the resulting two-dimensional spectra the inhomogeneous broadening is redistributed along tilted lines, preventing signal cancellation and enabling the extraction of high-resolution spectral slices.

We further showed that in magnetic resonance imaging experiments the antiphase signal must be converted into inphase magnetization, otherwise the magnetic field gradients otherwise cause mutual cancellation of its absorptive and emissive components.

In parallel, we developed NMR-compatible reactors based on hollow glass tubes and hollow alumina spheres. These rectors reduced susceptibility distortions and enable high-resolution and spatially resolved NMR even at thermal polarization levels, which is essential for quantitative studies of catalytic reactors.

These results demonstrate integrated solutions for operando NMR of heterogeneous and multiphase catalytic systems.

E.S.K. thanks RSF (grant #25-13-00053) for financial support.

From Signal to Cell in the Human Brain: MRI at the Crossroads of Physics and Neurological Disease

Magnetic resonance imaging has transformed our ability to visualize the human brain, yet its dominant contrasts remain largely macroscopic and only indirectly reflect the cellular processes that drive neurological disease. Bridging this gap between signal and biology represents a central challenge for the next generation of MRI.

In this keynote lecture, I will discuss how recent advances in diffusion MRI are beginning to enable in vivo characterization of brain microstructure, including features such as axonal geometry, soma size and density, and extracellular space. I will highlight how innovations in gradient hardware, acquisition design, and computational modeling are expanding the range of measurable scales in the human brain.

A key example is the Connectome 2.0 platform, which leverages ultra-strong gradients to access previously inaccessible diffusion regimes, enabling improved sensitivity to cellular and sub-voxel architecture. I will discuss how these capabilities, combined with advances in model design and machine learning, are reshaping our ability to probe brain tissue organization with greater specificity.

Importantly, I will place these developments in the context of neurological disease. Using examples from multiple sclerosis and Alzheimer’s disease, I will illustrate how microstructure-sensitive MRI can reveal early and clinically relevant changes that are not captured by conventional imaging. I will also discuss ongoing efforts to translate these advances to widely available clinical systems, enabling scalable and robust deployment.

Finally, I will address key challenges, including model identifiability, validation, and cross-platform reproducibility, and argue that the future of brain MRI lies in a fundamental shift: from producing images of disease to making quantitative, biologically meaningful measurements of human brain tissue, with direct implications for precision neurology.

Nuclear Cross Effect

Tan, **Kong Ooi**, Pang, Zhenfeng, and Lumsden, Jake

Dynamic nuclear polarization (DNP) has significantly enhanced the sensitivity of nuclear magnetic resonance (NMR) by transferring polarization from electrons to nuclei. While the Overhauser DNP has a well-established nuclear counterpart in the nuclear Overhauser effect (NOE), the cross-effect (CE) DNP has traditionally been considered an exclusively electron-mediated process. In this work, we present experimental evidence of a nuclear counterpart to the cross effect, termed the nuclear cross effect (NCE), which mediates polarization transfer in a three-spin nuclear system. The NCE mechanism occurs when the chemical shift difference between two homonuclear spins (^{13}C) matches the Rabi field applied to a third heteronuclear spin (^{15}N), i.e., $\delta_{C1} - \delta_{C2}$=$\nu_{15N}$. We experimentally verified this effect using a single crystal of ^{13}C-^{15}N-labeled glycine. The experimental results, including NCE field profiles and polarization transfer from the ^{13}C polarization difference to the ^{15}N spin, show excellent agreement with both analytical theory and numerical simulations. Furthermore, we utilize the NCE as a model system to explore pulsed DNP strategies. While pulsed CE DNP theoretically allows for enhancement factors that double the conventional continuous-wave (CW) limit, experimental realization is often challenging due to hardware limitations in the microwave regime. By using readily available RF hardware, we demonstrate that the NCE can be "turned on or off" with pulses to avoid depolarization, providing a foundation for future developments in advanced magnetic resonance techniques across NMR, EPR, and DNP.

Photochemically Induced Dynamic Nuclear Polarization for Dye Sensitized Solid-state NMR

Máté Visegrádi,[a] Marcel Levien,[a] Federico De Biasi,[a] Ganesan Karthikeyan,[b] James W. Wipham,[a] Christian Reiter,[c] Sebastian Wegner,[c] Armin Purea,[c] Michael R. Wasielewski,[d] Olivier Ouari,[b] Lyndon Emsley[a*]

[a] Institut des Sciences et Ingenierie Chimiques, École Polytechnique Fedérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland
[b] Aix-Marseille University, CNRS, Institut de Chimie Radicalaire, 13013 Marseille, France
[c] Bruker BioSpin GmbH & Co KG, 76275 Ettlingen, Germany
[d] Department of Chemistry, Center for Molecular Quantum Transduction, Institute for Quantum Information Research and Engineering, Northwestern University, Evanston, Illinois 60208-3113, United States

NMR spectroscopy is an extremely versatile technique for atomic level structural investigation. One of the major disadvantages of NMR spectroscopy is its poor sensitivity, due to the low polarization of nuclear spin-flip transitions. To address this, throughout the past decades, significant advances have been made to hyperpolarize nuclear spins.

Emerging pathways to generate bulk nuclear hyperpolarization are optically based techniques such as photochemically induced dynamic nuclear polarization (photo-CIDNP). Recently, we have shown that it is possible to generate bulk hyperpolarization using small donor-acceptor molecules (D-A) as polarizing agents (PAs). Following the initial demonstration of ^{1}H bulk hyperpolarization at low magnetic field, we extended the approach through rational design of small D-A systems to magnetic fields as high as 21.15 T, reaching enhancements up to a factor of 100.

To further guide the rational design of new photo-PAs, we recently introduced a kinetic photocycle description of the solid-state photo-CIDNP process, and here we report the next generation of photo-PAs, that reach unprecedentedly high ^{1}H enhancements at high magnetic fields. We will present experimental and theoretical results on the photo-CIDNP mechanisms that facilitate higher signal enhancements. We also present additional optimization of the hardware to improve irradiation efficiency and reproducibility of the experimental results under MAS at 100 K. Furthermore, we will also discuss theoretical and experimental progress on understanding polarization transfer via spin diffusion to the bulk to increase bulk enhancements.

^{1}H Bullet-Dynamic Nuclear Polarization and its application for drug screening experiments

Dmitrii Zasukhin[1], Masoud Minaei[1], Kajum Safiullin[1], Karel Kouřil[1], Nils Lorz[2], Alvar Gossert[2], Anna Sonnefeld[3], Geoffrey Bodenhausen[3], Benno Meier[1]

[1] Institute for Biological Interfaces 4, Karlsruhe Institute of Technology, Karlsruhe, Germany

[2] Department of Biology, ETH Zurich, Zürich, Switzerland

[3] Laboratoire des Biomolécules, LBM, Département de Chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, Paris, France

The low sensitivity of NMR spectroscopy limits its application to biochemical and pharmaceutical material, which is often available only in limited amounts. Hyperpolarization methods that increase nuclear spin polarization are the most promising methods for substantial sensitivity boosts. The broadly applicable methods of dissolution-DNP (D-DNP) and bullet-DNP (B-DNP), have indeed achieved liquid-state ^{13}C polarization levels of tens of percent, enabling the detection of ^{13}C-labeled substances at nanomolar to low micromolar concentrations. For protons, however, the observed gains have thus far been limited. In D-DNP, fast relaxation during the liquid-state transfer has limited ^{1}H enhancements to values often well below 100, and substantially higher enhancements have only been observed at the expense of >100 fold dilution. With bullet-DNP we previously estimated ^{1}H enhancements of up to 400 when using deuterated methanol as a solvent.

Here we show that the sample composition has a dramatic effect on the attainable signal enhancement and that optimization boosts the enhancements approximately 5-fold. We observed up to two thousand-fold signal enhancements in liquid-state NMR at 9.4 T in aqueous solutions on fast relaxing ^{1}H resonances of a range of small molecules.

These enhancement factors allow us to observe ligand-binding in water using unlabeled ligands. Moreover, the use of such strong signal enhancements in combination with the fully automated Bullet-DNP system allows us to run automated ^{1}H NMR drug screening experiments. We conducted experiments with different proteins, such as bovine serum albumin (BSA) and trypsin. As hyperpolarized ligand samples, we used either individual compounds from a defined ligand library, or mixtures of 8 substances.

Exploring α-Ketoisocaproate for Real-Time Metabolic Magnetic Resonance Spectroscopy using PHIP-SAH

May, **Leander K.**, Börschmann, Lena, Moll, Denis, Fries, Lisa M., Rodriguez, Gonzalo G., Santi, Maria D., Leonov, Andrei, Glöggler, Stefan

Increasing signal intensities in nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) offers advantages, such as the ability to monitor real-time metabolism *in vitro* and *in vivo*, providing i.e. information about disease progression and localization non-invasively. As a powerful technique, *para*hydrogen induced polarization (PHIP) enhances magnetic resonance signal intensities via hyperpolarization of nuclear spins. [1-^{13}C]Pyruvate is the most extensively studied hyperpolarized metabolite and the focus of current efforts to translate PHIP to the clinics. Expanding the scope of traceable metabolic pathways using specially tailored metabolic precursors increases the influence of this field.

Here, we report the successful synthesis of a new PHIP by sidearm hydrogenation (SAH) substrate, vinyl-d_3 [1-^{13}C]α-ketoisocaproic acid-3,3-d_2 (VαKIC), and demonstrate polarization levels of up to 39% of the resulting biocompatible [1-^{13}C]α-ketoisocaproate (αKIC). αKIC is metabolized by branched-chain amino acid aminotransferase (BCAT), whose activity is associated with cancer and neurodegenerative diseases, such as Alzheimer's or Parkinson's disease.[1-3] Through initial *in vitro* studies with cancer cells, we demonstrate this hyperpolarized metabolite's ability to monitor BCAT activity by converting αKIC to leucine. Our goal is to use this precursor for *in vivo* imaging studies to further demonstrate its significance and potential for metabolic diagnostics.

References:

[1] S. Siivanand, M. G. Vander Heiden, *Cancer Cell* **2020**, *37*, 147–156.

[2] Q. Ma, H. Li, Z. Song, Z. Deng, W. Huang, Q. Liu, *Metabolism* **2024**, *161*, 156016.

[3] A. Dimou, V. Tsimihodimos, E. Bairaktari, *Int. J. Mol. Sci.* **2022**, *23*, 4022.

Topics:

1. Hyperpolarization
2. Small molecules/ Drugs

Pulsed dynamic nuclear polarization: Spin engineering *in silico* and *in situ*

Nielsen, **Niels Chr.**, Carvalho, José, Jensen, Filip Vestergaard, Goodwin, David L., Baligacs, Eniko, Untidt, Thomas S., Vosegaard, Thomas, Tosner, Zdenek, Wili, Nino, Nielsen, Anders B.

Pulsed dynamic nuclear polarization (DNP) has important potentials for sensitivity enhanced NMR and quantum sensing. At the same time, it represents a wonderful challenge for spin engineering calling for combined control over electron and nuclear spin interactions with widely different amplitudes and timescales. This presentation will address different aspects of pulsed DNP spin engineering combining- state-of-the-art instrumentation, theory, and numerical calculations. With focus on pulse sequence design, this includes (i) Exact and single-spin vector effective Hamiltonian theory, (ii) optimal control, (iii) constrained random walk, (iv) Bayesian-based AI learning, and (v) new aspects of SIMPSON. These tools are used to formulate efficient spin engineering protocols for *in silico* and *in situ* pulsed DNP experiment design. It is demonstrated that DNP experiments with 100 MHz bandwidth and robustness to instrumental errors can be designed solely on basis of a single, static crystallite and that these experiments can be translated directly into solid-state MAS NMR dipolar recoupling experiments thereby hugely simplifying solid-state NMR experiment design. This unifies spin engineering over different disciplines. The presentation includes analytical, numerical, and experimental results.

Biomolecular dynamics by ultrafast high-resolution relaxometry

Fabien Ferrage

Measuring NMR relaxation rates provides quantitative information on molecular dynamics experimentally. Relaxation rates depend on the spectral density function at the eigenfrequencies of the Zeeman Hamiltonian, make magnetic-field dependent measurements particularly insightful. Fast field-cycling relaxometry measure relaxation rates over many orders of magnitude of magnetic field, alas, with no spectral resolution. Our objective is to design an instrument to measure relaxation range on a range broader than fast field cycling, while preserving the high resolution and sensitivity necessary to investigate complex systems. We introduce ultrafast high-resolution relaxometry (UHRR) on an instrument built by Bruker Biospin, in collaboration with CERM, in Florence. A commercial high-field magnet is equipped with a sample shuttle to move at high speed the sample to a field-cycling coil. To preserve magnetization during the sample-shuttle transfer, the magnetic field is maintained above 1 T by a magnetic tunnel positioned inside the bore of the high-field magnet. Field cycling from 0.8 T down to 100 µT and back is achieved in about 2 ms, allowing the measurement of fast relaxation.

We show the first applications of UHRR to biomolecular systems, with a particular focus on the dynamics of the mitogen-activated protein kinase p38γ and the enzyme imidazole glycerol phosphate synthase (IGPS). We measured carbon-13 relaxation in methyl groups in both enzymes between 100 µT and 28 T. In the kinase p38γ we investigated the effect of binding of the inhibitor BIRB796 on dynamics throughout the protein. We developed a dedicated framework for analysis, combining molecular dynamics simulation, explicit models of motion, providing a detailed description of molecular motions in these enzymes.

Quantitative hydrogen exchange in intrinsically disordered proteins by ^{15}N lineshape analysis

Ceccolini, Irene, Mulder, Frans A.A.

Hydrogen exchange (HX) provides residue-specific insight into solvent accessibility and transient structure in proteins. However, quantifying HX in intrinsically disordered proteins (IDPs) remains challenging, especially under physiological conditions, where exchange rates approach timescales of 10^1–10^3 s^{-1}. We present a method that exploits exchange-induced self-decoupling of the ^{15}N–^{1}H scalar interaction to quantify hydrogen exchange directly from ^{15}N lineshapes. When proton exchange occurs on the millisecond timescale, the scalar coupling interaction is dynamically averaged, leading to a characteristic collapse of the ^{15}N doublet. Rather than suppressing this effect, we exploit it as a direct reporter of protein backbone amide exchange rates. The method is implemented using a carbon-detected (HACA)CON-IPAP experiment, which allows its application to IDPs with good resolution and sensitivity. The resulting lineshapes are described using a Liouvillian formalism, and the exchange rate k_{HX} is determined through a numerical fitting procedure. Application to α-synuclein at different pH values demonstrates that residue-specific exchange rates in the range of $\sim$ 30–1000 s^{-1} can be extracted. Comparison with established HX approaches establishes the robustness of the presented method. Exchange-induced ^{15}N self-decoupling therefore provides a simple and physically transparent strategy to quantify hydrogen exchange in intrinsically disordered proteins.

FREESIA - FREquency Encoding via Selective Irradiation with Accordion spectroscopy

Banaś, **Bartłomiej**, Zawadzka-Kazimierczuk, Anna, Koźmiński, Wiktor

University of Warsaw, Faculty of Chemistry, Biological and Chemical Research Centre, Warsaw, Poland

The immense need for increased sensitivity of NMR measurements results in rise of popularity of strong magnets (over 23 T). However, in some situations the increase of sensitivity caused by stronger field could be outweighed by fast transverse relaxation. It is relevant especially for nuclei which exhibit high chemical shift anisotropy (CSA), as relaxation caused by CSA accelerates proportionally to the square of the magnetic field strength. In extreme cases, the use of ultra-high magnetic fields could prevent the conventional determination of resonance frequencies of such nuclei.

To mitigate this problem we designed a new indirect method of measuring chemical shifts - FREESIA - FREquency Encoding via Selective Irradiation with Accordion spectroscopy. This method relies on perturbation of the signal amplitude which occurs when the frequency of the irradiation pulse matches the resonance frequency of the nucleus involved in the created spin state. To make such a measurement time-efficient, we implemented the accordion spectroscopy approach, by co-incrementing the frequency of the irradiation pulse with evolution time in one of the indirect dimensions. The information is then embedded into the lineshapes of peaks, and can be extracted from the spectrum based on the properties of the Fourier transformation.

We demonstrate an application of this method to carbonyl ^{13}C nuclei in proteins. We modified the standard 4D HNCACO experiment, creating a new pulse sequence 3D HNCA(COdec). The measurements performed on the maltose-binding protein (MBP, 41 kDa) at 18.7 T (800 MHz) allowed to determine chemical shifts of carbonyl ^{13}C nuclei for 90.54 % of residues of the protein.

Porous biocompatible hydrogels and their crosslinking process measurements with fast NMR restricted diffusion methods

Czarnota, **Marek**, Domański, Sylwester, Urbańczyk, Mateusz

Restricted diffusion measurements using Nuclear Magnetic Resonance spectroscopy provide useful information about porous structures. A disadvantage of this measurement is its duration, which can be significantly shortened by applying fast methods. Restricted diffusion measurements utilize the behaviour of molecules whose movement is limited by porous structures, as their diffusion coefficients are lower than expected for unrestricted movement. An example of a porous structure can be a gelatin methacrylate (GelMa) hydrogel. GelMa is a biocompatible material with numerous applications in medicine. One of the most promising applications is as a scaffold for bionic organs such as the pancreas. Two types of methods were used to examine the GelMa hydrogels by measuring the dependence of the water diffusion coefficient on diffusion time. One method is Time-Resolved (TR) Restricted Diffusion NMR, which speeds measurements by using a repeating set of magnetic field gradients while incrementing the diffusion time with each measurement. Then, the obtained data is divided into overlapping subsets to obtain good temporal resolution in the diffusion time dimension. The second method is Ultra Fast (UF) Laplace NMR, which employs spatial encoding. This method allows determining the diffusion coefficient or even its dependence on diffusion time in a single scan. The UF method makes the measurement much faster at the cost of sensitivity, which is not a problem for hydrogels due to their high water content. The TR method was used to accelerate the measurements of a static sample of GelMa hydrogels, and the UF method enabled monitoring of the hydrogel's crosslinking process in situ.

Intrinsically disordered proteins studied by NMR spectroscopy: exploring interactions

Isabella C. Felli

Magnetic Resonance Center (CERM) and Department of Chemistry “Ugo Schiff”, University of Florence, Italy

Highly flexible intrinsically disordered proteins as well as regions of complex multi-domain proteins introduce an additional dimension in protein function, still exploiting simple modules (globular domains and highly flexible regions themselves). This high extent of disorder and modular protein architecture is shared by many proteins involved in recognition, signaling and regulation, all processes in which structural and dynamic heterogeneity plays a fundamental role. Protein malfunction, linked to the onset of incurable diseases, is often related to highly flexible regions.

NMR represents a unique tool for their investigation at the atomic level. Most studies to date focus on backbone nuclear spins; however, amino acid side chains are the main determinants of the weak and transient intra- and inter-molecular interactions tuning the properties of IDPs. New approaches for direct monitoring of amino acid side chains reveal the driving forces modulating IDPs’ interactions. When globular and disordered domains are simultaneously present in a protein, the NMR spectra can become quite complex. ^{13}C detection offers an elegant approach to study them not only in isolation but also when part of complex multi-domain proteins.

Extending the Capabilities of Benchtop NMR for Continuous Flow Applications

Münnemann, Kerstin, Mross, Sarah, Salgado, Billy, Phuong, Johnnie

Benchtop NMR spectroscopy is increasingly used for reaction and process monitoring due to its compact size, low cost, and user-friendly operation. These advantages make the technique particularly attractive for routine analysis outside specialized NMR laboratories. A representative example is the monitoring of grape quality and fermentation processes in wine production, where benchtop NMR enables rapid and direct quantification of key compounds such as sugars, acids, and rot indicators [1], as will be shown in the first part of the lecture.

However, more demanding applications may require the monitoring of faster reactions or the observation of heteronuclei. In complex mixtures, 1H NMR spectra often suffer from severe signal overlap, which complicates spectral analysis. Detection of heteronuclei such as 13C provides greater chemical shift dispersion and therefore improved spectral resolution. Nevertheless, the application of benchtop 13C NMR under continuous flow conditions is challenging due to low sensitivity and insufficient polarization build-up at high flow velocities. Paramagnetic relaxation enhancement (PRE) offers a strategy to overcome this limitation by accelerating relaxation and enabling higher polarization levels even for fast-flowing samples, as will be presented in the second part of the talk.

If sensitivity remains a limiting factor, hyperpolarization methods can provide an additional boost in signal intensity. Overhauser Dynamic Nuclear Polarization (ODNP) is particularly well suited for online NMR spectroscopy under continuous flow conditions because the polarization build-up occurs on very short timescales. This allows signal enhancement even at high flow rates [3]. However, ODNP efficiency strongly depends on parameters such as solvent composition, radical type, temperature, and the observed nucleus, which complicates quantitative analysis. Reliable quantification can be achieved through appropriate calibration strategies [4], enabling the use of ODNP-enhanced NMR for reaction and process monitoring, as will be shown at the end of the lecture.

References:

[1] J. Phuong, et. al.,Food Research International 203 (2025) 115741.
[2] J. Phuong, et. al., Anal. Chem. 97 (2025) 4308-4317.
[3] J. Phuong, et. al., ChemPhysChem 26 (2025) e2401052.

Time-Resolved Diffusion NMR Monitoring of UV-Induced PET Depolymerization on a Benchtop Spectrometer

Khalid, Farwa, Urbańczyk, Mateusz, Malangi Gajendramurthy, Chunchesh

Polyethylene terephthalate (PET) is widely used in bottles, textiles, and packaging because of its transparency, strength, and durability. Its extensive use, combined with its natural degradation under sunlight and UV rays, slowly and uncontrollably contributes to environmental pollution. However, the large amount of PET waste has become a serious environmental problem. Developing efficient methods to break down PET into useful products is therefore important for sustainable plastic recycling.

In this study, we investigate the UV-induced depolymerization of commercial PET samples dissolved in a mixture of Trifluoroacetic acid and Chloroform (1:50). The reaction is monitored in real time on an 80MHz benchtop spectrometer. A flow-based experimental setup allows the solution to be continuously exposed to UV light while measurements are recorded simultaneously.

Time-Resolved Diffusion NMR (TR-DOSY) experiments are used to track changes in the molecular diffusion coefficient during the reaction, enabling us to monitor polymer chain scission. These diffusion measurements provide information about the size and mobility of molecules in the solution. As depolymerization proceeds, the real time diffusion data reveal the depolymerization kinetics, indicating the breakdown of large PET polymer chains into smaller fragments, which is quite useful for providing information about the molecular mass and the dispersity of the polymer.

This study demonstrates that low-field benchtop diffusion NMR is a very simple and effective method for monitoring polymer degradation in real time. This method offers valuable insights into the mechanistic pathway of PET depolymerization, potentially improving sustainable plastic waste management. Additionally, the presented methodology can be adopted to any depolymerization process, making it a compelling tool for polymer science.

In situ ^{1}H and ^{13}C SABRE for mixture analysis using 1D and 2D benchtop NMR spectroscopy

Hehir, **Izzy**, Taylor, Daniel A., McCall, James, Sehrawat, Neelam, Yule, Gregory, Halse, Meghan E.

Benchtop NMR spectrometers can be advantageous over high-field spectrometers on account of their portability, low maintenance needs and affordability. However, these benefits come at the expense of lower sensitivity and increased peak overlap. Hyperpolarisation methods allow the barrier of sensitivity to be overcome by generating large signal enhancements irrespective of the magnetic field of the spectrometer.

This work concerns SABRE (Signal Amplification By Reversible Exchange), in which spin order from *para*-hydrogen (p-H_2) is transferred onto a substrate via reversible binding to an iridium catalyst. SABRE can create large amounts of polarisation in seconds and allows repeated hyperpolarisation of the sample; the polarisation transfer is typically carried out in a weak magnetic field (nT to mT) and the sample is subsequently transferred to the spectrometer for detection.

Here, an entirely *in situ* method was developed by delivering the p-H_2 gas *via* a capillary and using radio-frequency (RF) irradiation to satisfy the polarisation transfer condition. The RF-SABRE method has previously been implemented at high-field, but was here adapted for the lower B_0 field of the benchtop spectrometer and extended to enhance ^{13}C *via* transfer from ^{1}H. The efficiency of this pathway enables ^{13}C experiments to be carried out on natural abundance samples in as little as one scan and the method has proved versatile, allowing magnetisation to be distributed throughout the substrate molecules or targeted to specific nuclei.

In this work, we will present the theoretical basis for the ^{1}H-^{13}C hyperpolarisation approach, demonstrate its efficiency and reproducibility and explore applications to mixture analysis using 1D ^{1}H, ^{13}C{^{1}H} and 2D ^{1}H-^{13}C NMR pulse sequences.

Free-radical generation and quenching detected in real time by NMR relaxation in Earth's magnetic field

Topor Alexandru[a,b,c], Voda Mihai A.[a,c], and **Vasos Paul R.**[a,b,c]

[a.] Biophysics and Biomedical Applications Group and Laboratory, Laser Gamma Experiments Department, LGED, Extreme Light Infrastructure, ELI-NP, "Horia Hulubei" National Institute for Physics and Nuclear Engineering IFIN-HH, Reactorului Street 30, Bucharest-Măgurele 077125, Romania
[b.] University of Bucharest, Faculty of Interdisciplinary Studies, 4-12 Regina Elisabeta Bd, 030018 Bucharest, Romania
[c.] C. D. Nenitzescu Institute of Organic and Supramolecular Chemistry, 202B Splaiul Independenței, Bucharest, Romania

Earth-field nuclear magnetic resonance (NMR) is improved with the scope of offering flexible approaches for biomedical investigations. In low magnetic fields, the formation of free radicals can be followed through NMR relaxation measurements of water protons. Oxygen formation from hydrogen peroxide creates paramagnetic contrast via the decreased transverse relaxation time constants of water magnetisation. In cells and living matter, Fenton reactions leading to oxygen formation are mediated by endogenous in iron concentrations specific to every organism. We show herein that the formation of O_2 can be followed in water, in glioblastoma and microglia cells, and used for imaging in hen egg models. The effect of antioxidants such as glutathione on the reaction rates was also quantified. This experimental method for magnetic resonance imaging in low fields can find applications in biomedical investigations of radiobiology mechanisms involving hydrogen peroxide and oxygen.

Financial support from the Romanian Research Ministry, UEFISCDI: PCE-2023-1836/ ELI-RO 2024-039, core program (PN), and European Union through NextGeneration EU-PNRR-III-C9-2022-I8 program are gratefully acknowledged.

High resolution ^{1}H Magic Angle Spinning benchtop NMR

Sakellariou; Dimitrios, De Oliveira-Silva; Rodrigo, Hugon; Cedric, Soleilhavoup; Anne, Alonso; Javier, Riley; Isabella, Aubert; Guy, Jacquinot; Jacques-François

Over the last fifteen years we have witnessed a dramatic increase in low-field benchtop NMR applications many of which benefited from high spectral resolution. Nowadays commercial instruments exist offering linewidths better than 0.5Hz on 5mm tube isotropic liquid samples for a variety of Larmor frequencies up to 90 MHz. The compromise to achieve strong field from permanent magnets is reduced free bore diameter. Although recent work to implement magic angle sample spinning have been reported [1,2], they focused on reducing the anisotropic broadening from dipolar couplings where modest resolution is sufficient.
Here we present a benchtop high-resolution NMR magnet (0.9T) developed since 2010, having a clear bore of 54 mm, which can be integrated with standard shim / gradient coils and standard liquids and solid-state MAS probes from superconducting NMR magnets. We demonstrate sub ppm high-resolution operation under fast and slow MAS conditions and reflect on the potential of this low-cost technology towards democratizing Magic Angle sample Spinning in applications with minimal sample preparation: metabolomics, materials, food science, etc.

[1] Sørensen et al, “Magic-angle spinning solid-state multinuclear NMR on low-field instrumentation”, JMR 238, p20-25 (2014).
[2] Sørensen et al, “Soil Analysis by Mobile Multinuclear NMR: Quantification of Phosphorus, Aluminum, and Sodium”, Anal. Chem 96, p17086-17091 (2024).

MAS and MACH

Ats Kaldma, Mai-Liis Org-Pang, Kalju Vanatalu, Ago Samoson

Tallinn University of Technology

According to our expermental findings, the most critical issue in magic angle spinning is ability to propel rotors. Theoretical resilience of technical ceramics is significantly higher than spinning frequency achieved by the momentum given by compressed air. The ultimate speed is limited by the sound motion. We demonstrate experiments in range of 250-320 kHz, using Helium as a propellant. Surface speed of rotor, supported radially still by air, achieves MACH 1.1

Surface-Only Nuclear Magnetic Resonance Spectroscopy by Dynamic Nuclear Polarization and ^{2}H-Dephasing

Tristan Georges, Ran Wei, Ray Cowen, Likai Zheng, Michael Grätzel, Lyndon Emsley

École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

The surface of a material governs many of its physicochemical properties, as it is the interface where interactions with the external environment take place. Accessing the atomic-level structure and chemistry of surfaces in functional materials is therefore crucial for understanding reaction mechanisms and improving material performance.

Magic-angle spinning dynamic nuclear polarization (MAS-DNP) dramatically enhances solid-state NMR sensitivity. Surface-enhanced DNP NMR can be performed when the hyperpolarization remains confined near the surface due to limited spin-diffusion. However, this surface selectivity is often lost for proton-rich or nanocrystalline materials.

Here we introduce a broadly applicable strategy for surface-only solid-state NMR using MAS-DNP. Partially deuterated vitrified solvents act as proximity probes, introducing ^{2}H–X dipolar couplings between the solvent and X nuclei in the target material. A ^{2}H-based dipolar dephasing filter then selectively suppresses signals from the bulk, thereby providing clear detection of nuclei located within the first one or two atomic layers at the surface. We demonstrate the method on several nanomaterials, including core-shell hydroxyapatite, tin dioxide, silica, and a passivated hybrid perovskite, revealing surface sites otherwise obscured by bulk signals. The approach also enables nanoscale domain size measurements, illustrated through the hydration of calcium silicate hydrate, the main component of cement, revealing growth through sheet stacking.

High-Sensitivity ^{19}F-^{19}F Dipolar Correlations with Adiabatic RFDR

Joseph Hurd,[1] Euan Bassey,[1] Tuan Nghia Duong,[1] Guido Pintacuda,[1] Anne Lesage,[1] Andrew Pell[1,2] and Judith Schlagnitweit[1]

[1]Centre de RMN à très haut champs (UMR 5082, CNRS/UCB Lyon 1/ENS de Lyon) Villeurbanne, France

[2]Institut Universitaire de France (IUF)

Fluorine is present in many pharmaceuticals, functional materials and can readily be incorporated into bio-molecules such as proteins and nucleic acids. ^{19}F is therefore a vital probe to detect structural changes and measure internuclear distances due as it is spin ½, has 100% natural abundance, a high gyromagnetic ratio, large chemical shift dispersion and large homonuclear dipolar couplings.

This large shift sensitivity, however, leads to challenges when acquiring spectra of solid-state materials under magic angle spinning with significant chemical shift anisotropy (CSA). As such, ^{19}F is described as entering a "semi-wideline" regime. Here, we use adiabatic pulses to counteract the challenges of large offset and CSA distributions for ^{19}F in the homonuclear recoupling sequence radio-frequency-driven recoupling (RFDR). We show that, despite spanning only ~10^2 kHz, rather than several MHz, these adiabatic pulses show excellent performance.

Specifically, we observe signal enhancements of more 20 times at long mixing times for all peaks. This enables the application of RFDR to both longer recoupling times as well as unlocking the potential of more complex recoupling experiments for nuclei with large anisotropies. We further demonstrate that our adiabatic RFDR sequence is effective over a range of MAS rates from 10-100 kHz, and leads to excellent sensitivity across a range of different systems, from organic molecules to inorganic materials. We compare our experimental data against numerical modelling simulations (Simpson), showing excellent agreement. Finally, we suggest additional experiments, including applications under MAS DNP conditions, that may employ our new sequence.

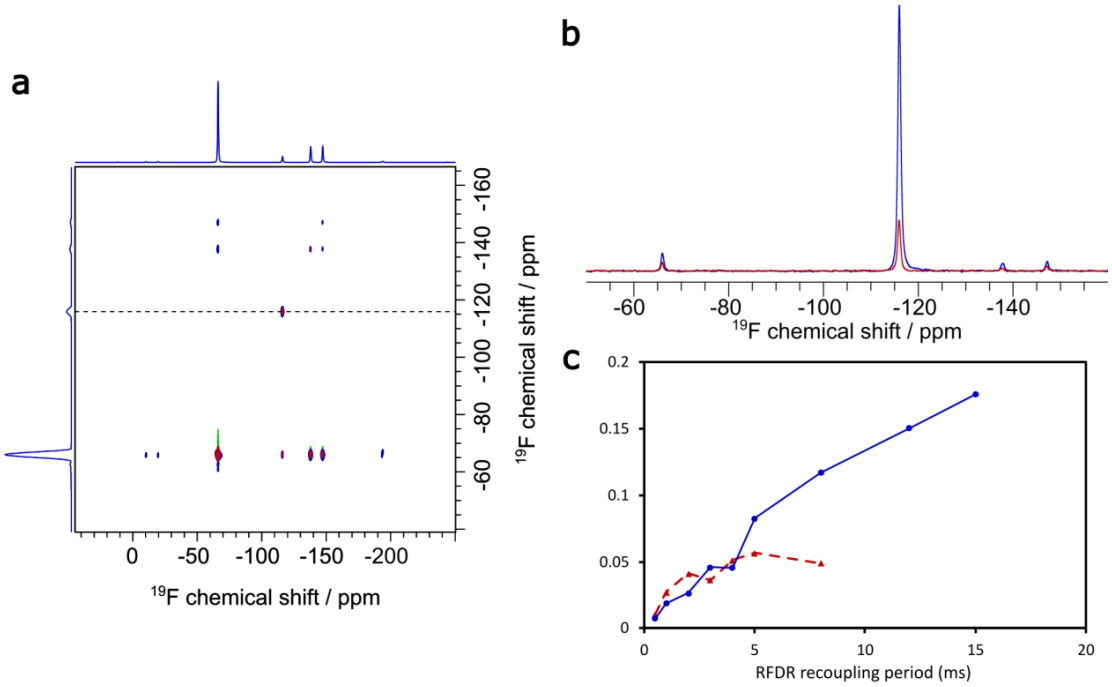


Figure – (**a**) 2D ^{19}F-^{19}F Adiabatic RFDR compared with conventional RFDR for the diabetes drug sitagliptin. (**b**) Cross sections along the dotted line for comparison of peak intensities and (**c**) build-up curves for both aRFDR (blue) and RFDR (red).

Toward ^{17}O Natural Abundance Nuclear Magnetic Resonance Detection in Solids

Franck Fayon1*, Alia Hassan2, Sebastian Wegner3, Pierre Florian1¶

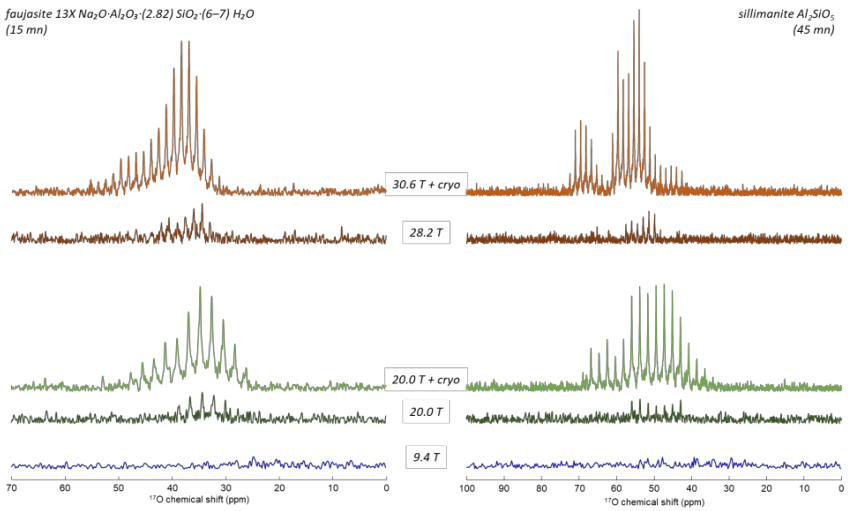
1 CNRS-CEMHTI, Univ. Orléans, Orléans, France

2 Bruker Switzerland AG, Fällanden, Switzerland

3 Bruker BioSpin GmbH & Co KG, 76275 Ettlingen, Germany

Oxygen is an essential component of a wide variety of inorganic and inorganic materials, natural or synthetic. The characterization of its chemical bonding state is therefore essential in a wide range of scientific fields. ^{17}O solid-state Nuclear Magnetic Resonance is known to be very sensitive to the oxygen local environment and bonding state, but also to suffer from a weak sensitivity due to both the low natural abundance and quadrupolar nature of the only NMR active ^{17}O isotope (0.037%).

We show that the limit of ^{17}O natural abundance NMR detection in solids can be overcome by using high (20 T) to ultra-high (30.6 T test magnet) magnetic fields in combination with MAS cryo-probes technologies and signal amplification strategies (DFS & CPMG). We demonstrate the method and explore its efficiency using various systems: geological minerals, porous catalysts, oxide glasses, Metal Organic Frameworks and micro-crystalline cellulose. This approach allows to obtain natural abundance ^{17}O MAS spectra with excellent signal-to-noise ratios and even to record 2D MQMAS ^{17}O spectra for environment with moderate quadrupolar (e.g. for oxide-based compositions).



Protein unfolding and aggregation at all stages as seen by NMR.

Heise, **Henrike** and all coworkers

Protein misfolding and aggregation is closely linked to many neurodegenerative diseases like Alzheimer's or Parkinson's disease, and understanding the underlying processes like protein (un)folding, aggregation and fibril formation is a rewarding challenge. Here we demonstrate the enormous potential of NMR-Spectroscopy at all time-scales ranging from solution NMR, HR-MAS via room-temperature solid-state NMR to DNP enhanced low-temperature NMR in frozen solutions for the study of protein folding, unfolding and aggregation [1].

On the one hand, the structure and molecular details of amyloid fibrils can be studied by solid-state NMR-spectroscopy [2-6]. We also apply a multidisciplinary approach combining solution-NMR, in-situ MAS NMR-spectroscopy and DNP-enhanced NMR solid-state NMR-spectroscopy of frozen solutions to follow and characterize the process of unfolding, oligomerization and protein aggregation of the model protein PI3K SH3 in real time and with high resolution [7]. In particular, we exploit DNP-enhanced solid-state NMR-spectroscopy of frozen solutions to determine structural ensembles of backbone and side-chain conformations of well-folded, unfolded, intrinsically disordered and fibrillated proteins [8-11], which are linked to aggregation kinetics and products at different conditions.

[1] König, A.; Schölzel, D.; Uluca, B.; Viennet, T.; Akbey, Ü.; Heise, H *Solid State NMR.* **2019,** *98*, 1-11.

[2] Gremer, L.; Schölzel, D.; Schenk, C.; Reinartz, E.; Labahn, J.; Ravelli, R. B. G.; Tusche, M.; Lopez-Iglesias, C.; Hoyer, W.; Heise, H.; Willbold, D.; Schröder, G. F., *Science* **2017,** *358* (6359), 116-119.

[3] Frieg, B.; Gremer, L.; Heise, H.; Willbold, D.; Gohlke, H., *Chem.Commun.* **2020,** *56* (55), 7589-7592.

[4] Willbold, D.; Strodel, B.; Schröder, G. F.; Hoyer, W.; Heise, H., *Chem. Rev*. **2021**, *121*, 8285-8307.

[5] Becker, N., Frieg, B., Gremer, L., Kupreichyk, T., Gardon, L., Freiburg, P., Neudecker, P., Willbold, D., Gohlke, H.; Heise, H. *J. Am. Chem. Soc.* **2023***, 145*, 2161-2169.

[6] Gardon, L.; Becker, N.; Gremer, L.; Heise, H., *Chemistry – A European Journal* **2024,** *30* (10), e202303007.

[7] Gardon, L.; Becker, N.; Rähse, N.; Hölbling, C.; Apostolidis, A.; Schulz, C. M.; Bochinsky, K.; Gremer, L.; Heise, H.; Lakomek, N.-A., *Frontiers in Molecular Biosciences* **2023,** *10*.

[8] Uluca, B.; Viennet, T.; Petrović, D.; Shaykhalishahi, H.; Weirich, F.; Gönülalan, A.; Strodel, B.; Etzkorn, M.; Hoyer, W.; Heise, H., *Biophys. J.* **2018,** *114*, 1614-1623.

[9] Viennet, T.; Wördehoff, M. M.; Uluca, B.; Poojari, C.; Shaykhalishahi, H.; Willbold, D.; Strodel, B.; Heise, H.; Buell, A. K.; Hoyer, W.; Etzkorn, M., *Communications Biology* **2018**, 1, 44.

[10] Siemons, L.; Uluca-Yazgi, B.; Pritchard, R. B.; McCarthy, S.; Heise, H.; Hansen, D. F., *Chem.Commun.* **2019,** *55* (94), 14107-14110.

[11] Levorin, L., Becker, N., Uluca-Yazgi, B., Gardon, L., Kraus, M., Sevenich, M., Apostolidis, A., Schmitz, K., Rüter, N., Apanasenko, I., Willbold, D., Hoyer, W., Neudecker, P., Gremer, L.; Heise, H. *J. Am. Chem. Soc*. **2025**, 147, 15867-15879.

Do MD Simulations and NMR Measurements Agree? Comparing Force Fields and ML/MM on Reproducing the Conformational Behaviour of Peptides, Macrocycles, and Proteins

The combination of classical molecular dynamics (MD) simulations and data from nuclear magnetic resonance (NMR) measurements has been studied over the past decades. The accuracy of MD results can be validated with NMR data (e.g., atom-atom distance bounds from NOESY or ROESY experiments and scalar couplings) and MD simulations can support the interpretation of NMR results. However, the choice of force field in the simulations can have a non-negligible effect on the level of agreement with the experimental data. We show our benchmarking results of classical force fields for the conformational sampling of cyclic peptides and non-peptidic macrocycles using NOE distance bounds in different solvents as well as for the description of protein motion using NMR relaxometry. Recently, machine-learning interatomic potentials (MLIPs) have been developed to replace QM Hamiltonians at much reduced computational cost, thus enabling simulations on the tens of nanoseconds timescale at near-DFT accuracy. Here, we show the results of using our MLIP with anisotropic message passing (AMP) to simulate peptidic systems and comparing the generated ensembles with experimental NOE distance bounds.

TOWARDS ULTIMATE NMR RESOLUTION WITH AI

Amir Jahangiri[a], **Tatiana Agback**[a,b], **Ulrika Brath**[c], and **Vladislav Orekhov**[a,c]

[a]Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, 40530, Sweden
[b]Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, 75007, Sweden
[c]Swedish NMR Centre, SciLifeLab, University of Gothenburg, Box 465, 40530 Gothenburg, Sweden

ABSTRACT

Artificial intelligence (AI), particularly deep neural networks (DNNs), has recently emerged as a powerful tool for NMR data processing. AI-based approaches enable rapid and high-quality non-uniform sampling (NUS) reconstruction, virtual homo-decoupling, and automated peak picking, providing robust alternatives to conventional processing methods. Collectively, these advances reduce spectral complexity and enhance effective resolution. Resolution in multidimensional NMR is fundamentally defined as the ability to distinguish and precisely localize signals in the presence of peak overlap, noise, and spectral artifacts. Achieving ultimate practical resolution, therefore, requires statistically validated peak localization rather than merely sharper intensity profiles. To address this challenge, we introduce Peak Probability Presentation (P^3), a novel statistical representation of NMR spectra (Jahangiri et al., 2026, Sci Adv., in press). Instead of describing spectra solely by amplitude, P^3 assigns to each spectral point the probability that a true peak maximum occurs at that location, reframing spectral analysis as a probabilistic inference problem.

Transformation of conventional spectra to P^3 is achieved using MR-Ai (Magnetic Resonance processing with Artificial Intelligence), a newly introduced physics-informed and computationally efficient deep-learning architecture designed for multidimensional NMR data. P^3 was validated on sixty experimental protein datasets for backbone assignment and further demonstrated on challenging systems such as Tau (44 kDa IDP) and MATL1 (45 kDa). Furthermore, using synthetic spectra, we show that peak-localization precision approaches the theoretical limits defined by the Cramér–Rao lower bound and Bayesian Monte Carlo estimators. Importantly, MR-Ai also enables joint co-processing of multiple spectra, allowing direct information exchange across datasets and significantly enhancing spectral quality, particularly under highly sparse sampling conditions.

Using a general MR-Ai architecture, we further implement advanced "smart" processing strategies, including high-fidelity NUS reconstruction, virtual decoupling, phase correction, peak narrowing, echo reconstruction, and solvent signal suppression. Together, these developments demonstrate that physics-informed deep learning reshapes NMR signal processing and analysis.

Keywords Deep Learnig · Peak Probability Presentation (P^3) · Signal Assignment · Signal processing · MR-Ai

Acknowledgement

The work was supported by the Swedish NMR Center (SNC) at the University of Gothenburg (Sweden). This study used NMRbox: National Center for Biomolecular NMR Data Processing and Analysis, a Biomedical Technology Research Resource (BTRR), which is supported by NIH grant P41GM111135 (NIGMS).

Bloch-Siegert Shift Management in Quantum Optimal Control

Dev, Aditya[1] and Kuprov, Ilya[1]

[1]Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot 7610001, Israel

The Bloch–Siegert shift (BSS) is a phenomenon in magnetic resonance that arises when the rotating frame approximation begins to fail. BSS is a second-order effect arising from the counter-rotating component of radiofrequency or microwave irradiation. Although typically small, it becomes significant when the pulse nutation frequency becomes comparable to the Larmor frequency. BSS pushes resonance frequencies away from the irradiation frequency and scales as $\sim \omega_1^2/\omega_0$.

In modern magnetic resonance and spin-based quantum device engineering, these shifts are no longer negligible. They introduce significant phase and frequency errors that degrade spectral resolution in multidimensional NMR and reduce magnetisation transfer efficiency [1]. To date, most strategies have sought to cancel out these effects using symmetry arguments, composite pulses, or time-scaling techniques within optimal control [2, 3]. While effective in weak-drive regimes, these strategies restrict the available control space and may reduce pulse sequence efficiency.

In this work, we move beyond simple BSS cancellation strategies and instead propose a formalism that incorporates Bloch–Siegert effects explicitly into the quantum optimal control framework. Using Magnus expansion to second-order, the BSS contribution is recast into a numerically efficient auxiliary matrix form that integrates naturally into the GRAPE gradient calculation. This enables the optimization of pulses whose trajectories explicitly account for BSS as a part of the Hamiltonian, rather than a bug to be compensated for. The resulting methodology, now implemented in Spinach, produces pulses that remain robust in the strong-drive regime, enabling shorter durations and potentially greater fidelities in systems constrained by rapid relaxation.

References:

[1] *Concepts in Magnetic Resonance*, 40A(6), 326-340 (2012), [2] *Journal of Magnetic Resonance*, p108030 (2026), [3] *Nature communications* 9(1):3014 (2018)

Calorimeter at Atomic Resolution: Theory and multiple thermodynamic states

Wolf, **Florian**, Mares, Jiri, Orts, Julien

Understanding the energetic landscape of proteins is fundamental to drug design and folding studies. However, current experimental techniques, such as calorimetry or global denaturation assays, yield only macroscopic thermodynamic parameters, averaging out the distinct contributions of individual residues and atoms. Consequently, experimentally resolving thermodynamics at the inter-atomic level remains a challenge. Here, we present a theoretical framework to derive inter-atomic thermodynamic values directly from temperature-dependent NOE cross-relaxation rates. Our derivation demonstrates that the observed NOE rate is an average of the conformational ensemble, where the contribution of any intermediate state is determined by its specific spectroscopic cross-relaxation rate. By accounting for the spectroscopic weighting of different conformational states, we successfully recovered input thermodynamic parameters from synthetic datasets, confirming the theoretical robustness of the approach. Additionally, we explore the possibility that correlations between these local melting curves could theoretically indicate energetic coupling between distant sites, offering a potential route to identify allosteric networks. This work establishes the mathematical foundation for extracting pairwise Gibbs free energies from spectroscopic data, supporting the experimental applications presented in the site-specific thermodynamic analysis of the WW-Domain and the bromodomains BRD4-BD2 and -BD1 presented elsewhere at this conference.

Broadband, Ultrabroadband, and fast pulsing NMR enabled by Optimal Control

Burkhard Luy

Optimal control enables the efficient optimization of shaped pulses, which in turn open new avenues for experiment design. Various approaches will be presented that have fundamental impact on traditional and novel pulse sequences. Broadband pulses for (IN)ADEQUATE-type sequences will be introduced, as well as ultrabroadband excitation pulses that allow the acquisition of 1D and 2D pulse sequences up to MHz bandwidths on conventional high resolution spectrometers. The same pulses at more conventional bandwidths but very low rf-power can also be used for fast pulsing experiments with severe restrictions with respect to the duty cycle. Next to simple pulses, also other optimal control-improbed elements like bilinear rotations, heteronuclear decoupling sequences as well as planar and isotropic mixing sequences will be shown to severely enhance fast pulsing sequences for utmost sensitivity and flexibility.

qNMR and CSI determination of small-molecule partitioning coefficients in phase separating systems.

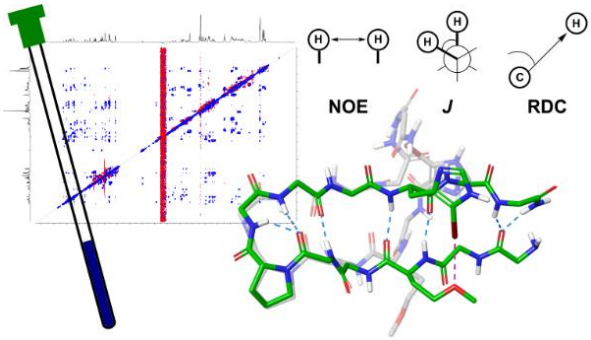
Mulder, Frans A. A., Anjum, M. Ali Raza, Baumgartner, Manuel, Bechmnann, Matthias, Maibøll Buhl, Julie, Kjaergaard, Magnus

Phase separation is a well-known concept in preparative and analytical chemistry and is a common method to define polarity scales and rank the hydrophobicity of drug molecules and pharmacophores in structure-activity relationships (SAR). More recently, intrinsically disordered proteins (IDPs) have also been shown to phase separate and form membraneless organelles. Because such condensates are increasingly becoming recognized as fundamental ways to orchestrate cellular chemistry in space and time, it is essential to investigate the properties of these condensates and to consider how their formation leads to sequestration of metabolites. To help in this quest, we report two distinct ways for multiplex determination of the relative concentrations of collections of small-molecule metabolites. First, we report a 2D qNMR method that is built on the $HSQC_0$ approach; it allows for the simultaneous determination of partitioning of molecules in a mixture, utilizing 2D NMR to disperse the signals. The elegance of the method lies in the fact that it extrapolates out the entire existence of the pulse sequence, and is suitable for molecules at natural ^{13}C abundance. The approach is demonstrated in a model two-phase system, measuring the concentrations of a panel of small molecules in the two phases independently. In a second application, we use chemical shift imaging (CSI) such that the two phases no longer need to be separated, but are observed simultaneously in the NMR tube using z-imaging. This approach is applied to ^{31}P NMR of metabolite mixtures that subdivide between the dense and dilute phases of a peptide condensate that is too viscous to be pipetted.

Characterising Weak Non-Covalent Interactions via NMR Ensemble Analysis

Herbst, Manuel A., Wu, Leyun, Woordes, Yannik T., Peintner, Stefan, Xin, Jian, Boeckler, Frank M., Xu, Zhijian, Zhu, Weiliang, Navarro-Vazquez, Armando, Erdelyi, Mate

The experimental characterisation of weak non-covalent interactions in dilute solution is cumbersome. Classical chemical shift titrations are rarely helpful due to the minimal chemical shift changes induced by the interaction and the low population of the bound form. The characterisation of weak interactions in dilute aqueous solutions, the environment of biological processes, remains especially challenging. We disclose a method applicable for solution-state NMR spectroscopic characterisation of very weak bonds in dilute aqueous solution, and demonstrate its applicability on the example of halogen bonding. We describe these interactions by detecting their influence on β-hairpin folding, in a model system that permits the formation of cross-strand halogen bonds upon folding.



The conformational space of flexible systems is not captured by a single population-averaged structure. We identify conformational ensembles by deconvoluting their population-averaged NMR observables, including isotropic NOE-derived distances and *J*-couplings, as well as anisotropic residual dipolar couplings (RDCs). The back-calculated parameters of computationally generated conformers are then fitted to the experimental data using Stereofitter (MestreLab). Increased β-hairpin folding was observed for peptides capable of cross-strand halogen bonding as compared to a non-halogenated reference. Refined side chain analysis revealed increased populations of geometries consistent with halogen bonding when substituting the halogen bond donor and acceptor atoms with their heavier homologues. We showcase the unique capability of solution-state NMR to characterise weak, transient interactions in water by incorprorating them into a cooperatively folding system, and demonstrate the power of combining isotropic and anisotropic NMR observables for describing the structure and dynamics of flexible molecules.

^{14}N solid-state NMR quadrupolar products as quantitative probes of hydrogen-bond environments in pyridinic solids

Sabena, **Chiara**, Nishiyama, Yusuke, Chierotti, Michele R.

Hydrogen bonds in molecular solids often span a continuum of interaction regimes, where subtle variations in proton position and hydrogen-bond geometry may lead to distinct solid forms and affect physicochemical properties. Probing these fine structural differences experimentally can be challenging. In this study, we explored the use of ^{14}N solid-state NMR as a sensitive probe of hydrogen-bond geometry, exploiting its quadrupolar nature and intrinsic sensitivity to local electronic environment. Since direct detection of ^{14}N is often impractical, we focused on indirect, ^{1}H-detected, ^{14}N experiments under fast magic-angle spinning. In particular, we identified the ^{14}N quadrupolar product (P_Q) as an easily accessible parameter, extracted by combining ^{1}H–^{14}N T-HMQC spectra with ^{15}N CPMAS. A series of crystalline systems containing pyridinic nitrogen atoms involved in different hydrogen bonds was investigated, spanning protonated, shared-proton, neutral, weakly hydrogen-bonded, and free nitrogen environments. Systematic variations of P_Q values were observed across the series, revealing a clear dependence on hydrogen-bond type. Moreover, independent ^{1}H–^{14}N distance measurements were performed using PM-S-RESPDOR and selective overtone-based (OT-RESPDOR) experiments. A strong linear correlation was found between P_Q values and the inverse third power of the experimentally determined N–H distances, providing direct experimental validation of the physical relationship between the quadrupolar interaction and hydrogen-bond geometry. These results establish ^{14}N quadrupolar products as robust and quantitative descriptors of hydrogen bond environments in solids, providing a valuable framework for NMR crystallography across the salt–cocrystal continuum.

Acknowledgements

The Italian Discussion Group on Magnetic Resonances (GIDRM) is sincerely acknowledged for financial support through the “Stefano Caldarelli” grant.

Solid-State NMR of Roxithromycin in Crystalline and Gel State

Svetlana Pavlović,[1,3] Maria Adobes Vidal,[2] Steven P. Brown,[3]

[1]*Department of Chemistry, University of Warwick, CV4 7AL, UK*

[2]*Novartis, Basel, Switzerland*

[3]*Department of Physics, University of Warwick, CV4 7AL, UK*

Roxithromycin (Active Pharmaceutic Ingredient – API) is a semi-synthetic drug that belongs to the group of macrolide antibiotics. It is broadly used for fighting infections caused by Gram-positive and Gram-negative bacteria, and atypical microorganisms. The structure of roxithromycin consists of a 14-member lactone ring with an N-oxime side chain, and two sugar molecules L-cladinose and D-desosamine, attached to the ring. It has been reported that a mixture of Roxithromycin and particular excipients forms a gel-like film during the dissolution process in acidic conditions. This phenomenon can slow down release of API from the formulation. Even though the gel formation behaviour has been reported, the mechanism behind it is still unclear, to our knowledge.

Recently, a ^{13}C-^{1}H HETCOR MAS NMR spectrum of Roxithromycin has been reported. In this study, we have used MAS NMR to look at Roxithromycin at its crystalline state and in a gel formed with specific excipients. The gel was studied by recording ^{13}C spectra using ^{1}H-^{13}C CP and INEPT and direct polarization experiments. Here, we present Gauge Including Projector Augmented Waves (GIPAW) calculations and ^{1}H detected experiments of Roxithromycin, acquired at 1 and 1.2 GHz with spinning frequencies of 150 and 199 kHz, using Samoson probes.

Cracking Nature's Recipes to Design Lipid-Targeting Antibiotics

Markus Weingarth

[1]NMR Spectroscopy Group, Utrecht University, Utrecht, the Netherlands

Antimicrobial resistance is a global health threat, calling for new antibiotics. Good candidates could be compounds that target special lipids that only exist in bacterial, but not in human cell membranes.

Recently, we determined the supramolecular killing mechanism of antibiotics that target the special lipid Lipid II in the bacterial plasma-membrane[1-5]. Here we present a general conceptional framework on the assembly mechanism of these antibiotics.

References:

[1] Medeiros-Silva, J., Jekhmane, S., Lucini Paioni, A., Gawarecka, K., Baldus, M., Swiezewska, E., Breukink, E., Weingarth, M. Nature Comm. (**2018**), 9, 3963, *High-resolution NMR studies of antibiotics in cellular membranes*

[2] Shukla, S., Medeiros-Silva, J., Parmar, A., Vermeulen, B.J.A., Das, S., Paioni, L.A., Jekhmane, S., Lorent, J., Bonvin, A.M.J.J., Baldus, M., Lelli, M., Veldhuizen, E.J.A., Breukink, E., Singh, I., Weingarth, M. Nature Communications (**2020**), 11, 2848, *Mode of action of teixobactins in cellular membranes*

[3] Shukla, R., Lavore, Sourav, M., F., Derks, G.N., Jones, C.R., Vermeulen, B.J.A., Melcrova, A., Morris, M.A., Becker, L.M., Wang, X., Kumar, R., Medeiros-Silva, J., van Beekveld, R., Bonvin, A.M.J.J., Lorent, J., Lelli, M., Nowick, J., MacGillavry, H., Peoples, A.J., Spoering, A.L., Ling, L.L., Hughes, Roos, W., D., Breukink, E., Lewis, K., Weingarth, M., Nature (**2022**) 608, 390*, Teixobactin kills bacteria by a two-pronged attack on the cell envelope*

[4] Shukla, R., Peoples, A.J., Ludwig, K.C., Maity, S., Derks, M.G.N, de Benedetti, S., Krueger, A.M., Vermeulen, B.J.A., Lavore, F., Honorato, R.V., Grein, F., Bonvin, A.M.J.J., Kubitscheck, U., Breukink, E., Achorn, C., Nitti, A., Schwalen, C.J., Spoering, A.L., Ling, L.L., Hughes, D., Lelli, M., Roos, W.H., Lewis, K., Schneider, T., Weingarth, M., Cell **(2023)** *A new antibiotic from an uncultured bacterium binds to an immutable target*.

[5] Jekhmane, S., Derks, M.G.N., Maity, S., Slingerland, C.J.,, Tehrani, K.H.M.E., Medeiros-Silva, J., Vermeer, B., Ammerlaan, D., Charitou, V., Weijde, M., Elenbaas, B.O.W., Fetz, C., Cox, R., Lorent, J.H.F.F., Baldus, M., Künzler, M., Lelli, M., Cochrane, S., Martin, N.I., Roos, W.H., Breukink, E., Weingarth, M., **(2024)** Nature Microbiology, in press, *Plectasin kills bacteria by a Ca2+-sensitive supramolecular mechanism*

NMR to probe the conformational landscape in enzyme evolution

J. Sun[1], S.F. Koene[1], V.E. van der Borg[1], M. Timmer[1], S. Brünle[1], A.S. Boyle[1,2], M. Ubbink[1]

(1) Leiden University, The Netherlands, m.ubbink@chem.leidenuniv.nl

(2) Bristol University, England

Both in natural evolution and directed evolution in the laboratory, enzymes evolve to acquire new functions. While in directed evolution gain of function is a straightforward, one-dimensional climb toward a fitness peak, the underlying journey through the conformational landscape can be quite complex. We use NMR to characterize the changes that occur during natural and directed evolution by studying resurrection of ancestral enzymes as well as performing laboratory evolution experiments on modern enzymes. As an example, the successive variants of directed evolution of the β-lactamase BlaC from *Mycobacterium tuberculosis* toward increased ceftazidime activity are described. Crystal structures show that the 3D ground state structure remains essentially unchanged except for increasing disorder in two loops that line the active site. NMR spectroscopy, however, shows that in each next variant new conformational states become populated and the free energy barriers between those conformations are high, given the slow exchange behaviour observed in the NMR spectra. This work shows that behind a simple gain-of-function in enzyme activity a complex travel through the conformational landscape occurs.

Emergence of high-field magnetic field effect in AsLOV2 through a cysteine bond-forming radical pair.

Salvia, **William** Straub, Joshua; Han, Songi

The spin-correlated radical pair (SCRP) mechanism in flavin-binding cryptochromes is essential for bird magnetonavigation and a cornerstone of the field of quantum biology. Cryptochrome is not unique among flavoproteins; MagLOV, engineered from AsLOV2, has highly magnetic field dependent fluorescence (>40%) and promising applications in quantum sensing. Both MagLOV and avian cryptochromes form a SCRP via electron transfer from a ~ 2 nm distant tryptophan. Therefore, the SCRP singlet-triplet interconversion is caused by differences in electronic hyperfine interactions and the MFE emerges at low fields, ~ 5-10 mT. Despite evidence that flavin-cysteine covalent bond formation occurs through a radical pair intermediate, no wild-type AsLOV2 MFE has been previously observed. We show that a fluorescent AsLOV2 MFE does emerge at ~80 mT. We hypothesize that its high-field onset is due to strong dipolar and exchange coupling, since the cysteine-flavin distance is short (~ 4 Å). At high fields, Zeeman splitting generates sufficient electronic frequency differences to drive singlet-triplet interconversion. We fit the field dependence of this MFE using a unique model incorporating dipolar interactions, exchange coupling, electron transfer rates and other kinetic parameters. Using changes in AsLOV2's characteristic UV-Vis absorption lineshape, we show that this fluorescent MFE translates to changes in AsLOV2 bond formation rates with magnetic field intensity. Our observation of a covalent bond-forming MFE is the first of its kind and connects the radical pair mechanism to AsLOV2 biochemistry. The high-field emergence of a protein MFE suggests that SCRP formation and magnetic field effects on biochemistry may be much more common in organisms than previously expected.

DNP Enhanced NMR of Biomolecules at High Magnetic Field and Very Fast MAS

Sharma Kshama[1], Le Marchand Tanguy[1], Duong Tuong Nghia[1], Ollier Claire[1], Sun Zhiyu[1], Tolchard James[1], Jaudzems Kristaps[2], Ouari Olivier[3], Lesage Anne[1], Pintacuda Guido[1]

[1]Centre de RMN à Très Hauts Champs de Lyon (UMR 5082 CRMN), CNRS, ENS Lyon, UCB Lyon 1, Université de Lyon, 5 rue de la Doua, 69100
[2]Latvian Institute of Organic Synthesis, Riga, Latvia;
[3]Institut de Chimie Radicalaire (UMR 7273 ICR), CNRS, Aix-Marseille Université, Av. Esc. Normandie Niemen, 13013, Marseille, France

Dynamic Nuclear Polarization (DNP) under Magic-Angle Spinning (MAS) has emerged as a transformative approach to enhance the sensitivity of solid-state NMR spectroscopy. While it has found impactful applications in chemistry and materials science, the full potential of DNP-enhanced MAS NMR in structural biology has not been yet realized. A key challenge lies in the reduced spectral resolution under the cryogenic conditions required for the experiments, limiting a broad applicability in biological systems.

In this work, we first investigate dedicated sample preparation to optimize both spectral resolution and sensitivity. Using microcrystalline GB1 protein as a model system, we compare two preparation strategies: the soaking method, in which pre-formed microcrystals are incubated in a radical-containing solution, and the co-crystallization method, where the biradical solution is present during protein crystallization. We evaluate these two approaches at high magnetic field (18.8 T) and fast MAS rates (40 kHz), conditions under which recent studies have shown that homogeneous broadening (a primary factor limiting resolution) is significantly reduced. We further demonstrate that proton-detected solid-state NMR can be effectively combined with cryogenic DNP, offering a powerful approach to improve both resolution (through an additional chemical shift dimension) and sensitivity (by leveraging the detection of the high-γ ^{1}H nuclei). By combining extensive deuteration with selective $^{13}CHD_2$ methyl labeling, and very fast MAS using 0.7 mm rotors spinning at 60 kHz, we obtain resolved DNP enhanced two-dimensional ^{1}H-^{1}H methyl correlation spectra of GB1. These results confirm that the benefits of ^{1}H detection established at room temperature are preserved under DNP conditions.

Bimodal cholesterol for correlative in-cell DNP solid-state NMR and confocal microscopy of the plasma membrane.

Overall, **Sarah A.**, Wilson, Ancy T., Pinotsi, Dorothea, Själsjö, César, Sigurdsson, Snorri Th. and Barnes, Alexander B.

Cholesterol plays a pivotal role in defining the structure and signaling function of plasma membrane lipid rafts, yet direct nanoscale measurements of cholesterol-rich domains in intact cells that enable atomic scale analysis remains challenging. Here, we present AsymPol-Chol-AF647, a bimodal cholesterol-based probe that integrates a biradical (AsymPol) for Dynamic Nuclear Polarization (DNP) with a fluorescent dye (AF647), enabling correlative in-cell DNP solid-state NMR and confocal fluorescence microscopy of the plasma membrane. The cholesterol anchor ensures insertion into the plasma membrane, providing spatially defined delivery of the polarizing agent. Confocal imaging in live cells confirms plasma membrane localization and revealed concentration-dependent perturbations to lipid raft integrity, with optimal conditions at 0.19 nmol/10^6 cells maintaining cell viability and discrete GM1-positive microdomains (associated with lipid-rafts). Under these conditions, significant global enhancements of natural abundance ^{13}C of $\varepsilon \approx 14$ were achieved. Analysis of signal buildup kinetics unveiled evidence for more complex hyperpolarization dynamics than the simple spherical model indicated by the microscopy. These findings lay the groundwork for future studies utilizing bimodal polarizing agents for use not just for selective cellular enhancements but also as tools for the determination of the spatial organization of cellular structures at nanometer resolution.

In-cell and Hyperpolarized ^{13}C NMR for Real-Time Metabolic Flux of Bacterial Infection and Antibiotic Tolerance

Jensen, Pernille Rose, Mathiassen, Thomas B. W., Wang, Ke-Chuan, Zahid, Alexandra L.N., Meier, Sebastian.

NMR is uniquely suited to follow in-cell metabolism noninvasively. Here we combine conventional ^{13}C NMR with sensitivity-enhanced hyperpolarized ^{13}C dDNP-NMR to increase the biological relevance of adherent cell studies using hydrogels and microfluidic chips. We demonstrate these approaches across three applications: infection biology, antibiotic tolerance, and perfused chip-based metabolic monitoring.

Using in-cell ^{13}C NMR with [U-^{13}C]glucose, we profiled central carbon metabolism in *Salmonella Typhimurium* and an antibiotic tolerant strain with 25-fold higher survival under ampicillin. The tolerant strain showed reduced metabolic activity yet maintained higher glucose consumption and metabolite production under antibiotic stress. Hyperpolarized [U-^{13}C,^{2}H]glucose enhanced by dDNP-NMR resolved rapid metabolic responses within minutes of ampicillin exposure, revealing distinct carbon influx into pyruvate and acetate in tolerant versus susceptible populations.

We further used hyperpolarized 2-^{13}C-pyruvate to probe intracellular infection and monitor conversion to [1-^{13}C]acetate as a specific signature from bacteria inside HeLa cells. Hyperpolarized [1,4-$^{13}C_2$]fumarate provided a noninvasive readout of host cell integrity, enabling separation of bacterial and host metabolism.

Finally, we present strategies for keeping adherent mammalian cells in the spectrometer. Feeding hyperpolarized [1-^{13}C]pyruvate to adherent mammalian cells in hydrogels enabled longitudinal drug-response measurements. A novel microfluid chip platform combined with a custom probe was shown to support perfused HeLa monolayers and allow reproducible pyruvate-to-lactate flux quantification using hyperpolarized NMR over 48 hours, including a two-chamber design compatible with organ-on-chip models.

Wednesday | Hall A | 12:10-12:40

Malene Jensen

Evolution of intrinsically disordered scaffold proteins in cell signaling: From new binding sites to regulatory mechanisms

Delaforge Elise, Orand Thibault, Tengo Maud, Lee Alexandra, Hoffmann Guillaume, Davis Roger, Brochier-Armanet Céline, Palencia Andrés, **Jensen Malene**

Intrinsically disordered regions (IDRs) provide a versatile platform for the evolution of protein interaction networks in cell signaling. This is particularly evident in scaffold proteins, which recruit multiple kinases to facilitate efficient signal transduction. In many cases, multiple scaffold protein paralogs are retained within the same signaling pathway suggesting functional specialization. Here, we combine evolutionary analysis with solution NMR spectroscopy to uncover the molecular basis of the functional divergence between the scaffold proteins JIP1 and JIP2 in the JNK signaling pathway. We show that, although both proteins share a conserved domain architecture, JIP2 originated from a gene duplication event and subsequently acquired a 120-amino-acid insertion domain within its IDR. NMR titrations, ^{15}N $R_{1\rho}$ and CPMG relaxation dispersion experiments reveal that the JIP1 IDR binds a single JNK1 kinase, while the JIP2 IDR simultaneously engages two JNK1 molecules. Functional assays in cells further show that while the first binding site of JIP2 promotes JNK1 activation and productive signaling, the second site, acquired during evolution, sequesters JNK1 in a non-productive state, thereby tuning signaling output. These results explain why closely related scaffold proteins are evolutionarily maintained: acquisition of new kinase binding motifs within IDRs enables functional diversification without loss of ancestral binding capacity. This work highlights the power of NMR spectroscopy to resolve the structural and dynamic consequences of evolutionary changes in IDRs.

Comparison of different signal enhancement approaches for the solid-state NMR analysis of polymeric micelles

Pöppler, **Ann-Christin**; Ulrich, Kersten; Żmuda, Justyna; Fleck, Anna

During the past years, solid-state NMR has been shown to be a versatile analytical tool to study polymeric micelles and learn about key intermolecular interaction upon loading with guest molecules such as hydrophobic drug molecules. This loading-dependent structural information could also be correlated with pharmaceutically relevant properties such as cellular uptake or dissolution properties. In most studies from our group, the guest molecule was present in high concentrations, e.g. between 17 and 54 wt%.

However, we recently encountered many examples, where lower guest molecule concentrations were pursued or where the chemical entities of interest were minor compared to the bulk polymer material: low loadings with drug molecules < 10 wt.%, loading with dye molecules, multicompartment micelles, surface functionalized micelles or specific polymer micelles that form hydrogels at 1 wt.% guest molecule concentration.

To obtain structural information on these minority-species phenomena by solid-state NMR spectroscopy, we have started to test a variety of signal enhancement tools: isotope labelling, dynamic nuclear polarization (DNP) and, most recently, first photo-CIDNP experiments. In both cases, we work with physically encapsulated as well as covalently attached biradicals/dye molecules. The insights obtained through these enhancement approaches will be highlighted and the approaches compared with respect to their practicability and general applicability for polymer micelle analysis.

Steady-State Free Precession in Solids under Magic Angle Spinning: Fact of Fantasy?

Lupulescu Adonis, **Jayanthi Sundaresan**, Grinshtein Julia and Frydman Lucio*
Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot, Israel

Steady-State Free Precession (SSFP) and Magic-Angle Spinning (MAS) are two seminal NMR methods discovered in the same year. SSFP can, under suitable conditions, deliver the highest sensitivity in liquid-state NMR, while MAS is essential for achieving high resolution in solids. We and others have shown that SSFP can become a method of choice for wide-line quadrupolar NMR of static powders. This study asks whether SSFP and MAS can operate together in a single experiment. To do so we investigate the formation of steady states under SSFP's rapid pulse trains for MAS samples experiencing arbitrary anisotropic interactions. The outcome of combining these techniques proves nontrivial. When the SSFP interpulse delay is rotor-synchronized, the resulting MAS dynamics reproduce the familiar steady-state behavior observed in solution NMR. In contrast, without rotor synchronization, theory and experiment show that single crystals fail to reach a steady state and can exhibit quasi-chaotic dynamics. Remarkably, experiments and simulations indicate that powdered samples can still reach a steady state under such mis-synchronization, with a value determined by the ratio of anisotropy to spinning rate. A theoretical explanation for this is proposed, involving the formation of an almost continuous train of steady-state rotor echoes within each rotor period for certain mis-synchronized SSFP–MAS conditions. Supporting experimental evidence from single-crystal MAS experiments are also provided.

Acknowledgments: Support from the Israel Science Foundation (grant 1874/22), the Minerva Foundation, ERC Advanced Grant 101200719 "SteadyNMR", and the Perlman Family Foundation, is acknowledged. SJ acknowledges the Weizmann Institute of Science for a sabbatical fellowship.

Boosting Sensitivity of High-Dimensional Solid-State NMR of Proteins by Optimizing Coherence Transfers

Klein, Alexander; Blahut, Jan; Hyberts, Sven G.; Tošner, Zdeněk; Linser, Rasmus

Solid-state NMR has emerged as a uniquely powerful approach for the resonance assignment of large protein systems. Unfortunately, assignment presents the rate-limiting first step towards any downstream applications such as protein dynamics. While higher-dimensional (>3D) correlation spectra facilitate streamlined resonance assignments also for small proteins, the assignment of high-molecular-weight targets is vitally dependent on these to resolve severe signal overlap. Experiments with multiple indirect dimensions, however, are partially impaired by the repeated necessity of quadrature detection and the associated losses in SNR.

Here we demonstrate that the extensive incorporation of transverse-mixing optimal control pulses (TROPs) into 4D and 5D experiments enables concomitant transfer of complex magnetization along multiple successive transfer steps. Exploiting this principle across 3 – 4 indirect dimensions simultaneously, we achieve SNR gains of ~3-fold in 4Ds and 4-fold in 5D experiments, implying measurement time reductions of close to an order of magnitude compared to established CP-based 4D and 5D experiments. The approach is robust across various spectrometer setups and labelling schemes, including perdeuteration and algal-extract preparations. We further show that sensitivity enhancement carries a non-linear bonus in the context of non-uniform sampling (NUS): Artifact noise suppression during spectral reconstruction is substantially more effective at higher initial sensitivity, and noise distributions in reconstructed spectra more easily approach Gaussian behaviour. Together, these effects make high-dimensional experiments well feasible, allowing practical timeframes of acquisition for protein systems that would previously have been inaccessible.

Boosting Sensitivity of High-Dimensional Solid-State NMR of Proteins by Optimizing Coherence Transfers

Klein, Alexander; Blahut, Jan; Hyberts, Sven G.; Tošner, Zdeněk; Linser, Rasmus

Solid-state NMR has emerged as a uniquely powerful approach for the resonance assignment of large protein systems. Unfortunately, assignment presents the rate-limiting first step towards any downstream applications such as protein dynamics. While higher-dimensional (>3D) correlation spectra facilitate streamlined resonance assignments also for small proteins, the assignment of high-molecular-weight targets is vitally dependent on these to resolve severe signal overlap. Experiments with multiple indirect dimensions, however, are partially impaired by the repeated necessity of quadrature detection and the associated losses in SNR.

Here we demonstrate that the extensive incorporation of transverse-mixing optimal control pulses (TROPs) into 4D and 5D experiments enables concomitant transfer of complex magnetization along multiple successive transfer steps. Exploiting this principle across 3 – 4 indirect dimensions simultaneously, we achieve SNR gains of ~3-fold in 4Ds and 4-fold in 5D experiments, implying measurement time reductions of close to an order of magnitude compared to established CP-based 4D and 5D experiments. The approach is robust across various spectrometer setups and labelling schemes, including perdeuteration and algal-extract preparations. We further show that sensitivity enhancement carries a non-linear bonus in the context of non-uniform sampling (NUS): Artifact noise suppression during spectral reconstruction is substantially more effective at higher initial sensitivity, and noise distributions in reconstructed spectra more easily approach Gaussian behaviour. Together, these effects make high-dimensional experiments well feasible, allowing practical timeframes of acquisition for protein systems that would previously have been inaccessible.

Synergy Between Solid-State NMR and Crystal Structure Prediction for Elucidating Structure of Molecular Crystals

Dudek, Marta K.

NMR crystallography, integrating solid-state NMR spectroscopy, powder X-ray diffraction, and first-principles calculations, provides a powerful strategy for determining crystal structures when single-crystal X-ray diffraction is not feasible, for example when crystals of sufficient size cannot be obtained. Despite its potential, the approach is often limited by the complexity of solid-state NMR spectra, which are frequently crowded and difficult to assign even with advanced multidimensional experiments. As a consequence, deriving a reliable structural model directly from experimental data can be extremely challenging. Crystal structure prediction (CSP) offers a promising way to overcome this limitation. By exploring the configurational space—commonly through quasi-random sampling—CSP generates large ensembles of plausible structures that define a crystal-structure energy landscape (Fig. 1). However, the corresponding search space can be enormous, as it must consider multiple space-group symmetries, conformational flexibility, and the possibility of more than one symmetry-independent molecule in the unit cell. In this context, solid-state NMR provides experimentally derived constraints that can efficiently restrict the CSP search space and guide structure selection. This lecture will highlight several modes of synergy between solid-state NMR and CSP, illustrated by real-world examples of challenging crystal structures.

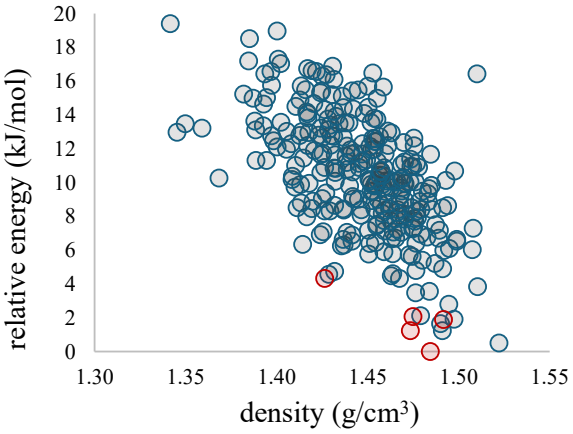


Fig. 1. Exemplary crystal structure energy landscape from CSP calculations. Each point in the plot corresponds to a different crystal structure.

This work was financially supported by the Polish National Science Centre under the 2022/46/E/ST4/00392 grant. We gratefully acknowledge Polish HPC infrastructure PLGrid (PLG/2025/018737)

ENDOR spectroscopy for distance measurements between electron and nuclear spins

Marina Bennati*

marina.bennati@mpinat.mpg.de

Max Planck Institute for Multidisciplinary Sciences and
Institute of Physical Chemistry, University of Göttingen, Göttingen, Germany

Latest developments in magnetic resonance spectroscopy are aimed at increasing sensitivity and resolution for nuclear spin detection. Paramagnetic centers are natural sources of spin polarization and selective spin probes for structural information, as recognized decades ago in the field of hyperfine spectroscopy. Particularly, electron-nuclear double resonance ENDOR can probe interactions as small as few kHz. The talk will illustrate recent progress in ENDOR polarization and coherence transfer experiments that can be used to measure intermolecular distances in biomolecules. We have recently introduced ^{19}F as a nuclear spin probe for biomolecules and demonstrated the utility in studies of enzyme mechanisms as well as the structure of nucleic acids. Studies with model systems have revealed the origin of ENDOR line broadening mechanisms, which can be circumvented by using time domain ENDOR pulse sequences. The latter are based on coherence transfer pathways and refocusing of nuclear dipolar couplings as well as CSA, giving access to hyperfine distance distributions at spectral resolutions down to 1 kHz. Information content in ^{19}F ENDOR can be complemented by broadband microwave chirp pulses, which increase resolution in orientation selection dimension. The methodology can be expanded to many other nuclei of interest, such as ^{31}P or ^{13}C.

Development of time-domain ENDOR experiments for electron-nuclear and nuclear-nuclear distance measurements

Sielaff, Lucca, Kuzin, Sergei, Kehl, Annemarie, Goldschmidt, Karoline, Aden, Anakin, Meyer, Andreas, Bennati, Marina

Measuring nuclear interactions in paramagnetic systems is essential for elucidating molecular structure and dynamics at the atomic scale. ^{19}F electron–nuclear double resonance (ENDOR) has emerged as a powerful technique for probing electron–nuclear distance distributions in the 5–20 Å range. In contrast to NMR, most ENDOR experiments are still performed in the frequency-domain, where hyperfine couplings are measured by scanning RF excitation frequencies. In this approach, the full magnetic interaction of the ^{19}F spin contributes to the spectrum, resulting in ENDOR line widths of 10–30 kHz that limit the resolution. Here, we present ENDOR experiments based on nuclear sublevel coherence spectroscopy that substantially enhance the spectral resolution. Central to this approach is the coherent time evolution and manipulation of nuclear spins, which enables the disentanglement and quantification of individual nuclear spin interactions. Using a series of rationally designed nitroxide model systems, we observe ENDOR line widths of approximately 2 kHz, corresponding to nearly an order-of-magnitude improvement in resolution. Applied to model systems containing multiple fluorine nuclei, time-domain ENDOR experiments directly report on homonuclear dipolar couplings and associated internuclear distance distributions, which were previously inaccessible with conventional frequency-domain ENDOR spectroscopy. Furthermore, similar to multiple quantum NMR, the detection of double- and multiple-quantum coherences enables correlation of hyperfine couplings. Overall, these results present time-domain ENDOR as a versatile platform for probing hyperfine and homonuclear dipolar interactions in multinuclear systems, paving the way for new structural studies using ^{19}F ENDOR.

Acquiring Focus on Paramagnetic Single-Atom Sites with Fast Magic-Angle Spinning NMR

Jaworski, Aleksander

Department of Chemistry, Stockholm University, Sweden

Email: aleksander.jaworski@su.se

Paramagnetic metal ions in proteins, catalysts, and other functional materials are essential for life and in chemistry. A detailed understanding of the local coordination environments of metal ions is often incomplete because of the limitations of available characterization techniques. We present a new approach for characterizing single-atom paramagnetic sites. It combines broadband fast magic-angle spinning (MAS) NMR data with *ab initio* computed paramagnetic NMR shifts using correlated wave functions (see *JACS* 2026, 148, 7, 6772–6778 for details). We present a challenging example of this. With Fe coordinated in a model compound, the PCN-224 porphyrin metal–organic framework (Fe@PCN-224 MOF) is used to elucidate the coordination geometry and electronic structure using ^{1}H and ^{13}C MAS NMR spectra of the ligand atoms. The computationally predicted ^{13}C NMR shifts on the paramagnetic Fe@PCN-224 MOF compare unprecedentedly well with experimental ^{13}C NMR shifts, as well as for the diamagnetic counterpart, the Fe-free PCN-224 MOF. This is despite the 25 times wider NMR shift range of 1200 ppm for the paramagnetic Fe@PCN-224 MOF. This approach is applicable to crystalline/noncrystalline materials and metalloproteins.

The use of EPR spectroscopy to study metal transcription factors *in-vitro* and *in-cell*

Sharon Ruthstein

Department of Chemistry, Faculty of Exact Sciences and the Institution of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat-Gan 5290002, Israel.

Bacteria are skilled survivors in environments with fluctuating and often toxic metal ion concentrations. While certain metals are essential for cellular function, their accumulation above threshold levels in the cytosol can be detrimental. To maintain metal homeostasis, bacteria have evolved sophisticated regulatory networks, central to which are metal-responsive transcription factors. These metalloregulators sense specific metal ions and modulate gene transcription to protect cells from metal-induced stress. Metal binding induces conformational changes in the transcription factor-DNA complex, triggering transcription of genes involved in adaptive and detoxification responses. High-resolution structural techniques such as X-ray crystallography and cryo-electron microscopy have provided valuable snapshots of metalloregulators in their metal- and DNA-bound states. However, transient and intermediate conformations that are critical for understanding the dynamic mechanisms of transcriptional regulation remain largely inaccessible to these approaches. Electron paramagnetic resonance (EPR) spectroscopy offers a powerful complementary tool to probe such states.

Our lab employs a range of spin-labeling strategies combined with continuous-wave EPR, relaxation analysis, and double electron-electron resonance (DEER) spectroscopy to investigate copper-responsive transcription factors from both Gram-positive and Gram-negative bacteria. These include *E. coli* and *P. aeruginosa* CueR [1-4], *M. tuberculosis* CsoR [5], and *S. pneumoniae CopY* [6]. By performing EPR measurements on both proteins and DNA, *in-vitro* and *in-cell*, we directly monitor metal-induced conformational changes that regulate gene transcription. Recently, we demonstrated that integrating multiple EPR modalities enables characterization of changes in protein oligomerization as a function of DNA binding [5], providing new mechanistic insight into how CsoR oligomerization contributes to transcriptional regulation.

[1] Sameach, H., Ghosh, S., Gevorkyan-Airapetov, L., Saxena, S., and Ruthstein, S. (2019) EPR Spectroscopy Detects Various Active State Conformations of the Transcriptional Regulator CueR, *Angew Chem Int Ed Engl 58*, 3053-3056.

[2] Sameach, H., Narunsky, A., Azoulay-Ginsburg, S., Gevorkyan-Aiapetov, L., Zehavi, Y., Moskovitz, Y., Juven-Gershon, T., Ben-Tal, N., and Ruthstein, S. (2017) Structural and Dynamics Characterization of the MerR Family Metalloregulator CueR in its Repression and Activation States, *Structure 25*, 988-996 e983.

[3] Yakobov, I., Mandato, A., Hofmann, L., Singewald, K., Shenberger, Y., Gevorkyan-Airapetov, L., Saxena, S., and Ruthstein, S. (2022) Allostery-driven changes in dynamics regulate the activation of bacterial copper transcription factor, *Protein Sci 31*, e4309.

[4] Yasin, A., Mandato, A., Hofmann, L., Igbaria-Jaber, Y., Shenberger, Y., Gevorkyan-Airapetov, L., Saxena, S., and Ruthstein, S. (2024) The Dynamic Plasticity of P. aeruginosa CueR Copper Transcription Factor upon Cofactor and DNA Binding, *Chembiochem 25*, e202400279.

[5] Igbaria-Jaber, Y., Mandato, A., Hofmann, L., Shenberger, Y., Gevorkyan-Airapetov, L., Sapir, L., Saxena, S., and Ruthstein, S. (2025) Tracking Copper sensing operon Repressor (CsoR) oligomerization in solution using Electron Paramagnetic Resonance spectroscopy, *Protein Sci 34*, e70303.

[6] Hirsch, M., Hofmann, L., Shenberger, Y., Gevorkyan-Airapetov, L., and Ruthstein, S. (2023) Conformations and Local Dynamics of the CopY Metal Sensor Revealed by EPR Spectroscopy, *Biochemistry-Us 62*, 797-807.

Mixture analysis using benchtop NMR spectroscopy with automated SABRE hyperpolarisation

Halse, Meghan E., Taylor, Daniel A., Hehir, Izzy, Yule, Greg, Hedges, Jonathan, Sehrawat, Neelam

Benchtop NMR provides a portable and more affordable alternative to high-field NMR, enabling new analytical applications outside of the traditional laboratory environment. Due to the moderate magnetic fields of benchtop NMR spectrometers (1 - 2.4 T) they suffer from lower sensitivity and increased peak overlap, presenting a particular challenge for the analysis of mixtures using ^{1}H benchtop NMR. Hyperpolarisation can be used to boost benchtop NMR signals by orders of magnitude, effectively decoupling sensitivity from detection field strength. Of particular interest is the *para*hydrogen-based signal amplification by reversible exchange (SABRE) method because it provides a route to renewable hyperpolarisation in seconds. Here we present an automated workflow for SABRE-enhanced benchtop NMR that can be used to generate repeatable hyperpolarisation during the recycle delay of any NMR pulse sequence, enabling multi-step experiments and signal averaging. The efficacy of automated SABRE-enhanced benchtop NMR for analysing mixtures at natural isotopic abundance is exemplified using a range of 1D and 2D ^{1}H and ^{13}C NMR experiments, including sequences that overcome peak overlap through homonuclear decoupling (e.g. pure shift NMR) or ultra-selective sequences (e.g. GEMSTONE) and 2D correlation experiments such as ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR.

In-cell and Hyperpolarized ^{13}C NMR for Real-Time Metabolic Flux of Bacterial Infection and Antibiotic Tolerance

Jensen, Pernille Rose, Mathiassen, Thomas B. W., Wang, Ke-Chuan, Zahid, Alexandra L.N., Meier, Sebastian.

NMR is uniquely suited to follow in-cell metabolism noninvasively. Here we combine conventional ^{13}C NMR with sensitivity-enhanced hyperpolarized ^{13}C dDNP-NMR to increase the biological relevance of adherent cell studies using hydrogels and microfluidic chips. We demonstrate these approaches across three applications: infection biology, antibiotic tolerance, and perfused chip-based metabolic monitoring.

Using in-cell ^{13}C NMR with [U-^{13}C]glucose, we profiled central carbon metabolism in *Salmonella Typhimurium* and an antibiotic tolerant strain with 25-fold higher survival under ampicillin. The tolerant strain showed reduced metabolic activity yet maintained higher glucose consumption and metabolite production under antibiotic stress. Hyperpolarized [U-^{13}C,^{2}H]glucose enhanced by dDNP-NMR resolved rapid metabolic responses within minutes of ampicillin exposure, revealing distinct carbon influx into pyruvate and acetate in tolerant versus susceptible populations.

We further used hyperpolarized 2-^{13}C-pyruvate to probe intracellular infection and monitor conversion to [1-^{13}C]acetate as a specific signature from bacteria inside HeLa cells. Hyperpolarized [1,4-$^{13}C_2$]fumarate provided a noninvasive readout of host cell integrity, enabling separation of bacterial and host metabolism.

Finally, we present strategies for keeping adherent mammalian cells in the spectrometer. Feeding hyperpolarized [1-^{13}C]pyruvate to adherent mammalian cells in hydrogels enabled longitudinal drug-response measurements. A novel microfluid chip platform combined with a custom probe was shown to support perfused HeLa monolayers and allow reproducible pyruvate-to-lactate flux quantification using hyperpolarized NMR over 48 hours, including a two-chamber design compatible with organ-on-chip models.

Hyperpolarization 2

Wednesday | Hall A | 16:00-16:40

Pernille Rose Jensen

Dissolution DNP of Biomolecules without Resolution Penalty - from IDPs to Condensates and Nucleic Acids

Dennis Kurzbach, Ertan Turhan, Milan Zachrdla

Hyperpolarization by dissolution dynamic nuclear polarization (DDNP) offers orders-of-magnitude signal enhancement, promising to overcome long-standing sensitivity bottlenecks. However, like most hyperpolarization methods, DDNP remains restricted in biomolecular applications due to spectral distortion and limited compatibility with large biomolecular systems.

Here, we demonstrate that this bottleneck can be overcome by combining hyperpolarized water (HyperW) DDNP with tailored instrumentation and machine-learning-assisted deconvolution. This strategy enables multidimensional hyperpolarized spectra whose line shapes are indistinguishable from those of their high-field thermal-equilibrium counterparts. In other words, hyperpolarization no longer imposes a severe trade-off between sensitivity and resolution.

We demonstrate the prowess of our strategy using three cutting-edge research questions: (i) biomineralization systems[1], (ii) condensed peptide phases[2], and (iii) kinetic partitioning in nucleic acids[3]:

(i) Biomineralizing intrinsically disordered proteins can be captured in action, i.e., with 2D spectra recorded within 10 s.
(ii) Surface-selective hyperpolarized NMR, selectively enhancing solvent-accessible residues in megadalton-scale peptide condensates.
(iii) We could boost the sensitivity of a wide array of polymorphic nucleic acids and directly observe low-populated folding intermediates.

In all three cases, DDNP provides residue-specific information that is inaccessible by conventional methods and lies at the heart of NMR structure determination.

1. Turhan, E.; Potzl, C.; Brandis, D.; Faglia, F.; Zachrdla, M.; Kurzbach, D., *Chemistry* **2025**, e02067. 10.1002/chem.202502067
2. Brandis, D.; Turhan, E.; Zachrdla, M.; Kurzbach, D., *J Am Chem Soc* **2025,** *147* (40), 36920-36927. 10.1021/jacs.5c15053
3. Zachrdla, M.; Turhan, E.; Buckova, M.; Hansel-Hertsch, R.; Trantirek, L.; Kurzbach, D., *J Am Chem Soc* **2025,** *147* (50), 46563-46572. 10.1021/jacs.5c17542

Revealing photochemically-driven self- and dis-assembly processes via MAS NMR combined with *in-situ* irradiation.

Van der Wel, Patrick; Lasorsa, Alessia; Van der Meulen, Pieter; Naumann, Ernst; Lerch, Michael; Marquez Garcia, Maria; Lan, Xiaohong; Loos, Katja; Feringa, Ben; Szymanski, Wiktor

Light is often the stimulus of choice for dynamically changing structure and function in materials and other contexts: in smart materials and photovoltaics, but also photochemical, biological and biochemical research (e.g., optogenetics). Proper understanding of the associated mechanisms requires insights into the underlying dynamic molecular and (photo)chemical transformations. NMR is a powerful tool for investigating these types of processes, providing unique chemical, structural, and dynamic information at the atomic level. Yet, while liquid-state NMR is increasingly regularly coupled with *in-situ* irradiation, it is limited to soluble samples. Here we report on the combination of *in-situ* illumination with magic angle spinning (MAS) solid-state NMR (ssNMR). I will describe our LED-based *in-situ* irradiation setup adapted to a customized Bruker MAS probe. Measurements on actinometer compounds allowed for quantification of sample illumination inside the sample, showing good efficiency in not only sapphire but also zirconia rotors. Using this setup we have characterized a variety of light-sensitive soft materials, probing self- and disassembly processes using dynamic spectral editing techniques. Different ssNMR polarization transfer techniques enable the real-time study of dynamic transitions that accompany light-triggered (dis)assembly and cross-linking processes. Tailored combinations of real-time 1D spectroscopy with targeted 2D NMR analysis allowed for dissection of chemical, structural or dynamic changes in diverse molecular systems (with and without isotope labeling). I will discuss perspectives on opportunities and limitations for applying these approaches to diverse research areas in photochemistry and photoresponsive materials research, and beyond.

Hyperpolarized ^{129}Xe NMR and MRI for the Elucidation of Structure, Function, and Dynamics in Industrial Materials.

Max Filkins[a,b], Stefano Collins[a,b], Arthur Harrison[a,c], Galina E. Pavlovskaya[a], Sean P. Rigby[b], and Thomas Meersmann*[a,c]

[a]Sir Peter Mansfield Imaging Centre, University of Nottingham, NG7 2RD, UK
[b]Department of Chemical and Environmental Engineering, University of Nottingham, NG7 2RD, UK
[c]Department for Strategic Development of Health Science and Technology, University of Nottingham, Ningbo, 315100, PR China

Hyperpolarized noble gas MRI, traditionally used in clinical studies, also offers a powerful but underexplored tool for materials science, chemical engineering and industrial applications. As a clinical tool, ^{129}Xe MRI has received a great deal of attention for pulmonary studies where it provides unique insights into the gas exchange dynamics and perfusion. On the other hand, hp ^{129}Xe NMR spectroscopy has been utilized for decades in material sciences and biochemical research, for example in work with specially designed biosensor molecules. The value of synergy between clinical and materials science applications is exemplified by the development of a new dissolution phase ^{129}Xe MRI lung phantom standard that emulates the hp^{129}Xe MR spectral profile and gas uptake dynamics in human lungs in health and disease.

Utilizing clinical hp ^{129}Xe MRI protocols and concepts, gas transport and reactive zones in diesel catalyst converters, that consist of hierarchical porous solids with ordered and disordered levels of hierarchy, can be explored. The methodology enables imaging of powder coating applied to enhance the converter performance, a coating that is invisible to current non-invasive methodology, such as micro CT. The gas flow, gas uptake and gas exchange in the porous phase of converters have been studied to provide the manufacturer with performance insights. Accessibility of the catalytic active sides can also be explored through changes in the ^{129}Xe relaxivity that indicates reactivity of the surface. A further application is a study of the dynamics of surface adsorbed water as it progresses into mesoporous alumina catalyst support pellets. The data that can be gained specifically through hp ^{129}Xe NMR and MRI, also shows water loss during this process.

Mapping metabolic aging and disease-associated acceleration using an interpretable NMR-based clock

Oscar Millet

CIC bioGUNE, Parque Tecnológico de Bizkaia, Bld. 800, 48160, Derio, Spain

Biological age captures cumulative physiological decline but remains challenging to quantify across the full adult lifespan with mechanistic and clinical interpretability. Here we develop an interpretable metabolic aging clock based on high-throughput serum nuclear magnetic resonance (NMR) profiling in 29,390 individuals aged 7–106 years across multiple cohorts. Using a machine-learning framework that integrates quantified metabolites and NMR-inferred clinical biomarkers, we achieve accurate age prediction ($r = 0.88$; RMSE ≈ 8.7 years) while resolving the multicollinearity that limits interpretability in spectral models. Feature attribution reveals a consistent biological signature dominated by chronic inflammation, kidney function, and energy metabolism, with albumin and erythrocyte sedimentation rate emerging as principal determinants of metabolic aging. Application to eleven pathogenic cohorts (analyzing serum from 3,882 additional patient donors that represent distinctive and most frequent causes of disease), shows that metabolic age acceleration reflects disease-specific distortions rather than uniform aging shifts, where acute inflammatory states, metabolic dysfunction, and organ-specific pathologies exhibit distinct signatures. In a prospective cardiovascular cohort, metabolic age acceleration precedes clinical events, highlighting its potential for early risk stratification. Together, these results establish an interpretable, scalable metabolic clock that links systemic biochemical states to aging trajectories and disease risk and provide a framework for precision medicine applications.

Phenotype classification of intact cells by NMR spectroscopy through machine learning approaches

Luchinat, **Enrico**, Mengucci, Carlo, Dell'Amico, Claudia, Del Giudice, Simona, Barbieri, Letizia, Mariottini, Alice, Onorati, Marco, Massacesi, Luca, Banci Lucia

NMR spectroscopy is a powerful non-invasive tool to analyze complex biological samples. *In vitro*, high resolution 1D NMR spectra of biofluids and cell extracts make possible to classify biological samples based on their metabolic fingerprint. However, such analysis is currently not possible with live cells or tissues, nor by spectroscopic imaging *in vivo*, due to the line broadening arising from the intrinsic inhomogeneity of such samples causing severe signal overlap. Here we show that machine learning approaches applied to poorly-resolved NMR spectra of live intact cells recorded at high fields allow classifying different physio-pathogenically relevant cell types cultured *in vitro*. We demonstrate the successful classification of neural progenitor cells, neurons and astrocytes, as well as classification of mixed cell type samples, and show that a classifier trained on high-field NMR spectra can discriminate cells analyzed at lower fields, approaching those of current MRI instruments. In the future, this approach could be further developed for MRSI data analysis applications, potentially offering a non-invasive diagnostic tool for lesions of the central nervous system and reducing the need for biopsies.

A Data-driven Approach Merging Untargeted and Targeted NMR Metabolomics Analysis

Klein, Matthias S; Saini, Anmol; Chen, Hongjin; Morcos, Keira; DeCloet-Mastrandrea, Kiano

Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, Québec, Canada. Correspondence: matthias.klein@mcgill.ca

Metabolomics, the comprehensive analysis of small molecule metabolites in living systems, can be performed by NMR either in a **targeted** way, allowing for quantitation of a predefined metabolite list, or **untargeted**, utilizing whole spectra to detect features of interest. Untargeted NMR allows for novel discovery by detecting unexpected features, however, metabolite identification is a time-limiting step and often requires excessive researcher time and expertise.

To address this issue, we aimed at developing a new data analysis pipeline, merging targeted and untargeted analysis. As most metabolites exhibit multiple detectable signals, the new algorithm analyzes correlations between expected peak regions to identify candidate metabolites. The algorithm then evaluates strength of correlation, number and ratio of correlated peaks, and expected metabolite concentrations to assign metabolite identities. Of note, the algorithm does not perform peak picking or peak shape analysis to avoid problems caused by peak overlap, a frequent issue in 1D ^{1}H spectra. Public databases were screened to create ranked lists of consensus metabolites for common sample matrices and restricted to metabolites expected to be NMR-visible. Spectral peak information was extracted from public databases and manually optimized.

The pipeline was implemented in R and tested on 1D ^{1}H NOESY and 2D ^{1}H-^{13}C HSQC spectra. Preliminary data show that the algorithm consistently identified over 70 metabolites with high confidence in serum, urine and milk. Results were in high agreement to orthogonal quantitative analyses. This highlights the novel approach's potential for deeper metabolic insights by reliable metabolite identification, and by freeing researcher time for identification of remaining unknown signals.

Using NMR and NMR-based Metabolomics to Study the Role of Carbasugars in Plant–Insect Interactions

Gabriel Anderson, Camila Bueno, Sol Tenca, Andrés López, Florencia Parpal, and Guillermo Moyna*

Laboratorio de Química Orgánica, Departamento de Química del Litoral, CENUR Litoral Norte, Universidad de la República, Paysandú 60000, Uruguay

Abstract

Soybean (*Glycine max*) is the most extensively cultivated summer crop in Uruguay, with the soybean looper (*Rachiplusia nu*) identified as a principal pest due to the substantial defoliation caused by its larvae. Conventional pest management practices depend on pesticides, which are linked to several environmental and agronomic disadvantages. As a result, efforts have increasingly focused on developing alternative strategies that reduce reliance on pesticide application. Priming, an induced defence mechanism that places plants in a heightened state of alert following exposure to environmental elicitors, is one of the strategies that can be incorporated into integrated pest management (IPM) for this purpose. In previous work we were able to establish that moderate herbivory by *R. nu* leads to increases in the level of pinitol in *G. max* leaves, and that the levels of this carbasugar in primed plants remained high even after recovery. In turn, these higher levels of pinitol correlate with lower levels of putrescine, a morphogenic hormone with an important role in insect development, in hemolymph of *R. nu* larvae. In this work we show that mechanical defoliation also causes changes in the profiles of carbasugars of *G. max* which could alter plant-insect interactions. However, NMR-based metabolomic analyses, selective ^{1}H NMR experiments, including 1D-NOESY and 1D-TOCSY, and ^{1}H-^{13}C correlation experiments carried out on different *G. max* leaf extracts show that mecanically damaged plants have comparatively higher levels of ononitol and/or squoyitol, two carbasugars that are intermediates in the biosynthesis of pinitol. These results indicate that despite mechanical damage leads to an increase in the levels of carbasugar in *G. max* leaves, herbivory pushes the biosynthetic route towards the production of pinitol. While preliminary, our findings help better understand induced plant defenses in the *G. max* – *R. nu* system and could be instrumental in the development of novel IPM strategies.

Tone Bathen

Despite advances in early detection and therapeutic strategies, breast cancer remains a leading cause of cancer-related death among women worldwide. Similarly, prostate cancer is one of the most prevalent malignancies among men and exhibits substantial clinical and biological heterogeneity. In both diseases, patients with similar clinical diagnoses may experience markedly different prognoses and responses to treatment, reflecting the complexity of tumour biology.

Metabolomics, the branch of “omics” technologies focused on the comprehensive identification and quantification of small-molecule metabolites, provides a powerful approach to characterizing these differences. Cancer cells must reprogram central metabolic pathways to sustain proliferation, enabling the conversion of nutrients into biomass while maintaining energy production. This metabolic reprogramming is recognized not only as a potential therapeutic target, but also as a rich source of biomarkers for risk stratification, prognosis, and treatment monitoring.

Importantly, metabolomic profiling can be performed on both intact tissue and biofluids, providing complementary insights. Analyses of intact tumour tissue offer a direct and spatially relevant view of cancer cell metabolism, capturing tumour-specific biochemical alterations such as changes in lipid synthesis and choline metabolism. In contrast, biofluid-based metabolomics (e.g., blood or urine) reflects a more systemic and holistic snapshot, integrating signals from the tumour, host metabolism, and microenvironment, which can be advantageous for non-invasive biomarker discovery and longitudinal monitoring in patients.

This talk will mainly focus on NMR-based metabolomics research in breast and prostate cancer, spanning applications from risk assessment and tumour characterization to treatment monitoring and prediction of clinical outcomes.

Optically induced spin polarization in open-shell molecular systems at variable magnetic fields

Yash Patel, Philip Weiss, Ilya Dergachev, Trent McHenry, Claudia E. Avalos

Molecular Design Institute, Department of Chemistry, New York University, New York, NY 10003

The ability to optically initialize spin states has applications in sensitive magnetic field detection as well as magnetic resonance signal enhancement. Photoexcited triplet states in organic chromophores or defects in solids can be combined with microwave irradiation to significantly enhance NMR signals. Alternatively, in the absence of microwaves, the magnetic field can be tuned to specific matching conditions to allow for all optical nuclear spin polarization. One may also take advantage of nuclear spin-dependent relaxation pathways which are present in select molecular systems (Chemically induced dynamic nuclear polarization, or CIDNP). What all of these approaches have in common is that the electronic transition rates often determine the ultimate 'cooling power'. How polarized, or how intializable are these systems under the best conditions? What are the molecular motifs that would maximize the ground-state spin polarization in a subset of these systems and within a reasonable amount of time? In this presentation I will provide a broad overview of different classes of optically pumped molecular systems (their pros and cons), their dependence on magnetic field and I will discuss our recent efforts to determine which combination of molecular motifs lead to desirable electronic and magnetic coupling parameters to maximize multiplet and doublet electron spin polarization in chromophore-radical systems. We combine electronic structure calculations with experimental datasets and state-mixing dependent kinetic models to predict expected spin polarization in the excited and ground-state spin manifolds as a function of magnetic field.

Improving the field homogeneity of cryogen-free superconducting magnets by removing induced eddy currents

E. Kryukov, A. Karabanov and J. Good

Cryogenic Ltd. London, UK

It is well-known that, during the field ramp, screening eddy currents are induced on the surfaces of superconducting filaments. These currents can decay very slowly and cause an undesirable extra inhomogeneity of the magnetic field created by a superconducting magnet.

Although using special twisted high-quality wire with fine filaments significantly resolves this problem, this makes the wire more expensive, and efficient ways of removing the eddy currents from the standard superconducting wire are still necessary.

In our talk, we will discuss the theoretical background of the eddy currents phenomenon in Type-II superconductors, relying on the theory of flux pinning and the critical state model. We will report experimental evidence of the effect of the induced eddy currents on the field homogeneity of homemade cryogen-free superconducting magnets wound with the typical NbTi wire. Finally, we will describe an efficient way to remove this effect, based on careful heating the magnet coils near the critical state between the superconductivity and the normal resistive behaviour. Some of our results are illustrated in Fig.1

We propose a new technique to remove the eddy current quickly to restore the magnet homogeneity to its designed value. This is especially important in multi-field NMR and other experiments, where frequent changes of the magnetic field are necessary

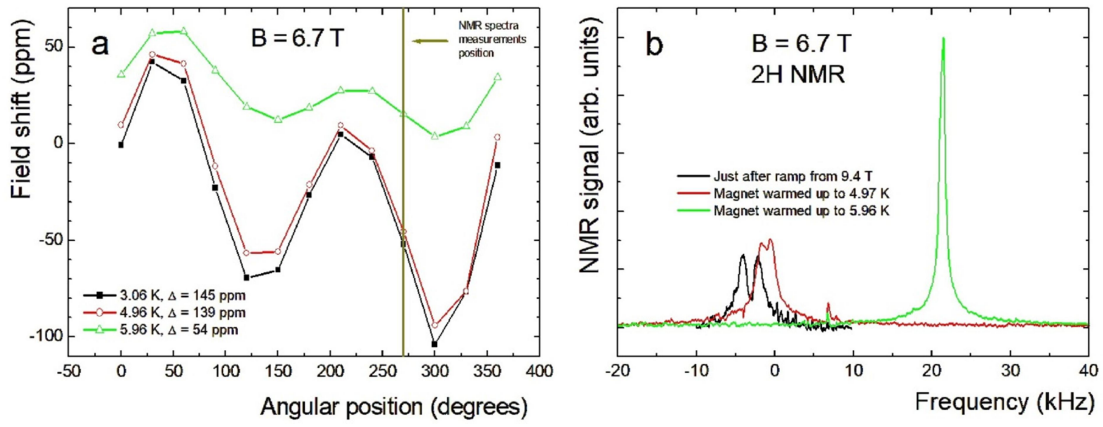


Fig.1. Removing the eddy currents as a source of magnetic field inhomogeneity, by heating the magnet near the critical state: a) magnetic field profiles at various temperatures, b) 2H NMR spectra under the same conditions.

Operando heterogeneous photocatalysis inside a light-irradiated MAS NMR rotor

Dorothea Wisser*, *Fei Ding, Karl König, Johannes Böhmer, Florian M. Wisser, Erdmann Spiecker, Benjamin Apeleo Zubiri*

Heterogeneous photocatalytic reactions are little explored in solid-state NMR due to the difficulty to achieve efficient light penetration in MAS rotors. Our group operates a unique, prototype MAS probe, suitable for 3.2 mm sapphire rotors, which are irradiated by four exchangeable LEDs. Using this probe, we present the first operando observation of photoreforming of methanol to formaldehyde in a transparent sapphire rotor, irradiated at 365 nm, at spinning rates up to 11.5 kHz.

Efficient light penetration into the entire rotor volume is crucial. Therefore, we introduce as catalyst support meso- and macroporous silica monoliths, shaped to perfectly fit the rotor. They exhibit a continuous network of macropores larger than the visible light, which shall support light incoupling into the bulk material. The monoliths provide a large pore volume to accommodate the liquid substrate and a high surface area. They are coated with particles or a continuous layer of TiO_2 (anatase) as supported heterogeneous catalyst, using a new technique, atomic layer deposition in solution.

Conversion of about 50 % of the methanol are achieved during approx. 90 h of catalysis. ^{13}C CP MAS NMR spectra show different distributions of surface-bound intermediates depending on the TiO_2 content. The improved penetration of light into the material is quantified by calculation of the photon flux inside the rotor.

Silica monoliths may be easily decorated with various photocatalysts. The combination of this new probe type and our optimized support material thus provides a versatile design principle for a newly emerging field, the online observation of photocatalytic reactions by solid-state NMR.[1]

[1]F. Ding, K. König, J. Böhmer, F. M. Wisser, E. Spiecker, B. Apeleo Zubiri, D. Wisser *Chem. Eur. J.* **2026**, *32:e03583*, DOI: https://doi.org/10.1002/chem.202503583.

Optically Addressable Spins in Proteins

Kun Meng, Linyan Nie, Johannes Berger, Nick R. von Grafenstein, Christopher Einholz, Roberto Rizzato, Erik Schleicher, **Dominik B. Bucher**

Optically addressable spins in solid-state materials have attracted significant interest for their potential in quantum sensing technologies. In my talk, I will highlight our latest research on optically addressable spins in proteins—specifically flavin-based proteins (*cryptochrome* and *iLOV*). Upon optical excitation, these proteins generate radical pairs that can be read out via photoluminescence and manipulated through radio-wave–controlled spin chemistry. This opens a new era of tunable and evolvable spin-quantum systems for optically detected magnetic resonance. I will explain how these systems differ from the well-established nitrogen-vacancy center in diamond and discuss their potential as genetically encoded quantum sensors for applications in imaging and spin chemistry.

Single-Cell Identification with Quantum-Enhanced Nuclear Magnetic Resonance

Author: Qian Shi

Co-Author: Zhiyuan Zhao

Identification of specific cells within heterogeneous populations is central to biomedical research and clinical diagnostics, yet current sorting strategies largely rely on external labels and predefined molecular markers. This field urgently requires label-free and non-destructive characterization methods. However, conventional nuclear magnetic resonance (NMR) is limited by sample volume and sensitivity and struggles to access the fL–pL regimes characteristic of single mammalian cells.

Here we present a single-cell identification platform based on quantum-enhanced nuclear magnetic resonance (NMR) using diamond nitrogen-vacancy (NV) centers, in which an array of pillars is etched on an ultrathin diamond substrate and high-fidelity spin initialization, control and readout are combined with ENDOR pulse sequences to extract weak nuclear magnetic signals. We demonstrate label-free detection of single-cell NMR signals and, by measuring differences in physical parameters between HeLa and MCF7 cells, show that the platform can resolve single-cell heterogeneity.

Our work establishes NV-enhanced single-cell NMR as a physics-based modality for cell identification and opens the way to multi-parametric, label-free phenotyping of heterogeneous cell populations and quantitative evaluation of therapeutic response at the single-cell level.

Determining the Surface and Subsurface Structure of Heterogeneous Catalysts using DNP-enhanced NMR Spectroscopy

Perras, **Frédéric A.**, Carl F. Fleischer III, Tommy Yunpu Zhao, Scott A. Southern, Guillaume P. Laurent

Understanding chemical structure forms the basis of modern chemistry and materials science. It has revolutionized chemical synthesis and biochemistry, however, significant challenges remain in the characterization of certain materials for which surface structure is often more important than bulk structure. Dynamic nuclear polarization (DNP) surface-enhanced solid-state NMR spectroscopy is unique in its potential to reveal rich structural details from externally facing interfaces. Care nevertheless needs to be taken in the design of the NMR experiments used to probe these surfaces.

In this presentation, I will describe recent efforts from Ames National Laboratory in leveraging internuclear distance measurements on oxide material surfaces of relevance to heterogeneous catalysis to reveal high-resolution surface site structures in addition to subsurface structure. Specifically, I will describe the use of REDOR and TEDOR-like experiments involving well-defined spin pairs, or groups of spins, to determine the structural configuration and conformation of single-site supported metal catalysts. Examples will be given for which high-resolution or orientational structural information revealed the origins of enhanced activity or enantioselectivity that would've been challenging to assess otherwise. The later half of the talk will discuss the surface reconstructions that occur in perovskite oxide materials, such as $SrTiO_3$, $LaScO_3$, and $LaAlO_3$, and how NMR can be used to help determine the preferred reconstructions. REDOR experiments are used to peer into the material from the interface to effectively count the number of surface layers that involve a given metal before the bulk crystallographic structure is established.

Debunking the Myths of ^{13}C MAS NMR of Cellulosic Materials

Brown, **Steven P.**, Cresswell, Rosalie, Kumar Deralia, Parveen, Yoshimi, Yoshihisa, Kuga, Tomohiro, Echevarría-Poza, Alberto, Franks, W. Trent, Dupree, Ray, Dupree, Paul

2026 marks the 50th anniversary of the first CP MAS publication, which included a spectrum of ebony wood. Already in the 1980s, it was identified that two sets of signals are observed for the C4 and C6 glucose resonances in cellulose in different algal, bacterial, and plant samples. For ^{13}C-labelled samples, the refocused INADEQUATE experiment is invaluable for tracing out the ^{13}C resonances in the sugar molecules of the different biopolymers, as exemplified by application to wood chips in the seminal 1999 publication by Lesage, Bardet and Emsley. Recently, we have presented (*JACS* 2025, *147*, 47223) refocused INADEQUATE and PDSD 2D MAS 13C NMR spectra of xylanase-treated holocellulose nanofibrils (hCNFs) from poplar wood that maintain the native plant cellulose secondary cell wall structure. These spectra reveal six major glucose species, three each in the two domains corresponding to the separated C4 and C6 resonances, that, building on Wang et al's publication for primary cell walls (*Biomacromolecules* 2016, *17*, 2210), are labelled a, b and c (domain 1) and f, g and j (domain 2). The ^{13}C chemical shifts for a and c match exactly those for crystalline Iβ (from tunicate), showing that these plant microfibrils have an exclusively Iβ structure, disproving a considerable literature that has hypothesised the additional presence of Iα. Moreover, the linewidths for the domain 1 and 2 peaks are similar disproving the widespread belief that domain 2 corresponds to amorphous cellulose and the interpretation of the relative intensity as a crystallinity index. Water-edited spectra show that peak b is close to water. Re-interpreting this as a surface residue together with the domain 2 peaks give an interior: surface glucose residue ratio of 1:2 consistent with an 18-chain microfibril that current understanding of the biosynthesis suggests to be likely.

Unraveling Calcium Silicate Hydrate Local Structure and Symmetry Using Eu^{3+} Probe

Rao, **Nikita**, Tyagi, Astha, Hmar, Lalrempuia, Shivakumara, C., Jain, Sheetal

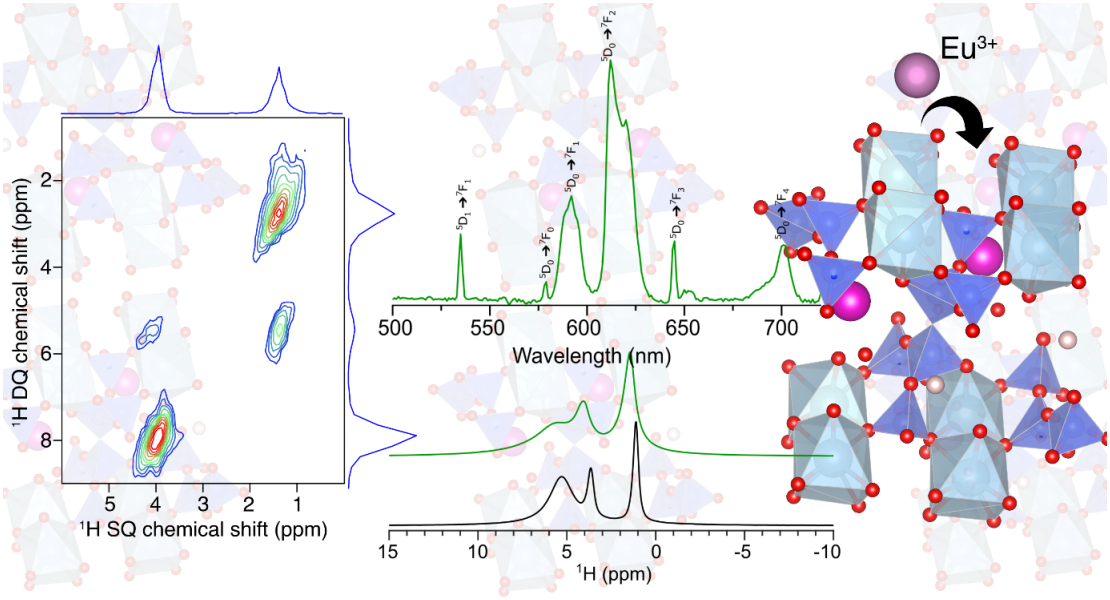
Elucidating the local structures in calcium silicate hydrate (C–S–H) phases and tracking their evolution under varying environmental and thermochemical conditions is crucial for developing alternative sustainable construction materials. However, the inherent nanoscale disorder in these materials limits precise structural characterization.

We combine solid-state NMR and Photoluminescence (PL) spectroscopy, utilizing Trivalent europium (Eu^{3+}) as a dual-functional probe. Eu^{3+} acts as a sensitive spectroscopic probe and structural proxy for Ca^{2+} due to its similar ionic radius. The intensity ratio of the electric dipole transition $^5D_0 \rightarrow {}^7F_2$ to the magnetic dipole transition $^5D_0 \rightarrow {}^7F_1$ in Eu^{3+} is sensitive to the symmetry and order in the surrounding environment. A high ratio confirmed Eu^{3+} incorporation into low-symmetry sites, indicative of disorder in the C-S-H phases. We mapped local chemical environments and nanophases using ^1H and ^{29}Si solid-state NMR on systematically Eu^{3+} - doped C-S-H gels (0–9 mol%). A quantitative analysis of Si-OH, Ca-OH, and adsorbed water was completed using ^1H NMR, while ^1H T_2 relaxation revealed the sites located in proximity to Eu through paramagnetic relaxation enhancement (PRE). The relative positions and clustering of various sites were probed using ^1H DQ-SQ NMR. ^{29}Si and ^1H-^{29}Si CPMAS NMR characterized Q^1 and Q^2 species, providing insight into silicate framework chain lengths.

Thus, by combining PL and solid-state NMR, we have unraveled the symmetry around Eu^{3+} (hence Ca^{2+}) sites, identified their framework connectivity, and local structural nanophases in C-S-H. We have shown that doping with ions like Eu^{3+} enables use of PL and paramagnetic solid-state NMR to gain key insights into disordered systems. Beyond this work, this study is vital for understanding the nuclear waste immobilization mechanism of radionuclides by the cementitious materials.

Thursday I Hall A I 11:30-11:50 Nikita Rao

Graphical Abstract



Coordination environments of single Pt atoms from solid-state NMR

Koppe, **Jonas**, Yakimov, Alexander V., Gioffrè, Domenico, Usteri, Marc-Eduard, Beshara, Gian-Marco, Anferov, Sophie W., Johansen, Christian M., Vosegaard, Thomas, Pintacuda, Guido, Lesage, Anne, Pell, Andrew J., Mitchell, Sharon, Pérez-Ramírez, Javier, Copéret, Christophe

Single-atom catalysts (SACs) are a relatively recent class of heterogeneous catalysts, with isolated metal centers stabilized on the surface of a suitable support material. While synthesis and performance evaluation of SACs have become routine, experimental elucidation of metal site structures remains notoriously challenging. Recently, we have used ^{195}Pt NMR to directly probe the local Pt site structure of single Pt atoms dispersed on nitrogen-doped carbon supports at loadings as low as 1 wt%.

We employ ultra-wideline NMR spectroscopy using advanced frequency-swept pulse schemes and multiple-echo acquisition to obtain ^{195}Pt NMR spectra under static and MAS conditions, which reveal a distribution of Pt surface sites. By combining a tailored numerical model with Monte Carlo simulations, we are able to convert the ^{195}Pt NMR spectra into two-dimensional distribution maps that describe Pt-site heterogeneity in terms of local coordination chemistry and bonding geometry. This approach enables us to monitor how synthetic parameters influence the final SAC structure, and how Pt coordination environments evolve under reaction conditions.

We are currently expanding our analysis to Pt single atoms stabilized on CeO_2, another industrially relevant SAC system. Pt on CeO_2 adds intrinsic complexity to the analysis of the Pt site structure, as Pt is widely assumed to exist in multiple oxidation states, particularly Pt(IV), in addition to Pt(II). Resolving the overlapping ^{195}Pt NMR signatures associated with different Pt oxidation states and coordination environments constitutes a major challenge and has so far precluded determination of the identity, distribution, and interconversion of these Pt sites at the atomic level.

This work is supported by the Alexander von Humboldt Foundation.

^{17}O ssNMR: an opportunity for understanding and improving carbon capture materials?

Laurencin, Danielle; Fabregue, Nicolas; Niasse, Yaye; Noël, Philippe; Peach, Austin; Métro, Thomas-Xavier; Leroy, César; Gajan, David; Lemarchand, Tanguy; Duong, Nghia; Florian, Pierre; Trébosc, Julien; Chanal, Trevys; Azaïs, Thierry; Pourpoint, Frédérique; Gervais, Christel.

With temperatures rising around the planet, and climate disasters increasing in frequency, scientists have been devoting an increasing amount of time to improve our understanding of carbon capture processes, and reduce CO_2 emissions. In this context, it has become critical to develop tools enabling to characterize in detail the local environment and reactivity of CO_2 within a variety of materials.

Long-considered as a niche analytical technique, oxygen-17 ssNMR spectroscopy has recently re-emerged as a valuable spectroscopy, including in the context of CO_2-capture. In this presentation, we will focus on some of our recent developments in this field, at the interface of *(i)* selective isotopic labeling procedures, *(ii)* advanced high resolution ^{17}O MAS ssNMR analyses, including at ultra-high magnetic fields (up to 28.2 T), and *(iii)* computational modeling. Specific examples related to CO_2 physisorption and chemisorption in metal organic frameworks will be shown.

Configurational and conformational analysis of flexible natural products and macrocycles based on anisotropic NMR spectroscopy

Anton Florian Ketzel[1,2], Caspar Jonas Schattenberg[1], Han Sun[1,2]

[1]Leibniz Institute for Molecular Pharmacology, 13156 Berlin, Germany
[2]Technical University of Berlin, 10623 Berlin, Germany

Configurational and conformational analysis of drug-like small molecules and macrocyclic peptides is essential for understanding their biological activity and cellular uptake properties. However, experimental methods that enable precise determination of the stereochemistry and conformational ensembles of these molecular systems remain limited.

In this talk, I will introduce experimental approaches that enable the straightforward extraction of anisotropic NMR parameters, including residual dipolar couplings (RDCs) and residual chemical shift anisotropies (RCSAs), in both aqueous and organic solvents using oligopeptide-based liquid crystalline phases [1,2]. Building on these developments, I will present several examples in which the combination of anisotropic NMR spectroscopy and advanced computational methods enabled the successful determination of the configuration and conformational landscapes of flexible natural products [3-5]. For macrocyclic peptides, I will demonstrate examples where anisotropic NMR-based approaches allow conformational analysis in both fast- and slow-exchange regimes on the NMR timescale, thereby significantly extending the structural insights obtainable from conventional NMR methods based on Nuclear Overhauser Effects (NOEs). In particular, these approaches provide a more faithful representation of solution-state conformational ensembles and molecular dynamics. Finally, I will discuss our recent efforts to determine the atomic structures of oligopeptide-based liquid crystalline phases using cryo-electron microscopy, providing new insights into the molecular organization of these alignment media and the underlying mechanisms governing analyte alignment and enantiodiscriminiation.

References:

[1] Li, X.-L.; Chi, L.-P.; Navarro-Vázquez, A.; Hwang, S.; Schmieder, P.; Li, X.-M.; Li, X.; Yang, S.-Q.; Lei, X.; Wang, B.-G.; Sun, H. *J. Am. Chem. Soc.* **2020**, *142* (5), 2301–2309.
[2] Lin, Y.; Li, J.; Qin, S.-Y.; Sun, H.; Yang, Y.-L.; Navarro-Vázquez, A.; Lei, X. *Chem. Sci.* **2022**, *13* (20), 5838-5845.
[3] Doro-Goldsmith, E.; Song, Q.; Li, X.-L.; Li, X.-M.; Hu, X.-Y.; Li, H.-L.; Liu, H.-R.; Wang, B.-G.; Sun, H. *J. Nat. Prod.* **2024**, *87* (2), 381–387.
[4] Chi, L.-P.; Li, X.-L.; Ketzel, A. F.; Navarro-Vázquez, A.; Schattenberg, C. J.; Li, X.-M.; Li, X.; Sun, H.; Wang, B.-G.; *Mar. Life Sci Technol*, **2025**.
[5] Ketzel, A. F.; Hu, Y.; Li, X. L.; Li, J.; Lei, X.; Sun, H. *Magn. Reson. Chem.* **2025,** 63(5-6), 417-423.

Structural insights into the chameleonic behaviour of PROTACs

Wieske, **Lianne**,[1,2] Esubalew Abeje, Yordanos,[1] Salerno, Alessandra,[2] Kihlberg, Jan,[1] Ciulli, Alessio[2]

[1]Department of Chemistry for Life Sciences, Uppsala University, Sweden

[2]Centre for Targeted Protein Degradation, School of Life Sciences, University of Dundee, U.K.

A Proteolysis Targeting Chimera (PROTAC) is a drug modality leading to the degradation of a protein of interest (POI) rather than inhibition. PROTACs consist of two pharmacophores with affinity for the protein of interest (POI) and an E3 ligase connected by a linker, resulting in relatively large compounds. Despite their size, many have shown passive cell permeability.

Extensive NMR studies have been used to obtain conformational information of several PROTACs to explain the observed passive cell permeation. Quantitative NOE experiments were conducted to obtain the solution conformations of a series of VHL PROTACs in apolar environment, mimicking the interior of the cell membrane. The obtained conformational ensembles helped explain the observed trends in membrane permeability. To explore whether the introduction of a ferrocene moiety to the linker would enhance molecular chameleonicity, qualitative NOE experiments were performed in polar and apolar environments.

Ligand-protein interaction studies by NMR to support the development of a PET diagnostic agent for cancer imaging

Timári István, Farkas László Bence, Kónya Zoltán, Gáti Tamás, Szücs Dániel, Képes Zita, Trencsényi György, Fekete Anikó

Cancer is one of the leading causes of death worldwide and its early detection can significantly increase the chances of survival. For this purpose, the improvement of the selectivity and sensitivity of various diagnostic methods, such as positron emission tomography (PET) is essential. The Galectin-3 (Gal-3) protein is a β-galactoside binding lectin with carbohydrate recognition domain, which regulates tumor progression through different signaling pathways, including tumor cell adhesion, migration and invasion, and contributes to tumor cell survival and resistance to anticancer therapy. Since many tumor types express Gal-3, such as melanoma, colon cancer, prostate cancer, and breast cancer, this protein may be considered a suitable diagnostic and therapeutic target for anticancer research. With this aim, we designed and synthesized a ^{68}Ga-labeled DOTA chelator-conjugated (2-naphthyl)methylated lactosamine derivative, a promising PET diagnostic agent for melanoma. The binding of this potential diagnostic agent to Gal-3 protein has been investigated by multiple NMR methods. The binding affinity (K_d) of the compound was determined by competitive Saturation Transfer Difference (STD) NMR experiment using thiodigalactoside (TDG), a well-known ligand of Gal-3, as a reference. Chemical shift perturbation induced by the studied compound in ^{1}H-^{15}N HSQC NMR spectra of ^{15}N-labeled Gal-3 protein has provided residue-specific information allowing to map the binding site. Moreover, *ex vivo* biodistribution and *in vivo* PET studies have indicated that our carbohydrate-based radiopharmaceutical is suitable for the highly selective imaging of Gal-3-expressing melanoma by PET.

ViscY NMR experiments coupled with chiral discrimination enable rapid analysis of amino acid mixtures.

Budai, **Anthony**, Lameiras, Pedro.

Nuclear Magnetic Resonance (NMR) is a key analytical technique for mixture analysis. However, conventional NMR experiments cannot differentiate enantiomers because they exhibit identical chemical shifts. Chiral discrimination by NMR usually relies on converting enantiomers into diastereomers, either through covalent derivatisation with chiral reagents or non-covalent complexation with chiral solvating agents (CSAs).

Our group introduced ViscY (Viscosity-enhanced spectroscopY) NMR, a set of experiments exploiting viscous solvents to enhance spin diffusion. Increasing viscosity slows the molecular tumbling of small- to medium-sized molecules, shifting them into the negative nuclear Overhauser effect (NOE) regime. Under optimized temperature conditions, magnetisation can propagate across all ^{1}H resonances of a molecule, enabling the individualisation of each component's 1D ^{1}H spectrum in a mixture using a simple 2D ^{1}H–^{1}H NOESY experiment.

Here, both strategies were combined to elucidate structures under spin diffusion using the viscous solvent mixture DMSO-d_6/H_2O (7:3, v/v) and to discriminate the chirality of a mixture of five racemic amino acids. (–)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid was used as the CSA and progressively added until distinct enantiomeric signals were observed.

At least one non-overlapping resonance (^{1}H or ^{13}C) was obtained for each enantiomer, enabling unambiguous assignment and structural characterisation by ViscY NMR. A 2D ^{1}H–^{1}H NOESY experiment recorded at intermediate temperature provided spatial correlations allowing determination of the α-carbon configuration of each amino acid. These results show that ViscY NMR can be effectively combined with chiral discrimination strategies to analyse chiral compounds in complex mixtures.

Apoptosis regulating Bcl-2 protein family in neurodegeneration

Ådén, Jörgen, Sophie Ayscough, Hanna Wacklin-Knecht, Tamás Nagy, Haiyan Wang, Tobias Sparrman, Jonathan Gilthorpe, Luke Clifton, Gerhard Gröbner

Programmed cell death (apoptosis) is essential for human life and its tightly by the Bcl-2 protein family. Onset of apoptosis causes the perforation of the mitochondrial outer membrane (MOM) by apoptotic Bcl-2 members like Bax while blocking of apoptosis by overexpression of the cell-protecting Bcl-2 in the MOM is the hallmark of many incurable cancers. Here, we hypothesize is that in neurodegenerative disorders premature neuronal death occurs through blocking cell-protecting Bcl-2 protein by misfolded, amyloidogenic proteins. However, the molecular mechanism by which Bcl-2 misfunctions at the MOM in neurodegenerative diseases remains elusive due to lack of structural and functional insight. Here, we prove our hypothesis using the. Here we use solid-state/liquid state NMR and neutron reflectometry (NR) to provide molecular and kinetic insight into the interference of amyloidogenic apoSOD1 protein - a key culprit in amyotrophic lateral sclerosis, ALS - recognition of Bcl-2 and sequestering of Bax from its Bcl-2 complex at the membrane level.

High-Throughput Bullet-Dynamic Nuclear Polarization

Benno Meier

Institute of Biological Interfaces 4, Karlsruhe Institute of Technology, Germany

In Dissolution-Dynamic Nuclear Polarization (D-DNP), near unity nuclear spin polarization is achieved by transferring electron spin polarization to nuclear spins at a field of several Tesla and a temperature of typically 1.5 K. The hyperpolarized sample is then dissolved with a jet of hot solvent, the solution is transferred to a liquid-state NMR instrument, and liquid-state NMR signals are recorded with signal enhancements of 3 to 4 orders of magnitude. In Bullet-DNP (B-DNP), the hyperpolarized sample is contained in a miniature plastic bucket (the bullet), transferred rapidly as a frozen solid, and the sample is dissolved only within the liquid-state NMR instrument. It turns out that the B-DNP approach is well-suited for fully automated experiments, in which also the loading of the DNP polarizer with "fresh" bullets is done automatically, enabling a throughput of > 30 samples per day. I will present the B-DNP system and show how it has been used to achieve ^{1}H signal enhancements of > 1000 at 9.4 T, and how those enhancements can be used to detect ligand-binding.

In-situ Laplace NMR (LNMR) analysis of aerosol cloud

Dawei Qi, Anne Selent, Petr Štěpánek, Sammeli Suominen, Otto Mankinen, Jack Lin, Nønne Prisle, Ville-Veikko Telkki

Understanding the effect of aerosols on cloud formation is a major frontier in atmospheric research, with crucial implications for health, weather, and climate. However, aerosol clouds are highly dynamic, dilute nano- to microscale objects in air, making *in-situ* characterization difficult for conventional methods such as adsorption spectroscopy, vibrational spectroscopy, and scattering. Recent studies have shown that LNMR can probe water species across distinct chemical environments through their relaxation profiles. Here, we present the first *in-situ* LNMR analysis of aerosol clouds.

Aerosol clouds were generated using a pressurized generator and directed through a 7 T NMR spectrometer. While flowing through the coil region, before observable condensation, we measured the proton spectrum, spin-lattice relaxation (T1), spin-spin relaxation (T2), and T1-T2 correlation. The clouds were then transferred to a scanning mobility particle sizer for real-time particle size monitoring.

Signal changes arising from flow rate were corrected during relaxation analysis. All measurements consistently revealed two water components in the cloud flow, one of which exhibited slower T1 and T2 relaxation. As the volume-averaged particle size decreased with increasing generation pressure, the amplitude of the fast-relaxing water increased, whereas that of the slow-relaxing water remained nearly unchanged. No clear change was observed in the relaxation times of either component. To clarify the origin of this two-component behavior, T2-T2 exchange analysis and molecular dynamics simulations are underway.

Our setup enabled the first *in-situ* NMR measurement of an aerosol cloud. This approach may broaden our understanding of atmospheric physics by LNMR and many other methods from the NMR toolkit.

A Comparative Study of an Optimized Fetal MRI Protocol Versus a Standard Protocol for Diagnostic Quality and Efficiency

Qiu Wei

Objective: To assess whether an optimized magnetic resonance imaging (MRI) protocol enhances image quality and acquisition efficiency for fetal central nervous system (CNS) evaluation.

Methods: In this prospective study, 60 pregnant patients referred for fetal MRI were randomized to either a Standard Protocol (Control, n=30) or an Optimized Protocol (n=30). The optimized approach included: (1) single-shot turbo spin-echo (SSTSE) with tailored slice thickness and interslice gap to limit partial volume effects; (2) accelerated T2-weighted sequences with motion-robust reconstruction; and (3) calibrated adjustments to signal averages and field of view to balance signal-to-noise ratio and scan duration. Two blinded radiologists independently graded overall image quality, CNS anatomical delineation, and motion artifacts using a 5-point Likert scale. Total examination time was recorded. Inter-observer agreement was calculated using the intraclass correlation coefficient (ICC).

Results: The optimized protocol significantly shortened mean scan time versus standard imaging (22.4 ± 3.1 min vs. 35.7 ± 4.5 min, $p < 0.01$). Image quality scores were higher with the optimized protocol (4.5 ± 0.5 vs. 3.2 ± 0.7, $p < 0.01$), with notable improvements in visualization of the corpus callosum and cerebellar vermis. Motion artifacts were significantly reduced ($p < 0.01$). Inter-observer agreement was excellent (ICC = 0.89).

Conclusion: The optimized fetal MRI protocol delivers superior diagnostic quality for CNS anatomy while markedly improving workflow through reduced acquisition time. These findings support adoption of the optimized protocol for routine prenatal imaging to achieve faster, more reliable diagnoses.

NV-centre-based deuterium NMR: chemical information at low field with interfacial sensitivity.

Hooper, **Riley W.**, Singh, Dileep, Banerjee, Utsab, Bucher, Dominik B.

Nitrogen-vacancy (NV) centres in diamond are emerging as powerful atomic-scale magnetic field sensors capable of performing NMR spectroscopy on spin systems orders of magnitude smaller than those accessible by conventional techniques. These sensors combine optical initialisation and readout with quantum control to enable non-invasive, room-temperature detection of nuclear spins, circumventing the sensitivity limitations of Boltzmann polarisation and opening prospects for single-cell NMR and single-molecule detection.

Here, we demonstrate nanoscale detection of deuterium (^{2}H) nuclei, a moderate-gamma quadrupolar nucleus, using shallow NV centre ensembles in diamond. Employing correlation spectroscopy protocols, we resolve ^{2}H quadrupolar Pake patterns from deuterated samples on the diamond surface and track their evolution with temperature through characteristic phase transitions for three model systems, with direct comparison to conventional bulk powder NMR.

Our results demonstrate the impressive sensitivity of NV-NMR for the deuterated solids and the spectral differences that can manifest when probing nanoscale volumes at the diamond surface compared to bulk powders. Further, NV-NMR measurements retain the rich spectral information encoded in the quadrupolar interaction and can be fit using standard solid-state NMR program packages, opening future opportunities for spatially resolved nanoscale dynamics measurements. This work establishes NV-based quadrupolar NMR as a new sensing modality for exploring molecules at surfaces and interfaces.

Investigation of the late-Roman burial chamber at Reichertsberg in Trier by NMR depth profiling

Blümich, Bernhard; Golini, Carlo; Yang, Qing; Waldecker, Maximiliane; Haber-Pohlmeier, Sabina; Brizi, Leonardo; Tomassini, Paolo; Frick, Jürgen; Anders, Jens; Heimerl, Ferdinand

Trier is the oldest City in Germany with many relicts of the Roman empire. One of them is the burial chamber at Reichertsberg. Since its discovery in 1967 it has been excavated and secured with protecting outer walls. Located largely intact in a mountain slope at the back of a school yard it had been neglected until a detailed study 30 years later. In an effort to draw attention to its deteriorating state of preservation, the interior decorations were investigated in September 2025 along with NMR depth profiling of the plaster layers applied at three different occasions in the 3rd to 4th century AD (Fig. 1). The NMR depth profiles and their analysis in terms of the known historical building phases are reported along with an estimation of the moisture flux inside the plaster cover of a naturally wet inner wall based on Fick's first law.

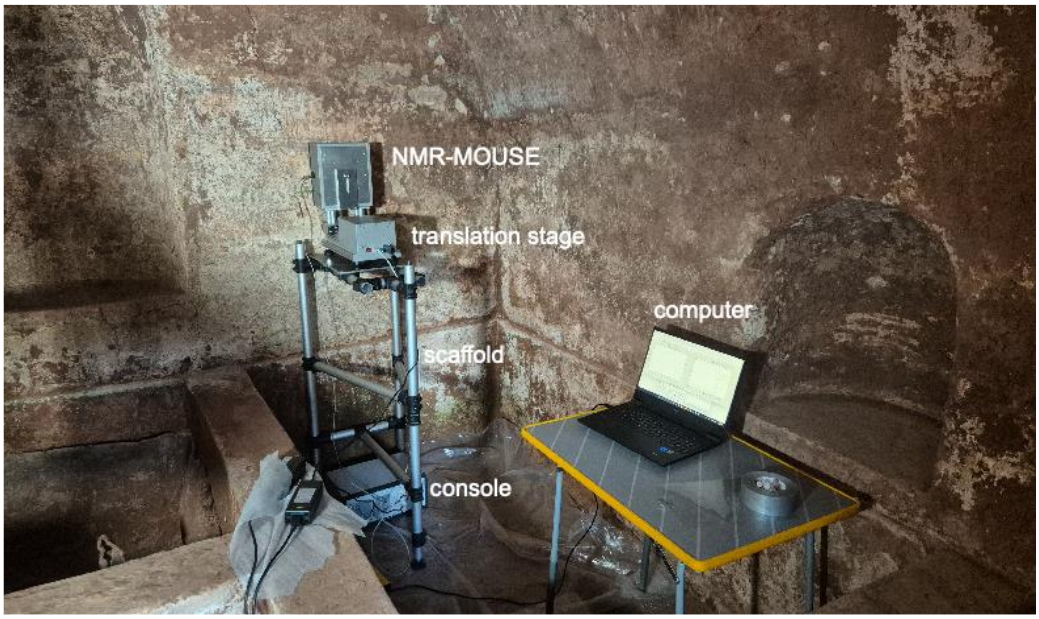


Figure 1: NMR setup for measuring depth profiles. The NMR-MOUSE is mounted on a precision translation stage, which rests on an adjustable scaffold. The sensor and the translation stage are connected to the console, which is operated by the computer.

Thursday | Hall C | 12:10-12:40 Bernhard Blümich

qing.yang@kit.edu

Parahydrogen-enhanced magnetic resonance to study biological systems

Stefan Glöggler[a,b]

a) Advanced imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas, USA
b) Department of Biomedical Engineering, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Over the past years, the use of parahydrogen to enhance magnetic resonance signals has seen many exciting developments to study biological systems including work on enzymes, cells and in vivo. I am going to present the recent developments of my lab to make parahydrogen induced polarization a tool that can provide new insights into biochemistry. I will present our results on designing molecular probes that can be used for in cell and in vivo applications to e.g. track the metabolism of tumors. Furthermore, I am going to present the implementation of parahydrogen to study hydrogenases. Hydrogenases are an important class of enzymes that can serve as a blueprint for eco-friendly hydrogenation catalysts. I will demonstrate that parahydrogen is an excellent tool to provide mechanistic insights into hydrogenases and allows us to obtain knowledge about the hydrogen activation process of enzymes that no other technology can provide.

Ultrabroadband and Multinuclear NMR: Pushing the boundaries of existing hardware with Seedless optimal control pulses

Bercovici, **Jack**, Turner, Abigail L., Davis, Benjamin G., Baldwin, Andrew J.

Applications in high resolution nuclear magnetic resonance (NMR) require radio frequency (RF) pulses that excite uniformly over a frequency range. We have recently developed Seedless, an engine that rapidly produces RF pulses using optimal control theory that can be specifically tailored to a desired application. We demonstrate here that Seedless can generate ultrabroadband pulses, including exciting the entire 59Co spectral width (20,000 ppm, 1.9 MHz bandwidth) on a 9.4 T spectrometer. Extending this, we develop a method by which one can simultaneously excite multiple nuclei, separated by up to 10 MHz using a pulse generated by a single coil, including 31P-7Li, 23Na-13C-59Co, and 37Cl-43Ca-25Mg. We further show how this methodology can be extended to produce pulses that can generate 2D HMQC experiments covering multiple nuclei, using a single coil. To generate these pulses effectively, we demonstrate the need to perform a calibration that shows how the B1 field varies as a function of offset from the tuning frequency and incorporate this into the design. This reveals that commonly used hardware can deliver appreciable B1 at frequencies far removed (in MHz) from the tune frequency and that our standard method for tuning (the ‘wobb’ curve) does not reveal the true capabilities of the probe. Taken together, by accounting in detail for the performance of specific hardware and samples, Seedless can generate pulses for ultrabroadband and multinuclear applications, expanding capabilities of existing hardware.

Magnified (Woggle)Bugs of Small Coherent Perturbations: The Heteronuclear Decoupling / Steady-State Free Precession Case

Jayanthi Sundaresan, **Osifová Zuzana,** Shif Mark, Lupulescu Adonis, and Frydman Lucio*

Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot, Israel

^{1}H-decoupled FT-NMR has been a cornerstone of analytical spectroscopy for over five decades. Recently, however, we showed that Steady-State Free Precession (SSFP) experiments— which depart from the Fourier acquisition scheme—can, under certain conditions, deliver higher sensitivities than their FT-NMR counterparts. SSFP is one of the earliest NMR pulse sequences, widely used in MRI and described in ~30,000 publications; yet most studies focus on water or other single-spin systems. This work shows that significant departures from classical SSFP behaviour arise when the steady state must be established in coupled spin systems. In fact, pronounced artefacts ("bugs") can appear even for the simple case of a single ^{13}C *J*-coupled to a ^{1}H under conventional WALTZ decoupling. Experiments and theory show that the small coherent perturbations introduced by ^{1}H decoupling—which in conventional FT experiments produce only weak, often harmless sidebands—can substantially alter the ^{13}C SSFP response. These unexpected effects are analysed here under different SSFP and decoupling conditions, and strategies to exploit or mitigate them are presented.

Acknowledgements: Support from the Israel Science Foundation (grant 1874/22), the Minerva Foundation, ERC Advanced Grant 101200719 "SteadyNMR", and the Perlman Family Foundation is acknowledged. ZO thanks the Azrieli Foundation for a postdoctoral fellowship; SJ thanks the Weizmann Institute of Science for a sabbatical fellowship.

EUROMAR 2026

Patent application pending

Line-Narrowing by Polychromatic Selective Spin Locking in NMR

Wiame, Coline, Loutfi, Hadi, Sheberstov, Kirill, Bodenhausen, Geoffrey

Selective Spin Locking (SSL) targets the magnetization vectors of selected singlets or multiplets in high-resolution NMR spectra using mono- or polychromatic selective RF irradiation. This approach can reduce linewidths to the fundamental limit defined by homogeneous T_{1rho} relaxation. The SSL sequence works by applying *M* small flip angle pulses within each dwell time, so that *M* peaks can be selectively spin-locked simultaneously in one experiment. This method not only removes the effect of the inhomogeneity of the static magnetic field B_0 but at the same time decouples scalar interactions that give rise to multiplet structures. Using mono- or polychromatic SSL on a 5 mm Bruker NEO iProbe at 11.7 T (500 MHz for ^{1}H), linewidths of peaks in conventional one-dimensional spectra are decreased by a factor as high as 8 for ^{1}H (i.e., linewidths decrease from 2 Hz to 0.25 Hz with SSL). For ^{19}F in per- and poly-fluoroalkyl substances (PFAS), the gain can be as high as 24, and the advantage can be typically a factor of 40 for ^{31}P in Adenosine Triphosphate (ATP). Consequently, the peak heights are greatly enhanced in these experiments, therefore boosting spectral quality significantly. An octachromatic SSL experiment, irradiating as many as 8 resonances simultaneously, was applied to a sample containing a mixture of 6 small molecules. In this experiment, overlapping multiplets, with central lines only 6.5 Hz apart, were line-narrowed and decoupled. Other common NMR experiments such as Inversion-Recovery (IR), Carr-Purcell-Meiboom-Gill (CPMG) echo trains, and the excitation and reconversion of Long-Lived States (LLS) into magnetization by Spin-Lock Induced Crossing (SLIC) benefit considerably from their combination with mono- or polychromatic SSL.

IDPs/IDRs by NMR and beyond

Bodor, Andrea

Intrinsically disordered proteins (IDPs) constitute a large part of the proteome and perform a wide range of biological functions. They are involved in liquid-liquid phase separation and represent important targets in disorder-based drug design. However, identifying their structural propensities - often linked to molecular recognition, dysfunction, and therapeutic targeting - remains challenging.

Our research combines *global* characterization methods, such as hydrodynamic measurements, with *local* descriptors including, for example, chemical shifts and residue-specific dynamics. We develop experimental and statistical approaches, as well as databases, to improve the characterization of IDPs.

As IDPs exhibit reduced spectral dispersion, simplified spectra with utmost resolution are essential for obtaining well-resolved resonances. We developed BIRD-filter-based acquisition schemes with extensive homo- and heteronuclear decoupling for both $^{1}H^{N}$ and $^{1}H^{\alpha}$ detection, enabling more accurate chemical shift determination and improved automated peak-picking.

Identifying regions with transient structural preferences is commonly addressed through secondary chemical shift analysis. Given the availability of numerous random-coil prediction methods, we introduced statistical tools that facilitate objective selection of the most appropriate predictors, such as the Sum of Ranking Differences and Discordance Ratio approaches. The SPIT (Structural Propensity Identification via t-statistics) method allows more confident identification of structural propensities.

Proline residues are abundant in IDPs, and besides the dominant *trans* conformer, the minor *cis* form typically accounts for 5–15% of the population. By systematically compiling literature data, we established the ProMiSeDB database, identified factors influencing *cis*-proline populations, and characterized chemical shift perturbations of neighboring residues.

These methodologies and resources provide broadly applicable tools for studying IDPs and other dynamic biomolecular systems.

Deep Learning and Terabyte-Scale Experimental Data: New Perspectives for Biomolecular NMR

Klukowski, Piotr[1], Riek, Roland[1], Güntert, Peter[1,2,3]

[1] Institute of Molecular Physical Science, ETH Zurich, Zurich, Switzerland
[2] Institute of Biophysical Chemistry, Goethe University Frankfurt, Frankfurt am Main, Germany
[3] Department of Chemistry, Tokyo Metropolitan University, Hachioji, Tokyo, Japan

NMR spectroscopy plays an important role in macromolecular studies, making it possible to elucidate protein structure, dynamics, and interactions mechanistically at the atomic level. One of the major hurdles in the field is the laborious data analysis process that requires weeks or months of expert effort to transform multidimensional NMR spectra into protein structure models and biophysical conclusions.

To address this long-standing bottleneck, we developed ARTINA — the first deep learning approach that delivers cross-peak positions, chemical shift assignments and protein structures given only raw NMR spectra and the sequence as input. The method forms the core of NMRtist, our online platform that provides automated analysis tools for the NMR community and brings machine learning into routine NMR data interpretation.

To date, NMRtist has analysed more than 30,000 2D–4D biomolecular spectra, collecting a terabyte-scale corpus of experimental data and providing an opportunity to learn directly from the diversity and variability of experimental measurements.

Here we present our most recent progress in this area, focused on developing the next generation of the ARTINA framework by leveraging a terabyte-scale experimental dataset to improve the throughput, robustness and generalization of our deep learning approaches. As part of these efforts, we explore methods going beyond automation of data analysis, such as deep learning-driven protein shift assignment and structure determination based solely on 2D ^{1}H–^{1}H measurements — a potential route to eliminate the need for ^{13}C/^{15}N isotope labelling and substantially reduce measurement time.

Machine-learned Chemical Shift Biasing of Molecular Dynamics Simulations

Kimball, **James J.**, Holmes, Jacob B., Rodriguez-Madrid, Ruben, Emsley, Lyndon

Structure determination of amorphous pharmaceutical solids is critical for understanding the molecular interactions that govern the stability of drug formulations. However, the lack of long-range order in these materials makes atomic-level structure determination challenging. Recent advances in NMR crystallography have enabled structural characterization of amorphous systems through the integration of solid-state NMR measurements with computational modelling and machine learning. In current workflows, molecular dynamics (MD) simulations are used to generate large ensembles of candidate structures that are subsequently filtered according to agreement with experimental chemical shifts. In practice, only a small fraction of configurations sampled during the MD trajectory exhibit chemical shifts consistent with experiment. This limits the ability to perform quantitative analysis of structural features such as hydrogen bonding networks and torsional angle distributions, since these analyses rely on subsets of structures selected post hoc. Here we present a chemical-shift-biased MD approach in which experimental chemical shifts are incorporated directly into the simulation through site-specific biasing potentials. Chemical shifts are predicted on-the-fly using the machine-learning model ShiftML3, enabling the MD trajectory to evolve within regions of configurational space consistent with experimental NMR observables. This framework enables the direct sampling of structurally realistic amorphous ensembles and facilitates quantitative characterization of intermolecular interactions and conformational distributions in amorphous pharmaceutical solids.

Explainable Models for the Prediction of NMR Shifts

Dimitri Domnjuk, Jana de Wiljes, Christian Dreßler, Robert Geitner

Accurate prediction of NMR chemical shifts has become a central component of computer-assisted structure elucidation and high-throughput experimentation, yet most state-of-the-art models operate as black boxes, offering limited insight into how structural features influence predicted shifts. In this context, there is a clear need for models that combine high predictive accuracy with chemical interpretability, allowing researchers to understand which structural motifs, electronic effects, and substituent patterns drive the predicted NMR response. Our work addresses this gap by explicitly designing and analyzing a lightweight, explainable graph neural network for ^{31}P NMR shift prediction.

We introduce the Small-Data Graph Neural Network (SDGNN), a parameter-efficient MetaLayer-based architecture optimized for sparse experimental datasets. On the Ilm-NMR-P31 dataset (14,062 molecule-disjoint test cases), the SDGNN achieves a state-of-the-art mean absolute error (MAE) of 8.88 ppm. Beyond accuracy, we systematically analyzed model behavior using chemically informed data splits, functional-group-resolved error statistics, feature ablation, and GNNExplainer visualizations. The model demonstrates physically meaningful behavior: node-level features dominate predictions, especially local coordination descriptors around phosphorus. Importantly, linear fits to homologous alkyl series show that the SDGNN autonomously rediscovers classical substituent effects in ^{31}P NMR, quantitatively reproducing the established β-deshielding and γ-shielding increments. This confirms that the network internalizes chemically interpretable structure–property relationships rather than merely memorizing data.

These results demonstrate that explainable graph-based models can reach or surpass empirical and standard DFT-based approaches in accuracy while being orders of magnitude faster in inference. The combination of predictive performance, parameter efficiency, and interpretability enables integration into interactive structure elucidation tools.

Large-scale exploration of protein space by design and automated NMR

Hiller, **Sebastian**; Wicky, Basile IM; Müntener, Thomas; Abramson, Dylan; Stern, Elsa; Hertel, Ines; Folkers, Gert E

Protein structures can now be predicted and designed at scale, yet experimental access to dynamics and conformational heterogeneity remains limited in throughput. This gap prevents a systematic understanding of how protein sequences encode motion and functional flexibility. Here, we leverage protein design to sample the structural space generatively, and establish a scalable experimental pipeline combining automated production and nuclear magnetic resonance (NMR) spectroscopy to enable high-throughput characterization of protein structure and dynamics at atomic resolution. A single operator can produce and analyze hundreds of isotopically labeled proteins per week, with per-sample cost largely defined by DNA synthesis. To benchmark this approach, we experimentally characterized 384 de novo designed proteins spanning diverse regions of structure space. High-quality two-dimensional NMR spectra were obtained for 239 samples (62% of designs overall). NMR characterization confirmed that the designed proteins adopt their intended folds, and revealed unexpected local dynamics that are not captured by current computational models. Our approach establishes a foundation for data-driven modelling of sequence–structure–dynamics relationships and unlocks a new regime of statistical structural biology, where insight into protein biophysics is gained from experimental ensemble studies of suitably designed proteins.

Robust Reconstruction and Analysis of Magnetic Resonance Spectroscopy with Exponential Signal Modelling

Guo, Di

Xiamen University of Technology, China

Exponential is a basic signal model of the ex vivo and in vivo Magnetic Resonance Spectroscopy (MRS). This model has empowered the state-of-the-art fast data acquisition and quantitative spectrum analysis. In this talk, I will present our latest progress made in this field, low-rank Hankel matrix and synthetic data artificial intelligence learning. Specially, the proposed algorithms are designed to smartly handle the challenging low-intensity signal, over-lapped signal, hypercomplex signal, multi-dimensional signal or non-ideal exponentials, leading to robust non-uniformly sampling reconstruction, quantitative measure of ex vivo MRS and metabolite concentration quantification of in vivo MRS. These algorithms are further deployed on cloud MRS platforms with biomedical applications, such as brain gliomas.

Using cross-correlated relaxation of methyl groups to probe protein dynamics

Julie Maibøll Kaasen, Ahmed A. A. I. Ali, Daiana A. Capdevila, Frans A. A. Mulder

The importance of protein dynamics in structural stability and molecular interaction is well-established. To fully investigate the role, we must be able to record sensitive relaxation experiments that can be interpreted easily and recorded quickly.

This work investigates a fast and simple method to investigate the dynamics of methyl groups through *coupled* ^{1}H-^{13}C HSQC spectra. Here, the ^{13}C transverse relaxation rates, R_2, of the four methyl lines, corresponding to protons in ααα, ααβ, αββ and βββ energy states, contain valuable dynamic information for each methyl group, including the cross-correlated relaxation (CCR) rates from dipolar interactions of two CH bonds. The order parameters, S^2, are commonly used as a measure for dynamics and can be derived from the CCR rates. As previously demonstrated, a simple constant-time approach on uniformly enriched proteins is hampered by differential relaxation during the INEPT delays, spoiling the 3:1:1:3 intensity ratio of the ^{13}C quartet in the slow tumbling limit. By measuring the relaxation rates of the individual lines in the ^{13}C quartet, we accurately determine the CCR rates and S^2 using different isotope labeling schemes, highlighting the robustness and simplicity of the method.
The ability to obtain S^2 in a rapid, accurate and sensitive manner allow us to investigate several correlates of the local conformational entropy. By probing the temperature dependence of S^2 for each methyl group, the thermal coefficients of the methyl group order parameter, the characteristic thermal coefficients, and the local heat capacities have been determined.

Turning the guest into a spy: Fast-MAS and paramagnetic solid-state NMR on a guanidinium ion trapped in a π-container

Busch, **Hannah**, Günzel, Lennart, Zocher, Christian, Bartalucci, Ettore, Taube, Florian, Grohe, Kristof, Wegner, Sebastian, Das, Chandan K., Koppe, Jonas, Corzilius, Björn, Fyta, Maria, Ernst, Matthias, Kersting, Berthold, Wiegand, Thomas

Calix[4]arene lanthanide complexes provide a well-defined cavity for host-guest chemistry enabling to trap small molecules, such as a guanidinium cation. Solid-state NMR allows simultaneously to probe the underlying non-covalent interactions and to determine the magnetic properties of the lanthanide ion by using the guest molecules as highly sensitive spy molecules for the induced paramagnetic effects. In the first step, solid-state NMR at fast magic-angle spinning (MAS) frequencies has been utilized on the diamagnetic La^{3+} complex to investigate the underlying host-guest interactions. The guanidinium cation motion has been fully characterized by temperature-dependent MAS-NMR experiments down to 100 K, as well as by a variety of further solid-state NMR experiments and supplementing quantum-chemical calculations and molecular dynamics simulations. While cation-π interactions fix the cation in the cavity, our data reveal that it still rotates along its internal C_3-axis.

Turning to the isostructural paramagnetic Eu^{3+} complex, the paramagnetic solid-state NMR spectra are affected by severe line broadening caused by enhanced transverse nuclear relaxation times, pseudo-contact shifts (PCSs) and extensive MAS-sideband manifolds due to anisotropy of the hyperfine interactions. While these effects pose a challenge for the acquisition of the spectra and the resonance assignment, they at the same time encode for quantitative distance and angular information. Applying fast MAS up to 160 kHz and short high-power adiabatic pulses enabled to record well-resolved NMR spectra. With the guest molecule serving as spy, the magnetic susceptibility anisotropy tensor initially determined in solution was selectively scanned pointing to the conservation of the tensor between solution and solid state as well as the importance of inter-complex interactions in the solid. We envision that probing Δχ-tensors with spy molecules in the solid state will be of high interest for future studies on lanthanide-based magnetic materials, molecular qubits, and catalytically active species.

Paramagnetic NMR as a Quantitative Structural Probe: Refinement of Na Positions in Prussian Blue Analogues

Bassey, Euan N.,[1] Pintacuda, Guido,[1] Pell, Andrew J.[1,2]

[1]Centre de RMN à très haut champs (UMR 5082, CNRS/UCB Lyon 1/ENS de Lyon) Villeurbanne, France

[2]Institut Universitaire de France (IUF)

Paramagnetic Prussian blue analogues (PBAs) exhibit complex and highly broadened multinuclear NMR spectra that are extremely sensitive to local structure, Na coordination, and framework distortions. However, the quantitative relationship between Na displacement, polymorphism, and paramagnetic NMR shifts remains poorly understood. Here we investigate the effect of Na ion position, lattice strain, and polymorphism on calculated (Fermi contact) paramagnetic NMR shifts in Na-ion PBAs using first-principles calculations. Systematic distortions of the Na sublattice reveal strong, highly anisotropic NMR shift responses to both local ionic displacements and macroscopic strain. These results demonstrate that the Fermi contact interaction is a sensitive probe of short-range symmetry breaking and dynamic disorder in PBAs. To efficiently describe the coupling between lattice distortions, Na motion, and the resulting shifts, we develop cluster-expansion surrogate models that express the shift as a function of Na displacement and strain. The models closely reproduce first-principles calculations while enabling rapid exploration of configurational space. By combining the surrogate shift model with neutron pair distribution function (PDF) constraints, we demonstrate (for the first time) accurate refinement of Na positions in paramagnetic PBAs, resolving local displacements and strain distributions consistent with experimental data. This work establishes a quantitative framework linking paramagnetic NMR shifts to short-range structural distortions, providing a route to structural refinement of technologically relevant PBA battery materials beyond the limits of scattering methods alone.

From Radical Defects to Metal Centers: EPR in Carbon Nitride

Salvadori Enrico, Melchionna Michele, Fornasiero Paolo, Chiesa Mario

Carbon nitride materials are attracting increasing interest due to their applications in energy conversion and heterogeneous (photo)catalysis. Their catalytic and photochemical properties are strongly influenced by paramagnetic species arising from intrinsic radical defects, photoexcited states, or paramagnetic centers introduced after synthesis.

In this work, Electron Paramagnetic Resonance (EPR) spectroscopy is employed to identify and characterize paramagnetic species in carbon nitride frameworks. A combination of continuous-wave and pulse EPR techniques, including hyperfine and dipolar spectroscopies, is used to resolve the electronic structure and local environment of these centers. The experiments allow the identification of intrinsic radical defects and photoinduced paramagnetic states and provide insight into their role in charge separation and photochemical processes. In addition, the incorporation of first-row transition metals such as nickel and copper is investigated, enabling the determination of the coordination environment, electronic structure, and redox properties of the resulting paramagnetic metal sites.

The results demonstrate that advanced EPR methodologies provide direct spectroscopic access to defect structures, photoexcited states, and metal coordination environments in carbon nitride materials lacking long-range order. This work highlights the ability of EPR to resolve the structure and reactivity of paramagnetic species in carbon nitride and underlines its central role in understanding the relationship between defect chemistry, electronic structure, and catalytic function.

POSTERS

SPONSORS & EXHIBITORS

PLATINUM

BRUKER

GOLD

JEOL Solutions for Innovation

SILVER

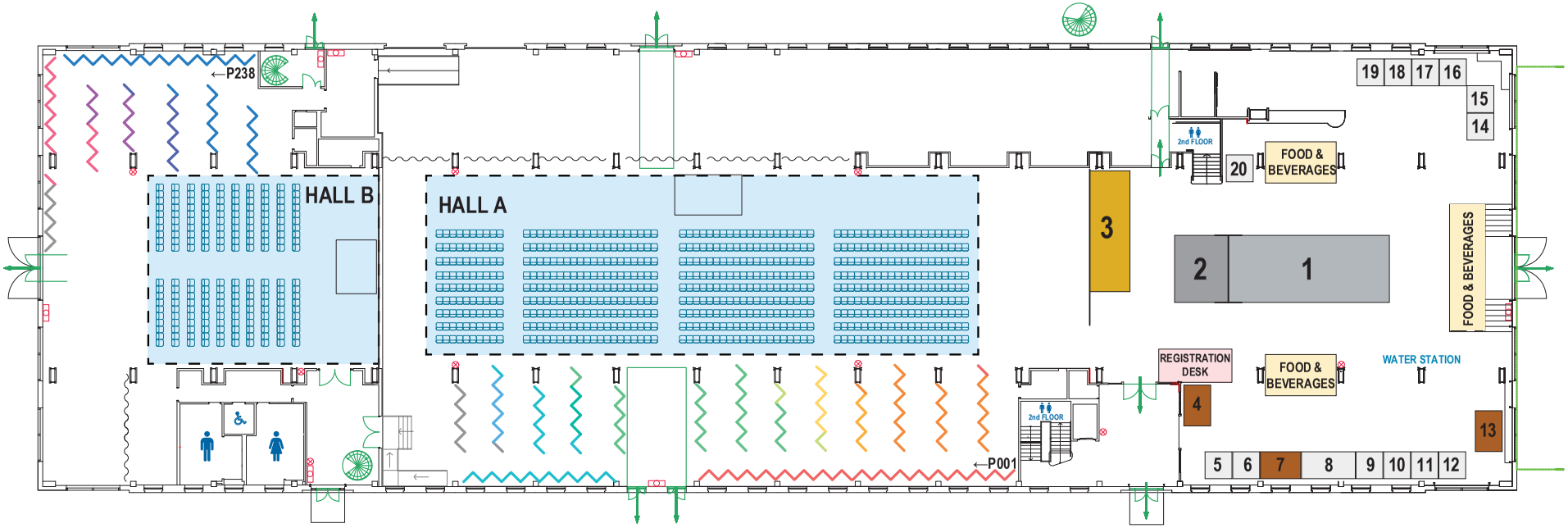
magritek

BRONZE

MERCK BLUEFORS CIQTEK

EXHIBITORS

Eurisotop, OXFORD INSTRUMENTS, QOne, CRYOGENIC, ZEOtope, ROTOTEC SPINTEC, QUAD SYSTEMS, PHOENIX NMR, NMR SERVICE, ePROBE, SciY, CORTEC NET, ARMAR ISOTOPES, SPIN-Doc, MRC, JMR, JMRO, City of Gothenburg, VÄSTRA GÖTALANDSREGIONEN, PANACEA, SwedNMR



To HALL C
"Teater Lärlingen"

POSTERS

AI/Theory/Computational	P001 – 035	Liquid methods	P188 – 223
BioNMR	P036 – 084	Low field/Benchtop	P224 – 237
Emerging technologies	P085 – 097	Materials NMR	P238 – 289
EPR/Paramagnetic NMR	P098 – 110	Metabolomics	P290 – 307, 348
Hardware	P111 – 118	Small molecules/Drugs	P308 – 326
Hyperpolarisation	P119 – 178	Solids methods	P327 – 346
Imaging/MRI	P179 – 187		

SPONSORS & EXHIBITORS

1. **Bruker**
2. **Magritek**
3. **JEOL**
4. **Merck**
5. Oxford Instruments GmbH
6. PhoenixNMR LLC
7. **Bluefors**
8. Bruker
9. Q.One Instruments Ltd
10. NMR SERVICE/SPIN-DOC
11. ZEOCHEM AG
12. HPC Standards GmbH
13. **Ciqtek**
14. PANACEA & SwedNMR
15. Rototec-Spintec GmbH
16. CortecNet
17. MR Latvia
18. QUAD Systems
19. Eurisotop
20. Cryogenic Ltd