

POSTER ABSTRACTS



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POSTERS

AI/Theory/Computational
BioNMR

Emerging technologies

EPR/Paramagnetic NMR

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Hyperpolarisation

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Liquid methods

Low field/Benchtop

Materials NMR

Metabolomics

Small molecules/Drugs

Solids methods

P001 – 035

P036 – 084

P085 – 097

P098 – 110

P111 – 118

P119 – 178

P179 – 187

P188 – 223

P224 – 237

P238 – 289

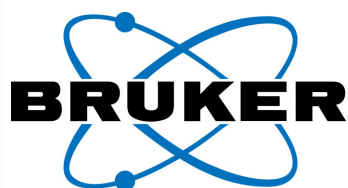
P290 – 307, 348

P308 – 326

P327 – 346

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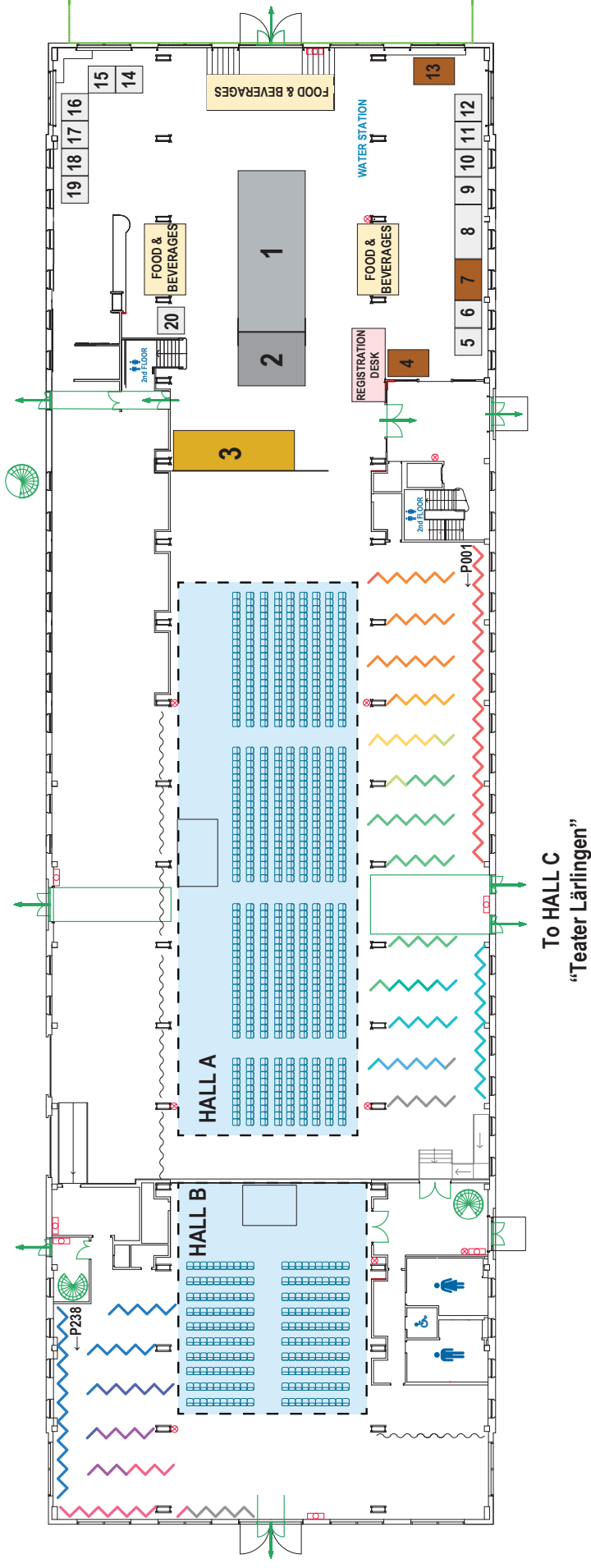


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EXHIBITORS





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Materials NMR

Metabolomics

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P188 – 223

P224 – 237

P238 – 289

P290 – 307, 348

P308 – 326

P327 – 346

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AI/Theory/Computation

Mapping of dynamic allostery within p38 alpha kinase via network analyses and NMR spectroscopy

Suchandra Roy Acharyya, Sara Medina Gomez, Jörn Weisner, Rafael.C.Bernardi, Rasmus Linser

p38 α kinase is a central component of MAPK signalling and remains a prime therapeutic target in inflammation and cancer. However, the high conservation of the p38 α catalytic region across the kinome has been limiting the development of selective orthosteric inhibitors. This has increased interest in possible distal regulatory sites and in dynamic allostery, by which function would be modulated through retuning of correlated motions across the kinase architecture. One such distal site (a cryptic pocket), the so-called lipid pocket, has been suggested as a starting point for allosteric effectors, but it has been unclear whether the important prerequisite of it being connect to the functional sites via any sort of allosteric communication in the first place is fulfilled. Here, we combine a complex array of NMR spectroscopy, MD simulations, dynamic network analysis, and mutational/functional experiments to map long-range communication pathways across p38 α and identify residues that motionally couple the N- and C-lobe. Our analysis reveals that small-molecule binding into the lipid pocket entails widespread chemical-shift perturbations and changes in local dynamics across almost the entire kinase. The experimental findings are supported by an extended dynamic network connecting the active site, activation loop, and the MAP kinase-specific lipid-binding domain, found by dynamic network analysis (an established molecular-dynamics-based assessment) and further validated through point mutations at key network sites. Together, these findings support p38 α function to be governed by distributed dynamic coupling and highlight allosteric locals in general and the lipid pocket in particular as promising entry points for selective allosteric modulation.

Combining Deep Learning and Quantum-Mechanical Modelling for Next-Generation Automated Analysis of NMR Spectra

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Routine 1D ¹H NMR spectra of small molecules are acquired in seconds, yet their interpretation still requires expert time: overlapping signals, strong coupling, and artefacts make automated extraction of chemical shifts, couplings, and proton counts difficult, especially in crowded aromatic regions and at the lower fields of benchtop NMR. This presentation covers more than four years of doctoral research combining deep learning with quantum-mechanical modelling to address this bottleneck across three stages, each extending and improving upon the previous one.

mldcon, a deep learning model trained on physics-informed synthetic spectra, deconvolves crowded, strongly coupled 1D ¹H spectra into accurate peak lists and is deployed as a production tool in Bruker TopSpin.

Building on these peak-level results, MolDETR, a detection transformer trained exclusively on quantum-mechanical spin-dynamics simulations, predicts complete spin-system descriptors (chemical shifts, scalar couplings, proton counts, line-width parameters) directly from raw spectra. On experimental benchmarks it achieves median errors of 0.89 Hz for shifts and 0.20 Hz for couplings with 93.5% proton-count accuracy, outperforming existing software and experienced spectroscopists.

The third stage feeds MolDETR predictions as informative priors into a Bayesian total line-shape framework under the full spin Hamiltonian, where sequential Monte Carlo and Hamiltonian Monte Carlo sampling yield posterior distributions over all parameters, providing physically grounded uncertainty estimates.

Together, the three stages form a coherent pipeline from peak deconvolution through spin-system inference to Bayesian refinement, applicable to structure verification, purity assessment, and high-throughput compound screening.

Deep learning approach for the reconstruction of pure-shift (PS) high-resolution relaxometry (HRR) spectra

Pejanovic Rajka, Siemons Lucas, Chacko Vineeth Francis Thalakkottor Jose, Ruda Alessandro, Garibay Jorge, Pelupessy Philippe, Bouvignies Guillaume, Hansen Flemming, Ferrage Fabien

High-resolution relaxometry (HRR) extends NMR relaxation measurements to fields far below those available with cryogenic magnets while preserving the spectral resolution and sensitivity of high-field detection. Proton HRR enables characterization of transient interactions between small molecules and macromolecules in complex mixtures through relaxation-dispersion analysis. However, quantitative interpretation is often hindered by spectral crowding, as proton resonances appear as multiplets from homonuclear scalar couplings. Pure-shift NMR experiments collapse multiplets into singlets and improve spectral resolution, but implementations often suffer from reduced sensitivity.

We present a deep-learning framework for reconstructing pure-shift HRR spectra from conventional HRR measurements. The approach uses a modified JTF-WaveNet architecture with five parallel output heads that process FIDs acquired at multiple relaxation delays simultaneously.

The network takes ten FIDs as input: five high-field FIDs recorded with a series of echo times and five HRR FIDs acquired at the shortest echo time but with five different relaxation delays at a given low field. High-field spectra encode multiplet structure, while HRR spectra provide relaxation-dependent amplitudes. By combining high-field reference experiments with low-field experiments, we aim to perform the pure-shift transform on an entire HRR dataset (typically 5 relaxation delays for 40 to 50 magnetic fields, so 200+ spectra) by acquiring only five additional FIDs.

Preliminary results on synthetic datasets demonstrate accurate reconstruction of pure-shift HRR spectra. The architecture predicts five spectra simultaneously and provides point-wise uncertainty estimates for each spectral point, enabling quantitative assessment of prediction confidence.

fiRelax – Novel fitting platform for relaxivity data of paramagnetic systems

Kubrak, Adam; Sebastiao, Pedro; Parigi, Giacomo

Fast-Field-Cycling (FFC) NMR relaxometry offers unique insights into the molecular dynamics of paramagnetic molecules or nanoparticles through the acquisition of longitudinal relaxation rates as a function of magnetic field, the so-called Nuclear Magnetic Relaxation Dispersion (NMRD) profiles. Through model fitting with numerical algorithms, structural, dynamic and electronic parameters can be extracted, providing information on motional processes and interactions within the system. The main model used for paramagnetic NMRD profiles is the Solomon-Bloembergen-Morgan (SBM) model, whereas for systems exhibiting static Zero-Field-Splitting (ZFS), the (modified) Florence models have been developed. However, a lack of software solutions combining all paramagnetic relaxation models persists. fiRelax is a web-based fitting application featuring an intuitive graphical UI, specifically designed for the analysis of FFC data. It can be used without in-depth knowledge of programming or paramagnetic theory, and is freely accessible at <https://firelax.cerm.unifi.it>. The software consists of a dynamic front-end enabling analysis of experimental profiles and relaxation curve simulations, and a fitting core — the One-Fit-Engine (OFE) — where calculations and minimization procedures are performed. OFE is developed as an open-source algorithm, successfully implemented also by other fitting programs. The platform has been tested with various datasets, including contributions from multiple relaxation processes for inner-, outer-sphere and fast internal mobility. Multiple datasets can be fit simultaneously by (1) performing fits with shared parameters or (2) using Arrhenius equations to calculate temperature dependencies.

Bridging from First Principles to Seconds: A Framework for Multi-Scale Quadrupolar NMR Simulations

Huss, Tabea; Köcher, Simone; Reuter, Karsten; Granwehr, Josef; Scheurer, Christoph

Solid-state NMR spectroscopy is a powerful probe of structure and dynamics in battery materials. However, for quadrupolar nuclei such as ^7Li , the experimentally observed spectra are often strongly influenced by dynamical averaging, making it difficult to disentangle local structure and ion dynamics. While computational approaches can provide access to the underlying electric field gradients (EFGs), they have historically been unable to capture dynamic effects at experimentally relevant complexity.

We present a multi-scale computational framework for simulating quadrupolar NMR spectra at experimental time and length scales while retaining *ab initio* accuracy. In previous work, we combined machine-learned EFGs derived from first-principles calculations with molecular dynamics (MD), demonstrating excellent agreement with motionally narrowed Pake patterns. Here, we overcome the intrinsic timescale limitations of MD by coarse-graining to kinetic Monte Carlo (kMC). kMC enables simulations of systems comprising thousands of atoms over timescales up to seconds, thereby reaching the experimental regime. Using the complex battery material lithium titanium oxide (LTO) as a model system, we simulate spectra across distinct dynamical regimes, including the static limit, fast diffusion conditions, and realistic room-temperature behavior. The simulated spectra show good agreement with experimental data, demonstrating that the framework captures both realistic ion dynamics and the impact of structural disorder.

Overall, combining machine-learned NMR observables with MD and kMC enables realistic simulations of quadrupolar NMR. The novel multi-scale simulation framework facilitates more detailed interpretation of experimental spectra, reducing reliance on heuristic or ambiguous assignments.

Spin response growth without the Overhauser effect in magnetic resonance

Aybas, Deniz, Abi Mershed, Silvana

In pulsed nuclear magnetic resonance (NMR), spin precession amplitude is expected to exponentially decay over consecutive pulses. However, if there is another type of nuclear spin ensemble present, the Overhauser effect can cause transfer of polarization and result in an increase of the spin precession amplitude over consecutive pulses. In this talk, we will show that such an increase can also be observed when the spins are in the presence of a static magnetic field gradient, even without another nuclear spin ensemble transferring polarization. This result is important for experiments that use precision magnetometry, where an ensemble of spins sensitive to weak perturbations are inevitably inside a static magnetic field gradient.

Modelling nuclear quantum effects in NMR

Lantto Perttu, Zakary Ouail, Laurila Ossi

Finite-temperature quantum effects, in addition to zero-point vibrational motion, play a significant role in nuclear dynamics. These effects influence structural properties and, consequently, NMR parameters of materials. Classical molecular dynamics is therefore often insufficient when a detailed and accurate description of material behavior is required. In particular, isotope shifts in NMR shielding are inherently quantum-mechanical phenomena. Here, we investigate these effects in the ^{13}C NMR chemical shifts of Buckminsterfullerene (C_{60}).

We first demonstrate that the negative thermal expansion (NTE) of C_{60} is a genuine quantum-mechanical phenomenon and cannot be captured by classical treatments, contrary to previous assumptions. Using a finite-temperature quantum-mechanical rovibrational framework, we identify the specific vibrational degrees of freedom responsible for the thermal behavior of both structural parameters and isotope shifts.

We then approach the same problem using neural-network machine-learning interaction potentials (MLIPs) combined with an NMR machine-learning model (NMR-ML) in both classical and quantum path-integral molecular dynamics (PIMD) simulations. The MLPIMD approach enables a systematic improvement of the quantum description and reveals its impact on the NTE of C_{60} . It also provides a practical way to assess the influence of different electronic-structure levels - basis sets, exchange–correlation functionals, and dispersion corrections - on the predicted properties. Owing to its generality, the MLPIMD method is applicable to a wide range of systems and can be used, for example, to rationalize monomer–dimer ^3He NMR shifts observed in fullerene complexes.

Boosting the efficiency of ENDOR simulations by using reduced Liouville spaces

Alexey Bogdanov, Ilya Kuprov

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

Pulsed electron–nuclear double resonance (ENDOR) is used for probing hyperfine-coupled nuclei through the transfer of polarisation and coherence from electrons [1]. Detection via electrons improves sensitivity and selectivity for nuclei located near the unpaired electron [2]. Recent applications of ENDOR include: (i) coherent control of electron-nuclear systems in quantum sensing applications [3] and (ii) particularly with ^{19}F nuclei, as a structural biology tool that provides distance and orientation constraints for spin-labelled biomolecules [4]. These applications require accurate spin dynamics simulations for quantitative interpretation, but those are computationally demanding because multiple nuclei must be considered – exponential complexity scaling with each added nucleus [5]. The use of soft pulses further increases computational complexity [6].

Here we propose several efficiency improvements for the ENDOR simulation workflow: screening powder grids by excitation efficiency, factoring electron state spaces in high-spin systems, and – most importantly – using reduced Liouville spaces for spin dynamics simulations. The latter limits the levels of spin correlation and coherence that are considered reachable during the evolution, substantially reducing the matrix dimensions. These strategies enable scalable and versatile simulations of ENDOR spin dynamics. Their performance is demonstrated on several realistic examples, including spin-labelled small molecules and proteins, as well as quantum sensing applications using diamond defects.

[1] J. Harmer, *eMagRes* 2016, 5(4), 1493; [2] C. Gemperle, A. Schweiger, *Chem. Rev.* 1991, 91, 1481; [3] J. J. L. Morton et al. *Nature* 2008, 455, 1085; P.-Y. Li et al. *Phys. Rev. Applied* 2020 13, 024080; [4] A. Meyer, S. Deschert, S. Dey., C. Höbartner, M. Bennati, *Angew. Chem. Intl. Ed.* 2020, 59, 373; F. J. Tucci, R. J. Jodts, B. M. Hoffman, A. C. Rosenzweig, *Nat. Catalysis* 2023, 6, 1194; [5] L. Sielaff, A. Kehl, A. Aden, A. Meyer, M. Bennati, *Science Adv.* 2025, 11, eady5665; [6] Lenjer et al. *J. Magn. Reson.* 2026, 382, 108005.

Multiscale modeling of spin relaxation and SPINOE hyperpolarization in ^{129}Xe NMR

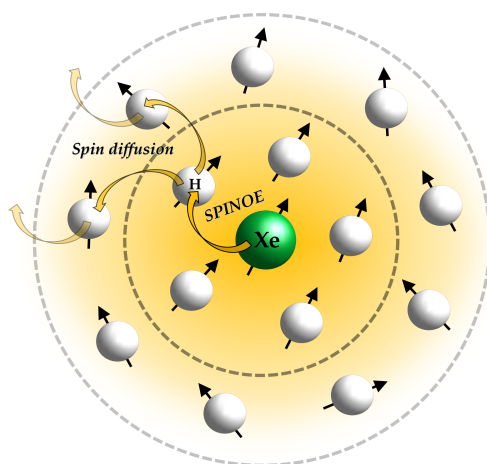
Perttu Hilla, Juha Vaara

The field of ^{129}Xe NMR has traditionally relied on the sensitive chemical shift of Xe as the primary source of chemical contrast. However, the relaxation of Xe is also very sensitive to local structure and dynamics. Despite this, Xe relaxation has received less attention as a source of contrast, partly due to the lack of robust methods for modeling Xe relaxation that would connect molecular-level details to experimentally observed relaxation rates.

Another interesting relaxation-related phenomenon is the flow of spin polarization from hyperpolarized Xe to surrounding nuclei via the spin polarization-induced nuclear Overhauser effect (SPINOE). Xe-proton SPINOE received considerable attention in the late 1990s and early 2000s but has since been less studied, partly due to difficulties related to proton-proton spin diffusion, which was impossible to model accurately at the time.

We have developed new multiscale modeling techniques for simulating relaxation dynamics in large molecular systems. This enables direct simulation of not only relaxation times, but also SPINOE polarization transfer, including spin-diffusion effects. The methods also allow explicit inclusion of chemical exchange across arbitrary exchange regimes and its effect on observable relaxation and SPINOE transfer. As a demonstration, we applied these methods to simulate relaxation and SPINOE in three model systems relevant to the field of Xe NMR biosensors.

In the presentation, I discuss the developed methodology, present interesting simulation results, and highlight future prospects of Xe relaxation as a source of chemical contrast, as well as the exciting potential of SPINOE for hyperpolarizing nuclei in various molecular systems.



Automated Simulation and Analysis of NMR Spectra with HQSpectrum

Wullschläger, Florian; Álvarez Barcia, Sonia; Enenkel, Nicklas; Hodecker, Manuel; Kleine Büning, Julius; Pinski, Peter; Schmitteckert, Peter; Schwenk, Iris; Vazigin, Artur; Zanker, Sebastian; Zech, Alexander

Accurate simulation of NMR spectra is vital for the rigorous evaluation of experimental findings. In this presentation, we introduce HQSpectrum, our cloud-based software solution for NMR spectrum simulation and analysis.

We have implemented a density functional theory-based workflow that uses conformer search and structural screening and is used for in-house high-throughput calculations of NMR parameters for small molecules. Moreover, we have developed a modern, fragment-based approach for fast prediction of chemical shifts and J -couplings alike, which was inspired by the seminal HOSE code method. The underlying database is an extensive set of NMR parameters for thousands of molecules computed with the in-house DFT workflow. This empirical prediction method is directly accessible in HQSpectrum, allowing users to predict parameters in a matter of seconds.

The spectrum simulation engine employs a sophisticated solver. Utilizing clustering approximations that minimize truncation errors and symmetry considerations, it efficiently computes NMR spectra with tens of spins. The solver shows a particularly strong performance even for the challenging simulation of NMR spectra in low magnetic fields, which is essential for the analysis of spectra obtained with a benchtop NMR device that often show overlapping peaks and broad multiplets.

With the simulated spectrum at hand, the HQSpectrum web frontend offers a variety of analysis tools for modifying chemical shifts and J -coupling constants in real time, and for comparing with an experimental spectrum. Thus, it can assist classical problems such as structure elucidation as well as more complex tasks like quality control and mixture analysis.

Optimal Control Framework for Spectral–Spatial RF Pulse Design

Spectral–spatial (SPSP) radio-frequency pulses play a key role in magnetic resonance imaging when both spatial localisation and chemical-shift selectivity are required, as in hyperpolarised ^{13}C experiments. We introduce an optimal-control approach for the design and simulation of SPSP pulses using the Qruise differentiable physics platform. The proposed method optimises RF and gradient waveforms by minimising magnetisation errors across the combined spectral–spatial domain while respecting practical hardware constraints such as RF amplitude limits, gradient slew-rate bounds, and gradient raster timing. The framework incorporates Bloch or Schrödinger equation simulations within a differentiable optimisation pipeline, enabling the generation of hardware-compatible pulse shapes. Preliminary results show homogeneous excitation for single-band targets, and current work focuses on improving spectral and spatial selectivity together with reducing pulse duration. This framework establishes a versatile basis for rapid, optimally controlled SPSP pulse design in metabolic and multinuclear MRI.

Deep-Learning: suppression of vibration artefacts in high-resolution relaxometry experiments.

Lucas Siemons, Rajka Pejanovic, Vineeth Francis Thalakkottor Jose Chacko, Alessandro Ruda, Jorge Garibay, Philippe Pelupessy, Guillaume Bouvignies, D. Flemming Hansen, Fabien Ferrage

High-resolution relaxometry (HRR) is a unique approach to study dynamics in large biomolecules and complex mixtures by coupling low-field relaxation with the polarisation and detection obtained from a homogeneous high-field magnet. This is achieved by rapidly transferring the sample, in ~ 60 ms, to the stray field or a fast field cycling coil, allowing us to measure low field relaxation rates with the sensitivity and resolution afforded by high-field cryogenic magnets.

Fast transfer times are critical to prevent polarisation loss. However, mechanical motor vibrations propagate through the magnet Dewar causing oscillations of the coil impedance, thereby modulating the free induction decay (FID). These vibration artefacts make quantifying relaxation rates challenging, particularly in crowded spectra.

To address this challenge we developed the deep-learning JTF-WaveNet architecture, which processes FIDs in both the time and frequency domains, in parallel. Training this model with on-the-fly simulations ($\sim 10^7$ unique examples) allowed us to develop a quantitative transform that efficiently removes vibration artefacts and gives well calibrated, normally distributed, point-wise uncertainty estimates. Our experimental validation, on a mixture of amino acids in the presence of Human Serum Albumin, indicates that processing the raw (HRR) experiments with this deep-learning model improves the accuracy and precision on the low-field longitudinal relaxation rates by a factor of ~ 2.5 .

This work highlights the high potential of deep learning for processing NMR data, as demonstrated here with high-resolution relaxometry experiments, and aiding in the extraction of information from complex spectra.

Machine Learning-Accelerated Molecular Dynamics and NMR Parameter Simulations in Porous Materials

Zakary, Ouail, and Lantto, Perttu

Porous materials (PMs) enable selective adsorption and chemical transformation of guest molecules, making them central to gas separation, catalysis, and energy storage. Their performance is governed by host-guest interactions, including binding sites, occupancies, and exchange dynamics, which remain poorly understood. Xenon NMR spectroscopy is a powerful tool for probing these interactions, as the ^{129}Xe isotope has a nuclear spin of 1/2 and a chemical shift highly sensitive to its local environment. However, extracting structural and dynamical information from ^{129}Xe spectra alone is challenging, making computational methods such as molecular dynamics (MD) simulations essential. These methods face an inherent accuracy-efficiency tradeoff, as accurate *ab initio* MD is limited to picosecond timescales and small system sizes, while efficient classical MD sacrifice accuracy.

Equivariant graph neural networks (EGNNs) have emerged as a promising solution, learning both interatomic potentials and NMR parameters at near-quantum-mechanical accuracy while maintaining high computational efficiency. Here, we present a dual-model EGNN approach consisting of (i) machine learning interatomic potentials that enable accurate and efficient MD simulations, and (ii) NMR machine learning models that predict magnetic shielding tensors efficiently and directly from MD trajectories.

We apply this approach to two porous materials, including porous liquids loaded with xenon gas, where we uncover three-site binding models and exchange dynamics, and xenon confined in carbon nanotubes, where nanotube geometry is shown to govern ^{129}Xe chemical shifts and diffusion. Our approach establishes EGNN-based modeling as a general tool for NMR interpretation at length and time scales previously out of reach.

Interpretable Neural Networks as Symbolic Regressors of Hamiltonian Eigenvalues

Sheberstov Kirill, Alexander Kharin

Deep neural networks are universal approximators of continuous functions, so that any physical process can be described by a trained neural network. Such an approach for modelling has limited practical value due to a lack of interpretability. Indeed, multilayer perceptrons (MLPs) typically apply the same nonlinear activation function across many layers. Consequently, the relation between the input and output of a network can be written as a composition of linear transformations and a nonlinear activation function. Such expressions usually cannot be considered as interpretable.

Kolmogorov-Arnold networks (KANs) are models with learnable activation functions. By training a network with a predefined set of activation functions, one can obtain a compact formula that consists of smooth algebraic operations. For problems for which it is difficult to derive an analytical solution but easy to perform numerical calculations (e.g., Hamiltonian diagonalization), a KAN-based approach can be used to obtain approximate analytical solutions.

The original KAN approach uses parametric spline functions. In many cases, polynomial regression is preferable. Here, we explore the ability to derive analytical polynomial expressions for the eigenvalues of a parameter-dependent Hamiltonian that drives collective spin dynamics of coupled protons in saturated aliphatic chains.

We present a hybrid MLP-KAN network, in which each neuron contains a set of three basis activations $(0, x, x^2)$. It is shown to be sufficient to produce expressions with an arbitrary degree of polynomial expansion. We further show that to get expressions with physically consistent units of measurement, one must train a network using unitless parameters, which are ratios of J -couplings defining eigenvalues of the Hamiltonian.

Wasserstein metric in NMR data processing

Kazimierczuk, Krzysztof; Domżał, Barbara; Grochowska-Tatańczak, Magdalena; Malinowski, Przemysław; Nawrocka, Ewa K.; Gołowicz, Dariusz; Ciach, Michał A.; Miasojedow, Błażej; Moszyński, Antoni; Goldstein, Antoni; Startek, Michał; Gambin, Anna

The Wasserstein metric $W(\mu, \nu)$ between two spectra can be thought of as the amount of work needed to reshape one spectrum (μ), into another (ν), as if they were piles of sand. This metric can be useful for data analysis in many contexts and is often more efficient than the standard Euclidean distance.

In this presentation, I will discuss a set of computational tools for quantitative NMR spectroscopy based on the Wasserstein metric. I will show how to use these tools to determine the relative amounts of components in a mixture. The presented software, called Magnetstein, is robust to overlapping or shifting peaks, lineshape distortions, noise, contamination, and low resolution. Magnetstein also deals with a series of spectra obtained during reaction monitoring. The examples of both processing cases (involving single and multiple spectra) will be demonstrated.

Magnetstein is freely available as an open-source Python package and, more recently, as an intuitive web application. I will provide a brief presentation of both.

Comparing spectra based on the Wasserstein distance is also effective for spectral alignment or peak-tracking tasks. I will demonstrate a tool called WNetAlign, which can handle a series of 2D-7D spectra and provide peak-specific chemical shifts.

Intrinsically Disordered Peptides (IDPs) are responsible for key functions in biological processes and up to 30% of the human proteome are IDPs. The disordered loops in proteins are often reactive centres and the question arises whether some catalytic key conformers are already present in conformer equilibria. The method of choice to study IDPs would be NMR but this method collects mean values on the time scale of milliseconds and more. The development of conformer equilibria of biopolymers proceeds on similar time scales. The interpretation of the NMR data requires computational methods that are able to represent the buildup of NMR mean values averaging over a multitude minima and transient structures. A bundle of say a dozen selected structures can only be a first approximation to the situation in solution.

Traditional molecular dynamics (MD) is not applicable for IDPs and therefore a multitude of accelerated MD methods have been developed. These methods rely mostly on force field potentials fitted to the problem. The new method of tensor driven MD presented in this paper works with original force-field potentials and to accelerate the dynamics rotational energy is disposed into mobile parts of the molecule. This is performed by tensorial constraints that represent the zero average of dipolar couplings in solution. These constraints require that all ^{13}C - ^1H and ^{15}N - ^1H dipolar coupling tensors have to approach zero in the simulation time. To get realistic occupation numbers of conformers we apply scalar constraints as chemical shifts, ^3J couplings or NOE distances on the fly of the MD simulation. The final result of the calculation is a trajectory and the calculated constraints should be in or near the error limits. The residual dipolar coupling should near zero Herz documenting that an averaged liquid state is reached.

As first test for IDPs the peptide amyloid β ($\text{A}\beta$) was selected because it plays a noxious role in human neurodegenerative diseases. In some aggregating IDPs there should exist a low populated conformer (<5%) that is suspected to be the first step in oligomerization of monomers. In the case of $\text{A}\beta$ it is discussed whether oligomers are pathogen or only the subsequent forming fibrils destroy the neuron cell structure. Experimentally this “dark” state is hard to identify and therefore computational methods are required.

Improved methodologies for quantum control in spintronics: solid-state NMR as an original toolbox

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In this work, we demonstrate how concepts and methodologies originating from solid-state NMR can be successfully transferred to the study and coherent control of electronic spin systems relevant to spintronics and quantum technologies. Using NMR-inspired frameworks, spin Hamiltonian engineering, pulse-based manipulation, Floquet analysis, and Liouville von Neumann dynamics, we developed and validated a unified approach for modeling and controlling interacting electronic spins.

We constructed generalized Ising-type models incorporating exchange coupling, Dzyaloshinskii-Moriya interactions, and external magnetic fields. Starting from analytically tractable three-spin chains, we extended the analysis to larger 1D systems and to 2D networks with frustrated geometries. Numerical simulations revealed how interaction strengths and lattice topology influence coherence, non-equilibrium evolution, entanglement generation, and the emergence of exotic magnetic textures.

A central contribution of this work is the design of tailored control pulses adapted from NMR and arbitrary-angle rotations to manipulate electronic spin magnetization with high fidelity despite shorter coherence times. Using Floquet theory, we transformed time-periodic Hamiltonians into effective time-independent operators, enabling stable and accurate simulations of driven spin systems and validating deterministic spin-rotation protocols.

Together, these results establish solid-state NMR as an effective conceptual and computational toolbox for advancing quantum control in spintronic architectures. The unified methodology developed here provides a foundation for spin-based logic elements, controllable spin textures, and emerging quantum-information schemes based on engineered spin networks.

Single-Spin Effective Rotations by Constrained Random Walk

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Periodic irradiation schemes comprising a repeating pulse-sequence element on the microwave (MW) channel have recently been shown to be able to achieve efficient broadband DNP transfers. Central to the success of this approach is recognizing the importance of the effective electron-spin rotation generated by the irradiation element, and how its robustness to electron offset and MW inhomogeneity impacts the efficacy and bandwidth of the DNP transfer.

The potential of this design strategy has also elevated our ambitions as pulse engineers, both in terms of the range of MW offsets for the electron spins contributing to DNP transfer and in our ability to compensate for MW inhomogeneity. This ambition, however, comes at a cost. As the number of optimization variables and targets increases, so does the computational cost of our optimizations and the complexity of the multi-parameter landscape.

In this talk, we will address how this limitation may be better understood by rethinking our optimization procedure. More specifically, we will show how, using constrained random walk, we can systematically design composite pulses for a given electron offset, with a set effective field, and rotation axis. Such pulses can then be used in dipolar recoupling experiments, potentially improving our fundamental understanding of the underlying spin physics, as well as serving as initial guesses for subsequent numerical optimization.

A first-principles Brownian dynamics approach to predict liquid-state NMR relaxation

Zerbetto, Mirco, Rampino, Sergio, Polimeno, Antonino

Gaining atomistic insight on slow molecular motions from nuclear magnetic resonance relaxation experiments requires molecular dynamics simulations (MD) capable of reaching the microsecond timescale. While all-atom MD simulations can now reach such timescales, they remain computationally expensive for medium-large biomolecules and difficult to interpret. Stochastic models offer a more efficient alternative: by separating relevant coordinates, treated explicitly, from irrelevant ones, acting as sources of fluctuation and dissipation, they dramatically reduce computational cost. However, traditional phenomenological Langevin and Fokker-Planck based approaches developed in the past, though effective at distinguishing (even complex) molecular systems, often lack true predictive power due to unclear links between model parameters and atomistic details. Here, we present a computational framework built on a rigorous, first-principles derivation of stochastic dynamics directly from the all-atom equations of motion under the relevant/irrelevant coordinates partitioning and implemented into the BD-NMR software package. Our approach combines the efficiency of stochastic modeling with the atomistic resolution of molecular dynamics, works in natural internal coordinates (bond distances, bond angles, dihedral angles) for a simplified physical interpretation, and leverages GPU acceleration for enhanced performance. Together, these features deliver quantitative prediction of NMR relaxation data for semi-flexible complex molecules. This strategy opens a path toward the effective interpretation of long-timescale dynamics, bridging the gap between rigorous theory and experimental NMR observables.

A method for determining turbulent flow diffusion spectrum distributions using multidimensional diffusion MRI

Clarke, Daniel, Topgaard, Daniel

Turbulent flows exhibit chaotic velocity fluctuations that enhance mixing in chemical reactors and similar process devices. Diffusion MRI can resolve anisotropic and spectral features of the turbulence via double rotation of the q vector. However this method is susceptible to sidelobe encoding that reduces the accuracy of the measured diffusion spectrum if the encoding strength is assigned to a single peak frequency. Here, we avoid this limitation by reconstructing distributions of subensemble-scale diffusion spectra using Monte Carlo inversion, analogous to recent work on anisotropic, restricted diffusion in biological tissues. Applying this method to a numerical model of turbulent flow through a periodic porous structure, we identified that the largest eddies were largely unidirectional, while smaller eddies were more isotropic in character. This method may allow for new insights into the mechanisms underpinning the energy cascade phenomenon intrinsic to turbulence, and inform the design of improved unit operations in chemical engineering.

Automatic Prediction of NMR Parameters from Quantum Chemistry via an Empirical Approach

Kleine Büning, Julius; Hodecker, Manuel; Pinski, Peter; Wullschläger, Florian

For computer-assisted analysis of NMR spectra, it is essential to predict NMR shifts and J -coupling constants both quickly and accurately. In this presentation, we provide insight into the technical details of the (empirical) NMR parameter prediction in HQSpectrum.

In a first step, we apply a density functional theory-based workflow to calculate NMR parameters in a high-throughput manner. To obtain realistic results, it is crucial to explore all conformational degrees of freedom within a molecule, allowing for the calculation of parameters across all accessible structures and their correct averaging. When using well-established conformer search software to generate structures for conformational isomers, the obtained ensembles are increasingly incomplete as the conformational complexity of molecules grows. Therefore, the workflow analyzes the structures and generates complete sets of energetically degenerate three-dimensional conformer structures. This enhancement results in cleaner simulated multiplet shapes that exhibit a profoundly improved agreement with experimental spectra.

In a second step, an empirical prediction approach is built on a large database of NMR parameters obtained with the NMR workflow. The method aims at generalizing the concept of hierarchically ordered spherical description of environment (HOSE) codes by using a graph representation instead of strings for greater flexibility and efficiency. It further extends the concept to the prediction of J -coupling constants and potentially further empirical parameters.

The empirical NMR parameter prediction is based on DFT data for more than ten thousand molecules and is integrated into HQSpectrum. The final NMR parameters can be utilized to simulate spectra under high-field and low-field conditions alike.

Pulse-Sequence Optimization in Solid-State NMR Based on Effective Hamiltonians

Agniva Ganguly, Matthias Ernst

Pulse-sequence optimization has predominantly relied on optimal control theory, which transforms an initial state into a desired final state in an optimal way. This framework has proven highly successful in several domains, including the design of selective and broadband pulses as well as optimized polarization-transfer sequences. It considers experimental imperfections such as B_1 inhomogeneity, phase transients and is a computationally efficient optimization strategy. Nevertheless, it requires time evolution of all considered spins in the full Liouville space, which becomes computationally expensive for larger spin systems. A further limitation is that optimal control generates fixed-length sequences that cannot be concatenated to produce longer mixing times.

Pulse-sequence optimization based on an effective Hamiltonian circumvents this latter restriction. Such effective Hamiltonians can be derived through average Hamiltonian theory, Floquet theory, and continuous Floquet theory. In many cases they can be obtained from single-spin trajectories, which makes the approach computationally efficient. Continuous Floquet theory further enables effective-Hamiltonian calculations for near resonance conditions. By incorporating resonance-offset terms into the interaction frame faster convergence of the effective Hamiltonian series can be achieved, providing an ideal framework for pulse-sequence optimization.

We have implemented such an optimization scheme for solid-state NMR under magic-angle spinning, with effective Hamiltonians computed and optimized in MATLAB. As a proof of concept, we optimized the basic element of the $C7_2^1$ sequence to achieve band-selective polarization transfer. Further, more general band-selective rotor-synchronized zero-quantum recoupling sequences were optimized. The optimized sequences were validated through effective-Hamiltonian calculations, full spin-dynamics simulations of a dipolar-coupled two-spin system, and experiments on model spin systems, confirming expected performance.

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.

Quantitative Prediction of Exchangeable Proton Chemical Shifts

Socha, Ondřej, Pavlišová, Jana, Manna, Debashree, Dračinský, Martin

Predicting NMR chemical shifts of exchangeable protons in solution remains challenging due to strong solvent effects, hydrogen bonding, and conformational dynamics. We present a workflow combining molecular dynamics simulations using the UMA and MACE machine-learning force fields with ShiftML3, an ML tool originally developed for solid-state NMR chemical shift prediction. The approach was validated on several challenging systems, including water in various solvents, nucleobases, glucose anomers, alcohols, and alkylated acetamides. Despite its training on solid-state systems, ShiftML3 within the combined workflow reproduces experimentally observed trends and captures key solvent- and hydrogen-bonding-dependent effects on exchangeable proton shifts. Furthermore, we compared the performance of UMA- and MACE-based simulations against traditional implicit solvent DFT, finding that ML-MD ensembles significantly outperform static DFT in reproducing experimental solution-state proton chemical shifts. By leveraging machine-learning force fields with near-DFT accuracy, this method provides a computationally efficient alternative to ab initio MD, facilitating the analysis of complex hydrogen-bonding environments in solution via NMR spectroscopy.

AI-assisted "virtual" decoupling of HaCa for In-Cell NMR spectra

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We are looking for solutions to make in-cell NMR possible. In-cell NMR can provide atomic-scale information on proteins in their native cellular environment, including their interactions and conformational equilibria. However, measurements must be performed under physiological conditions of pH and temperature in a highly crowded environment, which limits the choice of suitable pulse sequences.

We designed a $^{13}\text{C}\alpha$ -detected $^1\text{H}\alpha$ - $^{13}\text{C}\alpha$ pulse sequence to improve sensitivity under these conditions. During ^{13}C acquisition, the $^{13}\text{C}\alpha$ - ^{13}CO scalar coupling can be refocused, but not the $^1\text{J}(^{13}\text{C}\alpha$ - $^{13}\text{C}\beta)$, resulting in peak doubling. We showed that this coupling can be removed after acquisition using a deep neural network (DNN) trained on 500,000 synthetic spectra. This "virtual" decoupling performs well even on spectra with many overlapping peaks, which are common for the intrinsically disordered proteins (IDPs) we study (α -syn, tau). The resulting linewidths are just a few Hz in both dimensions, ensuring a close-to-comprehensive analysis of IDPs at every pH and T.

We also explored a variant in which ^1H decoupling is omitted during acquisition, shortening the interscan delay. Quadruplets appear due to $^1\text{J}(^{13}\text{C}\alpha$ - $^{13}\text{C}\beta)$ and $^1\text{J}(^{13}\text{C}\alpha$ - $^1\text{H}\alpha)$, which are appropriately refocused by an adapted DNN. However, weak $^2\text{J}(^{13}\text{C}\alpha$ - $^1\text{H}\beta)$ and $^2\text{J}(^{13}\text{C}\alpha$ - $^1\text{H}_\text{N})$ couplings cause peak broadening, which provokes peak overlaps in IDPs' spectra. We discuss the advantages and limitations of both approaches for in-cell NMR studies of disordered proteins.

Targeted Client Recruitment for Federated NMR Metabolomics: Privacy-Preserving Metabolic Syndrome Detection

Legaristi, Jon, Alberdi, Ane, Iriarte, Asier, Gil-Redondo, Rubén, Roman, Ibai, Ibañez de Opakua, Alain, Embade, Nieves, Ayala, Unai, Millet, Óscar, Larrinaga, Felix

Introduction. NMR-based metabolomics provides rich biomarker profiles for metabolic syndrome (MetS) prediction, yet clinical spectral datasets are distributed across institutions governed by strict data-sharing regulations. Federated Learning (FL) enables collaborative model training without pooling raw spectra; however, standard FL is hampered when centres exhibit severely heterogeneous scenarios.

Objectives. We aim to address inter-centre heterogeneity and label imbalance in FL, enabling privacy-preserving MetS detection from distributed NMR metabolomics data.

Methods. We propose a targeted client-recruitment strategy that performs a pre-training selection of a balanced federation pool based on label distribution and diversity criteria, with an optional data-volume weighting term.

Results. Applied to an NMR metabolomics cohort (N=13,200; 22 features; 1.9% MetS+) across 100 simulated centres using deep learning local models under Dirichlet-controlled heterogeneity, the proposed strategy selected 27 centres (8–15% of available data) and achieved AUROC = 0.955 ± 0.006 and sensitivity/specificity of 0.891/0.921, surpassing a centralised oracle on 100% pooled data (AUROC = 0.938) and standard FL with all centres (AUROC = 0.924), revealing that recruitment quality, not data quantity, is the key determinant of minority-class detection performance.

Conclusions. The proposed strategy offers a practical, privacy-preserving and computationally efficient path to multi-site MetS screening from NMR metabolomics: superior diagnostic performance and lower variance with fewer than one-sixth of available centres, substantially reducing communication and computational overhead.

Keywords: Federated Learning, Metabolic Syndrome, Client Recruitment, Data Heterogeneity, NMR Metabolomics

Zero-Training Foundation Model vs. Purpose-Built Classifier: NMR Metabolomics Detection of Metabolic Syndrome

Iriarte, Asier, Roman, Ibai, Legaristi, Jon, Gil-Redondo, Rubén, Alberdi, Ane, Ibañez de Opakua, Alain, Embade, Nieves, Larrinaga, Felix, Millet, Óscar, Ayala, Unai

Introduction: Metabolic syndrome (MetS) — the co-occurrence of abdominal obesity, hyperglycaemia, hypertension, and dyslipidaemia — affects ~25% of adults worldwide. NMR metabolomics of serum offers a rich substrate for predictive screening, but deployable classifiers conventionally require bespoke pipelines of feature engineering and regularised model fitting.

Objectives: We ask whether a tabular foundation model, pre-trained once with zero task-specific training, can match a purpose-built classifier.

Methods: We benchmark **MetSCORE** — an O-PLS-DA model on 20,662 donors distilling 153 NMR variables into 22 features — against **TabPFN**, a transformer performing in-context learning: the training set is fed as context and predictions emerge in a single forward pass. Both models were evaluated under 10×5-fold cross-validation, 100 permutation tests, and an independent holdout cohort (n=661, prevalence 12.7%), with thresholds fixed at the training-set Youden optimum.

Results: In cross-validation, TabPFN reached AUROC 0.975 [0.966–0.986] vs. 0.931 [0.900–0.963] for MetSCORE (both $p < 0.01$). On independent validation, discrimination was equivalent (TabPFN 0.932 [0.904–0.954] vs. MetSCORE 0.933 [0.910–0.956]). At matched sensitivity (99%), TabPFN converted 70 false positives into true negatives — a 27% reduction in unnecessary referrals.

Conclusions: A zero-training foundation model matches a domain-optimised NMR classifier on discrimination and clinical operating performance, supporting low-overhead deployment in data-scarce settings.

Keywords: Metabolic syndrome, NMR metabolomics, tabular foundation model, TabPFN, in-context learning, clinical classification

GPU IMPLEMENTATION OF THE GRAPE ALGORITHM FOR QUANTUM OPTIMAL CONTROL

Keitel, Maximilian; Kuprov, Ilya

Pulse sequence design for nuclear magnetic resonance (NMR) experiments has traditionally relied on practical experience and time-consuming quantum mechanical analysis. However, experimental factors such as hardware filter functions and B1 field inhomogeneities can significantly impact performance. Optimal control is an excellent tool to achieve efficient magnetisation transfer under these imperfections and additional user-defined constraints (such as pulse power or pulse length). The computational framework and various formalisms for simulating NMR experiments generated by Optimal Control are well established. The popular and flexible Gradient Ascent Pulse Engineering (GRAPE) algorithm is readily available in Spinach and other software packages. Therein, simulations of robust GRAPE generated NMR experiments require a large number (time evolution as well as ensemble averaging over rotor grids, powder grids, B1 field distributions, transmitter offsets, etc.) of repetitive matrix operations, making them ideal candidates for GPU-accelerated algorithms. We present a GPU-accelerated implementation of the GRAPE algorithm within *Spinach*, significantly improving the efficiency of simulations by keeping data transfer overhead to a minimum and using custom-built sparse matrix objects. Tests on large liquid-state spin systems and large rotor-rank solid-state systems showed improved performance and scaling behaviour. A few novel GPU-accelerated shortcuts within the GRAPE algorithm enable time-efficient development of previously unfeasibly complex optimal control pulse sequences.

Acknowledgements

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NMRflux: From Hilbert Space to Deep Learning; A Quantum Mechanical Ecosystem in Julia for NMR Simulation and Analysis

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Modern NMR workflows remain fragmented across vendor software, signal processing pipelines, and machine learning environments. Although NMR data are physically structured, coordinate dependent, and complex valued, they are often reduced to generic tensors before AI analysis, separating learning workflows from their spectroscopic context. We present NMRflux, an open source ecosystem written in Julia, a high performance scientific programming language, integrating quantum spin simulation, coordinate aware processing, and deep learning within a common type system.

At its core is SpectData, which couples spectral arrays with physical axes while remaining compatible with Julia arrays. SpinSim.jl supports Hilbert space simulation of coupled spin systems using sparse operator algebra, allowing FIDs and spectra to be generated from Hamiltonians. Apodisation, zero filling, Fourier transformation, phasing, and baseline correction are callable components forming transparent workflows. As a proof of concept for AI compatibility, we developed RINSE for one dimensional NMR spectral restoration. Here, NMRflux constructs paired reference and degraded spectra with controlled noise, phase distortions, and baseline artefacts. A one dimensional U Net trained on these pairs reduces stochastic and structured corruptions while preserving quantitative spectral information. In tests, RINSE reduced spin recovery error from about 25 to 34% in corrupted spectra to below 10% for SNR values of 200 and above. These results demonstrate NMRflux as a reproducible ecosystem for quantum simulation, signal processing, and AI enabled NMR analysis.

EasyNMR: Flow-based programming for versatile analysis of NMR spectra

Vosegaard, Thomas; Afrough, Armin; Pérez Mendigorrí, Maria

A common challenge in interdisciplinary studies across disciplines is that they often rely on interpretation of state-of-the-art experiments from e.g. NMR and EPR. Such data analysis typically requires complex data-processing and analysis workflows, often involving multiple software tools, nonstandard data formats, and advanced numerical modelling. Although commercial solutions exist and several academic tools are developed within individual research groups typically there is not one software solution for all analyses.

Here, we present EasyNMR, a web-based platform that applies principles of flow-based programming (FBP) to scientific data analysis workflows, with a primary focus on NMR spectroscopy. In EasyNMR, analysis steps are represented as interconnected flow objects with well-defined data interfaces, enabling users to graphically design, execute, and share complex workflows. This approach simplifies the construction of advanced analyses, automates data conversion between steps, and reduces the burden of software development and maintenance. Being web-based, EasyNMR avoids local installation and facilitates collaborative use, while a backend system allows seamless integration of external simulation and analysis tools executed in sandboxed environments. This includes popular software packages like SIMPSON, Spinach, EasySpin, NMRGlue, MRSimulator, and others.

To ensure self-contained and interoperable data handling, EasyNMR internally converts all supported scientific data into the Core Scientific Data Model (CSDM). This guarantees correct specification of multidimensional datasets and enables efficient remote data access, including partial data transfer for visualization and analysis. As a result, complete workflows involving large multidimensional datasets can be shared as lightweight workflow files, substantially improving reproducibility, collaboration, and long-term preservation of scientific analysis pipelines.

Controlling Coupling Evolution via Optimal Control

Weiß, Jannik, Luy, Burkhard

The control of scalar and dipolar coupling evolution represents a fundamental challenge in the design of radio frequency pulses for modern NMR spectroscopy as designed shaped pulses have to meet increasingly complex requirements.

Within the framework of optimal control, it is in principle possible to incorporate coupling evolution into the pulse design process, either to suppress it or to exploit it in a controlled manner. Nevertheless, the computational demands associated with simulating coupled spin systems present a significant obstacle. Here we aim is to achieve a significant reduction in the computing time required for pulse optimization through the targeted use of Average Hamiltonian Theory (AHT) and Optimal Control Theory (OCT), which allows the optimization of the task with single spin calculations.

An optimal control cost function for the targeted control of weak scalar and dipolar coupling interactions is developed on the basis of AHT. Through transformation into the appropriate interaction frame and subsequent averaging of the total spin Hamiltonian, a tractable form of the coupling Hamiltonian is derived that serves as the basis for corresponding cost functions in optimizations. Several strategies for constraining the energy of the optimized pulses were qualitatively evaluated, and the feasibility of achieving offset-selective control of coupling evolution using the proposed cost function were implemented successfully.

The optimized pulses demonstrate satisfactorily offset compensation and B_1 robustness. It was established that constraining the average power or total energy yields superior results in terms of maximum achievable coupling efficiency compared to amplitude-limited optimization. A further finding concerns the temporal structure of the optimized pulses. Within a single pulse, the control of magnetization and the control of coupling evolution in some studied cases appear to be temporally separated, and the two objectives frequently seem to act in opposition. As a direct consequence, full coupling efficiency cannot be achieved simultaneously with a reorientation of the initial magnetization. Fundamental differences were also identified between pulses with and without a centered inversion pulse applied to another channel. In pulses with centered inversion, magnetization control occurs predominantly in conjunction

with the ideal inversion pulse, whereas in pulses without centered inversion, magnetization control is confined to the terminal position in the pulse.

A coupling-selective refocusing pulse was furthermore designed specifically for the selective $\text{H}\alpha, \text{C}\alpha$ -HSQC experiment, in which the $\text{C}\alpha$ protons of amino acids are detected in place of the amide protons.

Solvent-Suppression in NMR Spectra Using Deep Learning

Parisa Hasani, Amir Jahangiri, Vladislav Orekhov

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Solvent signal in biomolecular NMR is an unnecessary and often dominant component that introduces artifacts. Its intense resonance obscures nearby peaks, distorts baselines, and complicates quantitative analysis. Traditional experimental suppression approaches, such as pre-saturation, WATERGATE, and excitation sculpting, attenuate the solvent signal but often leave significant residual solvent artefacts that hinder spectral analysis. Moreover, these methods assume prior knowledge of the solvent-signal position and are typically optimized for the water signal near the centre of the spectrum. In recent years, neural networks have been increasingly applied to NMR. Here, we present a deep-learning approach for solvent suppression in multi-dimensional NMR spectra. A version of our MRAi deep-learning architecture was trained to suppress residual solvent signals without predefined frequency assumptions, thereby enabling removal of multiple solvent signals. The model preserves noise characteristics and spectral peaks, enabling reconstruction of complete 2D ^1H - ^{15}N correlation spectra without manual parameter tuning. The model was tested on four proteins: azurin, tau, maltose-binding protein, and ubiquitin. Performance was evaluated using correlation coefficients and RMSD within a fully two-dimensional framework. The method generalizes across noise levels, spectral resolutions, solvent-peak positions, and solvent-artefact patterns, consistently achieving strong suppression while maintaining accurate signal preservation. These results demonstrate a frequency-agnostic, data-driven alternative to traditional algorithm-based water-suppression techniques, offering improved performance and robustness.

Keywords: NMR, Solvent Suppression, Deep Learning, Neural Network

FAIRMD: Open-access All-atom Biomolecular Simulations to Interpret NMR Experiments

Miettinen, Markus

While NMR spectroscopy offers unique insights into the structure and dynamics of biomolecules, interpretation of the experimental data often remains challenging. The FAIRMD Databank, successor of the NMRlipids Databank (*Nat. Commun.* **15** 1136 (2024)), openly available at fairmd.org, provides hundreds of all-atom molecular dynamics (MD) simulation trajectories and tools to intuitively interpret NMR parameters in complex biological systems, focusing on lipid bilayers and intrinsically disordered proteins (IDPs). To provide direct connection between experiment and simulation, it uses observables directly calculable from the MD simulation such as relaxation parameters (R_1 , R_2 , hetNOE), and for lipid bilayers, C–H bond order parameters. This enables automated systematic comparisons, facilitating validation of MD force fields and interpretation of experimental data. By implementing FAIR (Findable, Accessible, Interoperable, Reusable) principles, it enables rapid sharing and reuse of simulation trajectories, with analysis tools to connect raw simulation data to experimentally comparable parameters, benefiting both experimental and computational researchers.

BioNMR

Phospho-Regulated Intramolecular Communication Between the CTAD and FHA Domains of APLF in DNA Repair

KARAKI Reem, **BELLON** Theodore, **PELUPESSY** Philippe, **MODESTI** Mauro, **CARLIER** Ludovic, **FERRAGE** Fabien

DNA double-strand breaks are among the most cytotoxic lesions a cell can encounter, and their accurate repair by non-homologous end joining (NHEJ) is essential for genomic stability.

Successful NHEJ requires the coordinated assembly of repair factors at damage sites, a process heavily dependent on protein-protein interactions and post-translational modifications.

Aprataxin- and PNK-like factor (APLF) is a multifunctional scaffolding protein that plays an integral role in NHEJ by facilitating the accumulation of key factors such as XRCC4 at damaged chromatin. APLF contains an N-terminal forkhead-associated (FHA) domain, which typically recognizes phospho-threonine motifs, and a C-terminal acidic domain (CTAD) of unknown regulatory function. While the FHA domain is known to engage phosphorylated partners including XRCC4, whether the CTAD participates in regulatory interactions has not been explored.

In this study, we identify a direct interaction between the CTAD and FHA domain of APLF and investigate its regulation by site-specific phosphorylation. Using NMR spectroscopy, we demonstrate that the CTAD is phosphorylated by casein kinase 2 (CK2) at two distinct residues, T479 and S483. Time-series HSQC experiments reveal that phosphorylation occurs in a sequential manner with the phosphorylation of S483 followed by the phosphorylation of T479. Notably, T479 resides in a sequence context analogous to the canonical pT233 recognition motif of XRCC4, suggesting a conserved mode of FHA engagement. Consistent with this, CK2-mediated phosphorylation of the CTAD significantly enhances its affinity for the FHA domain. Quantitative two-way titrations experiments using isotopically labeled proteins confirm a binding affinity in the low K_d for the doubly phosphorylated CTAD.

Molecular mechanism of RocS-mediated DNA-membrane anchoring and its role in chromosome segregation in *Streptococcus pneumoniae*

Lefeuve, Martin, Alvarez-Mena, Ana, Morvan, Estelle, Lambert, Clara, Kallisseri Parambil, Anagha, El Mammeri, Nadia, Dufourc, Erick, Feuillie, Cécile, Grangeasse, Christophe, Habenstein, Birgit

Accurate chromosome segregation during bacterial cell division is a fundamental process for bacterial viability. In the human pathogen *Streptococcus pneumoniae*, the protein RocS has been identified as a key player in chromosome segregation, yet, its molecular mechanisms remain poorly understood. RocS combines a DNA-binding domain (N-ter wHtH domain), and a short membrane anchor (C-ter AH domain). Because RocS activity is tightly regulated in space and time, we hypothesized a stringent interplay with the bacterial membrane and first focused on the membrane-anchor interactions. Combined MAS and wide-line NMR together with real-time AFM imaging we revealed that the membrane anchor folds into a short membrane-inserted kink-helix motif connected to an extended linker, with membrane insertion locally perturbing lipid packing. Composition and fluidity of the lipid bilayer in turn influences the interactions of the anchor with the membrane. Consistently, mutational analysis showed that altering a single glycine residue significantly disrupts chromosome segregation and affects membrane properties in vivo. Together, our findings provide a mechanistic explanation for RocS membrane targeting and reveal general principles by which the highly conserved membrane anchor can coordinate protein targeting during cell division. To address the mechanistic aspects of chromosome binding, the wHtH domain of RocS was produced, purified, and refolded using a denaturation–renaturation strategy, and subsequently characterized by solution-state NMR. We will here present the mechanistic aspects of RocS membrane targeting as well as first results of NMR chemical shift assignment and secondary structure analysis of the DNA-binding wHtH domain.

Identifying Ligands that Disrupt the HuR–RNA Interaction through an Integrated NMR Approach

Gado Irene, Garbagnoli Martina, Listro Roberta, Collina Simona, Carlomagno Teresa, and Vasile Francesca

Hu/ELAV-L proteins are RNA-binding proteins (RBPs) that regulate gene expression at the post-transcriptional level by controlling mRNA stability and translation. Among them, HuR (ELAVL1) is a key regulator implicated in cell proliferation, tumorigenesis, invasion, and metastasis. Its overexpression and cytoplasmic accumulation are associated with poor prognosis in several cancers, making HuR an attractive therapeutic target.

Disrupting the interaction between HuR and its RNA targets represents an attractive strategy for the development of new therapeutic agents. In this work, we used an integrated NMR approach to identify and characterize small molecules targeting the HuR RNA-recognition interface.

First, ^{15}N -labeled HuR was expressed, and protein-based NMR experiments were performed to define the RNA-binding region. ^1H – ^{15}N HSQC spectra enabled residue-specific mapping of the interaction between HuR and its RNA target.

To facilitate ligand-binding studies, a decameric Peptide Nucleic Acid (PNA), designed to mimic the natural RNA sequence, was synthesized and evaluated as an RNA surrogate. Its interaction with HuR was confirmed by protein-based NMR experiments.

A series of rationally designed ligands was then investigated using complementary protein- and ligand-based NMR techniques, including Saturation Transfer Difference (STD) NMR.

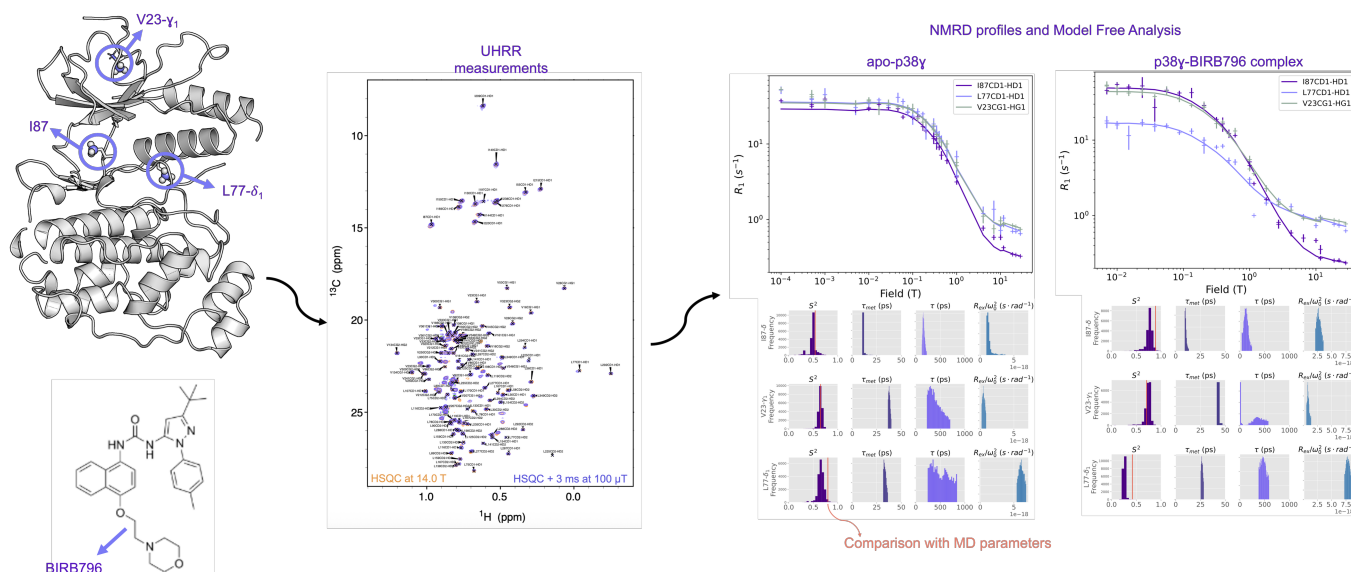
The results indicate that these ligands bind within the same RNA-recognition region identified for RNA and PNA, supporting their potential to interfere with the HuR–RNA interaction.

Overall, this study establishes an NMR-based strategy to probe ligand binding at the HuR RNA-recognition interface and provides a framework for identifying compounds capable of disrupting HuR–RNA complexes.

Insights into Molecular Motion

Aguilar Alva Ana Paula, Siemons Lucas, le Paige Ulric, Pelulessy Philippe, Bouvignies Guillaume, Teetz Jakob, Riniker Sereina, Wolff Nicolas, Cordier Florence, Garibay Jorge, Ferrage Fabien.

Relaxation is a unique tool to determine protein dynamics. Traditionally, relaxation experiments on proteins are performed at high fields (>10 T) yet, such measurements are not sufficient to fully characterize ns motions. In this work, we use high-resolution relaxometry to probe low ps to high μ s motions in great detail in the protein kinase p38 γ , for which evidence of allosteric pathways has been found. Our objective is to better understand such allosteric pathways through the observation of ns dynamics of side-chains in p38 γ in two states: its apo state and in complex with inhibitor BIRB796, with NMR relaxation and molecular dynamics (MD) simulations. To do so, we complement high-field relaxation measurements on ^{13}C methyl-bearing side chains with a new method, Ultrafast High-Resolution Relaxometry (UHRR). UHRR allows us to measure relaxation rates down to very low fields on a high-field spectrometer and get a full coverage of the spectral density function in a site-specific manner. We used a new apparatus to reach magnetic fields down to $100\ \mu\text{T}$ just above a 14.1 T magnet in as little as 79.5 ms. With these experiments, we obtain and analyze Nuclear Magnetic Relaxation Dispersion (NMRD) profiles to get a further understanding of the dynamics in p38 γ . We have measured a set of relaxation rates at over 28 magnetic fields covering 6 orders of magnitude. The analysis of relaxometry data of apo-p38 γ and the p38 γ -BIRB796 complex will be presented as well as the MD analysis performed to better understand the conformational dynamics of p38 γ . In conclusion, UHRR is a new technique which allows to study the dynamics of large proteins over a broad range of timescales (low ps to high μ s), allowing us, in combination with MD simulations, to fully characterize the dynamics of p38 γ .



Dynamics of microtubule-associated protein 2 c binding to protein kinase A from high-resolution relaxometry

Laníková, Alice; Jansen, Séverine; Padrta, Petr; Vařečka, Vojtěch; Bartošík, Viktor; Pelupessy, Philippe; Žídek, Lukáš; Ferrage, Fabien and Kadeřávek, Pavel

Microtubule-associated proteins 2 (MAP2) are a family of intrinsically disordered proteins (IDPs) regulating the dynamics of microtubules in neurons. The regulation of MAP2s' function relies on the interactions with their binding partners and post-translational modifications. Prime examples are kinases that bind and phosphorylate MAP2s, altering their affinity for microtubules. Due to the lack of stable structural arrangements, characterisation of motions in IDPs is a key step towards understanding the mechanisms underlying their functions.

We investigated how binding to protein kinase A (PKA) modifies the dynamics of the disordered N-terminal region of the MAP2c isoform. It has been previously found that the natively disordered MAP2c forms a stable helical arrangement when bound to the dimerisation and docking domain of PKA. To understand the change in MAP2c dynamics upon binding of PKA, we measured nitrogen-15 relaxation rates at multiple high fields, complemented by high-resolution relaxometry (HRR) measurements. The determination of relaxation rates over about three orders of magnitude, from 50 mT to 20 T, opens a wide window on motions on ns-ps timescales, typical for important segmental reorientations of IDPs.

The analysis of this large dataset gives more insight into the dynamics of MAP2c than conventional high-field NMR. The NMR longitudinal relaxation dispersion profiles elucidate residue-specific motional changes arising from the interaction. Interestingly, residues outside the binding site also show increased rigidity, highlighting long-range interactions in MAP2c. Overall, HRR combined with high-field relaxation provides a comprehensive description of the dynamics of the disordered MAP2c, offering insight into the mode of interaction with PKA.

Thermodynamic Insights and Allosteric Communication in Oncogenic KRAS G13D Revealed by ILVMT Methyl NMR spectroscopy

Silva, Micael S.; Coudeville, Nicolas; Orts, Julien

KRAS is widely considered undruggable, in part because shallow or transient pockets are coupled to distributed allosteric networks that reweight conformational ensembles. The structural and dynamic organization of these networks remains incompletely defined. Focusing on the clinically relevant G13D mutant, we implement an ILVMT methyl-labeling strategy that provides dense, high-sensitivity probes across both lobes and the partially disordered switch regions. By integrating Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion with exact nuclear Overhauser effects (eNOEs) and temperature-dependent build-up analysis, we quantify excited-state populations and exchange kinetics on the μ s–ms timescale and extract precise inter-methyl distances and thermodynamic parameters.

In the active (GMPPNP-bound) state of KRAS G13D, ILVMT methyl CPMG reveals lobe-synchronized exchange via a sparsely populated, higher-energy intermediate distinct from the canonical state 1 (open) and state 2 (closed), consistent with earlier isoleucine-only studies. At lower temperatures, the effector lobe, dominated by Switch I/II, exhibits slightly slower exchange than the allosteric lobe, indicating modest kinetic asymmetry. Temperature-dependent cross-relaxation rates and methyl–methyl eNOE-derived restraints report segment-specific entropic signatures and define atomic-level routes from the nucleotide pocket through the switches to distal surfaces, revealing temperature-dependent long-range coupling and allosteric pathways.

Together, these results provide a structural and thermodynamic framework for how GTP-state activation and the G13D mutation sculpt KRAS allostery, helping to explain its perceived undruggability and pointing to a potential new allosteric binding site.

NMR analysis of Transthyretin dynamics directly in human serum

de Lourenço, Isabella Ottenio, Cantarutti, Cristina, Mimmi, Maria Chiara, Verona, Guglielmo, Mangione, Palma Patrizia, Giorgetti, Sofia, Bellotti, Vittorio, Corazza, Alessandra

The amyloidogenic conversion of transthyretin (TTR) is strongly influenced by its natural biochemical environment, which determines the protein's stability, dynamics, and aggregation. Metal ions bind specific sites on TTR, modulating its stability, while human serum albumin (HSA) suppresses TTR amyloid formation, and reduced HSA levels correlate with accelerated deposition, suggesting a protective effect *in vivo*. Despite these insights, most structural studies of TTR have been performed in simple buffers, which fail to capture crowded molecular interactions and dynamic behavior present in physiological conditions. We characterized isotopically labeled human TTR directly in human serum depleted of endogenous TTR, enabling NMR analysis of its structural and dynamic properties in a native-like medium. We also investigated the influence of metal ions through titrations with EDTA, EGTA, and TETA. Complementary relaxation experiments were performed in buffer with and without HSA, including titrations of paramagnetic ions to probe flexible and transient interaction regions. Our results show that the overall conformation of TTR in serum is highly similar to that in buffer, even at physiological concentrations. Specific changes in chemical shift and the disappearance of signals from flexible regions were observed, consistent with paramagnetic effects. In the presence of HSA, an increase in peak intensity was detected for residues near the thyroxine-binding pocket, indicating weak and transient interactions. Relaxation measurements revealed a decreased in transverse relaxation (μs – ms) and slight increase in longitudinal relaxation (ps – ns), suggesting local conformational stabilization. Translational diffusion remained unchanged with HSA, confirming fast-exchange binding. These findings demonstrate that the native serum environment modulates TTR dynamics, stabilizes specific regions, providing new insights into how physiological conditions influence amyloidogenic processes.

Mapping the FF domain folding pathway via structures of transiently populated folding intermediates and understanding its interaction with ANS

Thapliyal Nemika, De Debajyoti, Tiwari Ved Prakash, Vallurupalli Pramodh

As the detection and characterization of fleetingly populated protein conformational states in solution remains a challenge, the protein folding problem remains unsolved in the sense that accurate atomistic models of how protein molecules fold into their native conformations from an unfolded ensemble are still elusive. Recent developments have made the NMR Chemical Exchange Saturation Transfer (CEST) experiment a powerful method to detect transiently populated protein conformational states. In this talk, I will discuss how we have used CEST NMR experiments to describe the folding trajectory of FF domain from human HYPA/FBP11. The CEST experiments provide a comprehensive four-state kinetic model for FF domain folding where the unfolded (U) state reaches the folded (F) state via two intermediates I1 & I2 (both populated to less than 1%). We have obtained the structure of the I2 state that along with the previously known intermediate I1, provides comprehensive description of FF domain folding trajectory. CEST derived urea *m*-values of the different states provide insights into the extent of compaction along the folding pathway. Our data establish that unlike the partially disordered I1 state, the I2 intermediate is well ordered and compact like the native conformer, while retaining nonnative interactions similar to those found in I1. Interestingly, the native state of the FF1 domain from human p190- A Rho GAP resembles the I2 conformation, suggesting that well-ordered folding intermediates can be repurposed by nature in structurally related proteins to assume functional roles. Moreover, I will discuss how these four states of the FF domain interact with 8-Anilino-1-naphthalene-sulfonic acid (ANS) which is traditionally used to detect the presence of molten globule states.

Probing Lipid Dynamics in Hypomyelinated *Ex Vivo* Brain Sections Using ssNMR with Dynamics-Based Spectral Editing

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Lipids constitute approximately 55% of the human brain and 78% of myelin dry weight, with a composition distinct from that of other tissues. Disruptions in lipid metabolism can alter membrane fluidity and integrity, as well as cellular signalling and energy storage, and have therefore been implicated in neurodegenerative diseases including multiple sclerosis (MS). Solid-state NMR (ssNMR) provides a unique approach for probing bulk lipid dynamics and membrane organisation in *ex vivo* tissue samples from relevant MS animal models.

In this work, tissue sections from the olfactory bulb, cerebral cortex, and cerebellum of wild-type (WT) and genetically modified (GM) hypomyelinated mice were analysed using ¹³C CPMAS and INEPT ssNMR experiments. WT and GM mouse brains were provided by the Káradóttir group at the Cambridge Stem Cell Institute. Brain tissue was harvested, sectioned, snap-frozen, and ground into a fine powder in liquid nitrogen prior to packing into the ssNMR rotor. This establishes a reliable sample preparation method that maintains the tissue in a frozen state throughout, minimising myelin degradation and preserving tissue integrity.

A significant increase in signal intensity was observed in T₁(¹H)-edited ¹³C CP and INEPT spectra of the GM cerebral cortex, consistent with decreased T₁(¹H) and prolonged T₂(¹H) relaxation times. These changes may reflect increased bulk lipid mobility arising from altered membrane fluidity and phase in oligodendrocytes, potentially linked to the impaired myelin maturation and compaction.

Dynamic NMR structures of G-quadruplex DNA modulated by cytosine methylation, ligand binding, and protein interactions

Janez Plavec

Cytosine methylation, a key epigenetic modification involved in the regulation of gene expression, raises intriguing questions regarding its role in the formation and thermodynamic stability of G-quadruplex (G4) structures. We investigated the impact of 5-methylcytosine (C^m) on the well-characterized bcl2Mid G4 structure, which forms within a highly GC-rich region known to influence BCL2 gene expression. Using solution-state NMR spectroscopy in combination with complementary biophysical techniques, we identified an unexpected and pronounced sequence-specific effect of C^m on the folding kinetics of the bcl2Mid G4.

Specifically, substitution of cytosine at position C6 with 5-methylcytosine ($C6^m$) significantly slows G4 folding kinetics, indicating that even a single methyl group can alter the conformational energy landscape of the structure. Moreover, this modification perturbs the equilibrium between major and minor conformations in the presence of K^+ ions, which are known to stabilize G4 structures. Notably, the increased population of the minor conformation enabled detailed structural characterization of its previously unidentified topology. In addition to influencing conformational equilibria, the presence of a single C^m residue induces local structural rearrangements within the major G4 conformation, subtly modifying base stacking interactions and loop geometry. These structural perturbations are accompanied by a measurable decrease in thermodynamic stability, further underscoring the sensitivity of G4 architecture to epigenetic modification.

Collectively, these findings demonstrate that C^m not only modulates G4 polymorphism but also reshapes the folding pathway and stability landscape of the structure. This highlights a previously underappreciated mechanism by which cytosine methylation may regulate G4 formation and dynamics, with potentially significant implications for the epigenetic control of gene expression.

Towards streamlined characterization of intrinsically disordered protein regions

Structural biology as a field has provided unprecedented insights into the architecture and function of well-structured proteins. Conversely, intrinsically disordered protein regions (IDRs), which represent as much as 40% of the human proteome, are still largely in the dark. Furthermore, IDRs act as hubs for multivalent protein-protein interactions and are heavily modified by post-translational modifications, which are both effective modulators of protein function.

It has become clear that IDRs' specific conformational ensembles respond to cell signalling and dictate protein function. Access to a large, curated, library of experimental ensembles would be transformative for the field, however, determining these ensembles experimentally remains challenging. It requires integration of multiple different data including SAXS, smFRET or EPR, and multiple NMR parameters. To date, this process is neither well-established nor streamlined.

NFAT is the transcriptional master regulator of T-cell activation. Transactivation of target genes relies on interaction between NFAT's N-terminal IDR and co-activators, a process that we have shown to be dependent on phosphorylation at Ser23. Phosphorylation rearranges NFAT's conformational ensembles, which dictates the affinity for the co-activator. Despite the wealth of semi-quantitative data, it has proven challenging to calculate a representative model. We are currently benchmarking ensemble generation tools and establishing a data-driven refinement platform. Taking the example of NFAT, I will discuss limitations of the process and potential opportunities for streamlining and uniformizing the community's efforts towards "unstructural" biology.

Decoding Non-Canonical Nucleic Acid structural paradigm by NMR: From solution to the cellular context.

Ghosh, Anirban, Lenarcic Zivkovic, Martina, Trantirek, Lukas.

Nucleic acids are inherently polymorphic and can adopt diverse non-canonical three-dimensional structures beyond the classical B-DNA helix. Among them, G-quadruplexes (G4s) are widely studied guanine-rich structures found in eukaryotic, and viral genomes. Their folding, stability, and structural interconversion are linked to gene regulation and disease mechanisms. Because of their biological relevance, small molecules (ligands) that modulate G4 folding are promising therapeutic candidates. However, G4 polymorphism and ligand specificity complicate rational drug design.

Focusing on human telomeric G4s, I showed that the benchmark ligand Phen-DC3 induces a structural switch from a hybrid-1 to an antiparallel topology via conformational selection. This work provided the first atomic-level structural insight into ligand intercalation and chaperonic activity within an intramolecular telomeric G4. In subsequent studies, I investigated bis-quinolinium ligands (Phen-DC3 and 360A) targeting quadruplex-duplex hybrids from the PIM1 oncogene. Here, both ligands bind at the quadruplex–duplex junction but display distinct binding dynamics. These findings challenge conventional models that assume ligand stacking onto fully folded, monomorphic nucleic acid targets.

To probe nucleic acid folding and ligand binding epitome in cellular environment, I developed a ^{19}F -based in-cell NMR methodology in cellular models. Our results show that non-canonical nucleic acids continuously sample multiple folded and unfolded states in the intracellular environment.

Collectively, these studies provide fundamental insights into non-canonical nucleic acid conformational modulation, which have applications in medicinal chemistry, drug design and screening, , biomolecular engineering, etc.

NMR-Based O-Chemotyping of Pathogenic *Escherichia coli* Strains from Uruguayan Cattle

Miranda, Juliana; Pérez-Tabárez, Cecilia; Cuchman, Sylvia; Lopez-Gambetta, Andrés; Corvo, Ileana; **Fontana, Carolina.**

Pathogenic *E. coli* strains affecting livestock are associated with severe conditions such as neonatal diarrhea and bovine mastitis. Accurate structural characterization of the O-antigen polysaccharide (O-Ag), which defines the serogroup, is essential when discrepancies arise between serological and molecular typing.

Here, we applied a streamlined NMR-based O-chemotyping strategy to characterize bovine isolates from Uruguay. The O-Ags were obtained by mild acid hydrolysis directly from bacterial pellets (100 mL cultures), followed by de-O-acetylation and a single desalting step. Structural analysis was performed at 11.4 T using a TXI probe, and assignments were supported by comparison with ECODAB database entries and published literature.

Strain JM-EDL35 was shown to express the previously described *E. coli* O20ab O-Ag structure, which has been postulated to be encoded outside the canonical O-antigen biosynthesis gene cluster (*galF-gnd* locus). In contrast, strain JM-EDL18 shares the same disaccharide backbone but carries a novel branching substitution, revealing previously unreported structural diversification within *E. coli* O20-related O-Ags. Importantly, an additional strain from our collection was proved to express an O-Ag structure fully consistent with its canonical gene cluster organization, which has been previously presumed non-functional and, thereby, confirming its functionality despite earlier reports suggesting otherwise. Together, these findings demonstrate the coexistence of at least three distinct O20-related O-chemotype variants locally. Beyond O20-related isolates, additional strains corresponded to classical O1C, O88, and O146 chemotypes, while strains JM-EDL15 and SC-UY4 shared backbone motifs with O139 and O99, respectively, but differed in branching substitutions.

Overall, these results highlight the power of NMR spectroscopy as a robust and cost-effective platform for resolving O-Ag structural diversity, refining O-typing, and strengthening genotype–phenotype correlations in epidemiologically relevant isolates.

Proton Transfer Kinetics in Protein Side Chains by pH-dependent NMR Relaxation

Weininger, Ulrich, Raum, Heiner N., Jordan, Paula L., Akke, Mikael

Ionizable amino acid side chains in proteins undergo continuous protonation and deprotonation, reshaping electrostatic interactions and hydrogen-bond networks. They directly impact key biological functions such as general acid–base catalysis, where individual proton-transfer steps can be rate limiting. Quantitative, site-resolved descriptions of protonation kinetics are essential for linking protein structure and mechanism.

Solution NMR spectroscopy has long enabled residue-specific characterization of protonation (and thereby the presence and absence of charges) equilibria: chemical shifts of certain atoms report directly on protonation state and thus allow determination of pK_a values. Here, this equilibrium perspective is extended to kinetics by combining pH-dependent chemical-shift analysis with pH-dependent NMR relaxation measurements, which provide access to protonation and deprotonation rate constants in a site-specific manner.

Using this approach, proton exchange dynamics have been quantified for the following ionizable groups: Asp, Glu, His, Tyr, and Lys. The resulting kinetic parameters show that proton transfer is generally very fast and can approach the diffusion limit. Moreover, systematic linear free-energy relationships connect kinetic rate constants to thermodynamic acidity (pK_a), capturing both hydronium-mediated exchange at low to neutral pH and hydroxide-mediated exchange at basic pH. Together, these findings establish a unified framework for describing and predicting protonation and deprotonation kinetics of protein side chains across the full pH range and for essentially any pK_a value.

Quantifying mitochondrial bioenergetics in human brain organoids using ^{31}P MR spectroscopy

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Understanding how MRI contrast reflects cellular processes underlying brain aging and neurodegeneration remains a major challenge in neuroimaging. Conventional MRI signals arise from complex interactions between neurons, glia, metabolism, and inflammation, making it difficult to identify the biological mechanisms responsible for observed imaging signatures in vivo. Human iPSC-derived brain organoids provide a controllable human model in which defined cell populations, genetic backgrounds, and metabolic states can be experimentally manipulated. We developed a high-field NMR/MRI platform enabling prolonged metabolic imaging of live brain organoids. A Bruker 14 T system was combined with an NMR-compatible incubation chamber that maintains physiological conditions through closed-loop perfusion of warmed (37 °C), gas-equilibrated culture media with continuous monitoring of temperature, pH, pO₂, and pCO₂. This setup enables stable long-duration measurements while preserving organoid viability. Organoids were imaged with T₂*-weighted MRI and monitored using ^{31}P magnetic resonance spectroscopy to probe mitochondrial bioenergetics through ATP, ADP, and inorganic phosphate signals. Stable ^{31}P spectra were observed for ~43 hours, confirming sustained metabolic viability during extended measurements. We then investigated mitochondrial metabolism in control and DJ-1 knockout midbrain organoids. Acute hypoxia (5% O₂) produced a 7–14% decrease in ATP/Pi ratios in wild-type organoids, while DJ-1 knockout organoids showed 5–13.5% lower ATP levels under normoxia. These results demonstrate that ^{31}P MR spectroscopy can detect mitochondrial bioenergetic changes in living brain organoids and provide a controllable framework for linking MR signals to cellular metabolic states relevant to neurodegeneration.

NMR characterization of CHCHD10 and its interaction with the TDP-43 C-terminal domain

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The mitochondrial protein CHCHD10 has recently emerged as a genetic factor associated with amyotrophic lateral sclerosis and related neurodegenerative disorders. Despite its increasing relevance in neurodegeneration, the structural organization of CHCHD10 and its molecular relationship with the aggregation-prone RNA-binding protein TDP-43 remain poorly understood. Here we combine solution nuclear magnetic resonance spectroscopy with complementary biophysical approaches to characterize the conformational properties of CHCHD10 and investigate its interaction with the C-terminal domain of TDP-43.

Backbone resonance assignments of full-length CHCHD10 were obtained using a standard set of triple-resonance experiments. Chemical shift analysis together with circular dichroism spectroscopy reveals the presence of a structured helical core consistent with the conserved coiled-coil-helix-coiled-coil-helix domain, whereas the N-terminal region displays increased conformational flexibility. Concentration-dependent NMR measurements indicate that CHCHD10 samples a monomer–dimer equilibrium in solution, suggesting a dynamic self-association behaviour mediated by the helical domain.

Protein–protein interaction studies monitored by chemical shift perturbation experiments show that CHCHD10 interacts directly with the C-terminal region of TDP-43 through residues located within the same helical region. Complementary aggregation assays indicate that the presence of CHCHD10 modulates the aggregation behaviour of TDP-43 in vitro, supporting the formation of transient soluble complexes between both proteins.

These results provide a structural framework for understanding the molecular interplay between CHCHD10 and TDP-43 and illustrate the power of solution NMR to characterize dynamic protein–protein interactions relevant to neurodegenerative disease mechanisms.

Conformational dynamics in the terminal Complex I membrane subunit Nqo12

Liebau, Jobst; Saura, Patricia; Badolato, Sofia; Gamiz-Hernandez, Ana P.; Beghiah, Adel; Kaila, Ville R. I.

To transduce energy, the membrane domain of Complex I actively pumps protons across the membrane, against an external proton gradient. However, the mechanism of this proton conduction and its regulation remain poorly understood. Here, we investigate the isolated, terminal antiporter-like subunit Nqo12 of Complex I from *T. thermophilus*. Employing ^{19}F -NMR, we observe that Nqo12 adopts distinct conformations, which show pH-dependent population changes. Consistently, the pH-dependence of the conformational ensemble is decoupled when a central conserved titratable residue is mutated into a non-titratable residue. Employing ^{19}F -CEST experiments, we demonstrate that the antiporter module undergoes conformational exchange between the pH-independent and the pH-dependent conformations. We further observe that mutation of a functionally crucial ion-pair residue that results in gate opening, gives rise to a shift in the conformational ensemble of the antiporter module, and an increased overall apparent pK_a . Our findings are consistent with constant pH-MD simulations, indicating that electric field effects control the conformational ensemble, and corroborate the central role of buried ion-pairs in controlling the proton pumping in Complex I. Based on the combined NMR data and MD simulations, we suggest a mechanism, in which the antiporter module samples various conformations that control proton conduction *via* electric field effects, modulating the pK_a of titratable residues along the proton pathways.

Investigation of Envelope protein from SARS-CoV-2 in complex with a human host protein

Small multifunctional viral membrane proteins called viroporins are attractive drug targets but are structurally difficult to characterize in a lipid-bilayer environment with techniques besides MAS-NMR. Considerable work has been done on viroporins, usually with truncated constructs. We have successfully reconstituted the full-length Envelope (E) protein from SARS-CoV-2 in a lipid-bilayer. The composition of the bilayer matches the native ER-Golgi Intermediate Compartment (ERGIC) membrane where E localizes in an infected cell. Furthermore, we have also co-assembled a native host factor that binds to E. We have elucidated structural details about the viral-host protein complex using 4-dimensional spectra, ^{13}C -detected experiments at 55 kHz MAS, and with differential labelling providing information about both components in the complex. We further investigated the overall dynamics of the complex.

The oligomer state of E is also of particular relevance to understand the proposed ion channel function of this viroporin. It remains unresolved, with both dimeric and pentameric states proposed for the protein. We have leveraged the enhancement afforded by dynamic nuclear polarization (DNP) combined with ^{13}C -based Centerband Only Detection of EXchange (CODEX) experiment to determine the ensemble-averaged oligomer state of E in and out of complex with the host protein. To achieve this result, we carried out ^{13}C - ^{13}C mixing for several minutes to allow spin diffusion across large distances. The potential of this experiment is also demonstrated using a GB1 sample where unprecedented distances on the order of 1 nm can be reached between ^{13}C spins using 25 minutes of mixing. Our results highlight the power of hyperpolarization techniques to explore challenging biological questions.

Selective NMR Experiments for Isotope-Labelled Glycans

Nestor, Gustav, Okmane, Laura, Wiesinger, Piera

Glycans used in structural studies by NMR are rarely ^{13}C -labelled, preventing exploitation of the ^{13}C spectral dispersion in 3D or higher order NMR experiments, which would alleviate severe resonance overlap in their ^1H NMR spectra. In this presentation, I will address the lack of suitable NMR experiments for isotope-labelled glycans and present about our efforts to design NMR experiments that are tailored for such compounds. Our aim is to provide NMR tools for detailed characterization of glycan moieties in glycoproteins, carbohydrate-binding proteins, and carbohydrate-active enzymes. Currently used NMR experiments for uniformly ^{13}C -labelled glycans, such as ^1H , ^{13}C -CT-HSQC and HCCH-TOCSY easily display substantial spectral overlap that prevents more detailed analysis. With inspiration from protein and nucleic acid NMR experiments, we are adapting NMR experiments for targeting certain positions in the sugar ring. Although triple-resonance experiments are not useful for sugars containing only ^1H and ^{13}C , we can selectively target C1 with ^{13}C -selective pulses and C6 with CH_2 -selective pulse sequences. A set of glycan NMR experiments selectively mapping C1, C2, C3, C4, C5 or C6 of aldohexoses will be shown, as well as examples of such applications on small sugars and a glycoprotein. These experiments, recorded on uniformly ^{13}C -labelled glycans, offer an attractive alternative to selectively labelled sugars, which require chemical synthesis. By minimizing spectral overlap, the assignment of complex glycans is facilitated and it opens the possibility for detailed characterization of certain positions in terms of coupling constants, residual dipolar couplings and relaxation.

Antioxidant Kinetics Detection by Long-Lived Coherences in immune Cells

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For in-cell ¹H NMR studies, spectral resolution is limited by the coherent and incoherent relaxation mechanisms as well as by spectral overlaps. Long-lived coherences (LLC's), i.e., coherent superpositions of nuclear spin states of different parities, allow improvements in signal linewidths by up to an order of magnitude compared to standard nuclear magnetic states. We leverage LLC's in the indirect dimension for improved detection of biomarkers in cells without recurring to isotope enrichment. This is demonstrated by following oxido-reduction kinetics for glutathione (GSH), the most abundant endogenous antioxidant in cells. LLC's allow the filtering of reduced-GSH and oxidized-GSH signals on the background of other cell metabolites. We show the in-cell application of 2D LLC's in a human B-cell lines derived from Burkitt's lymphoma, commonly used as *in vitro* models for studying B-cell metabolism and immune responses (RAMOS and RAJI cells). In these cells, we distinctly observe the presence of reduced and oxidized glutathione signals (GSH/GSSG), and the LLC method enabled the follow-up of GSH slow oxidation kinetics on the timescale of hours.

Keywords: long-lived coherences (LLC's), singlet states, glutathione antioxidant, immune cells.

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Protein-based materials - using structural biology methods in materials science

Lendel, Christofer

In nature, proteins are the building blocks of many materials with intriguing properties, with spider silk as a prominent example. These materials have served as inspiration for man-made structures for long time but the challenge to produce synthetic materials with comparable properties from biobased resources remains. The key to achieve this is to gain control over the assembly of hierarchical structures from the protein building blocks. In this work, we combine several different methods (NMR, mass-spectrometry, X-ray scattering, AFM, and cryo-electron microscopy) with the aim to connect the molecular and/or nanoscale with the macroscopic material properties. My presentation will highlight how we use NMR methods developed for structural biology in the elucidation of hierarchical material structures. I will describe this in two recent examples from our research; the first is the assembly mechanisms of spider silk [1] and the second is the determination of high-resolution structures of amyloid-like protein nanofibrils formed by the milk protein β -lactoglobulin [2, unpublished results].

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Biophysical approaches for the early diagnosis of neurological disorders: alpha-synuclein and TDP-43 as case studies.

Cerofolini, Linda, Susini, Bianca, Staderini, Tommaso, Ciullini, Arianna, Della Role, Ilaria Linda, Bacinoglu, Merve Begum, Fiorucci, Letizia, Guerra, Elisa, Polykretis, Panagis, D'Andrea, Cristiano, Matteini, Paolo, Ravera, Enrico, Cazzaniga, Federico Angelo, Moda, Fabio, Fragai, Marco

Clinical diagnosis of neurological disorders, like alpha-synucleinopathies and TDP-43 proteinopathies, is challenging especially in the early stages. Structural data suggest the presence of distinct misfolded protein aggregates specific for each disease, that could represent key disease biomarkers.

Seed amplification assay (SAA) has been widely used to amplify aggregates present in biological fluids of patients, and can be implemented to detect misfolded protein aggregates.

Nuclear Magnetic Resonance has been here proposed as a tool to obtain disease fingerprints from protein aggregates obtained by SAA. In particular, hydrogen-deuterium exchange combined to NMR analysis has been used to this aim.

Aggregates of alpha-synuclein and TDP-43, obtained in well described experimental conditions and already structurally characterized at atomic detail by SSNMR or Cryo-EM, have been employed to develop novel methodologies. Then the analysis has been applied to amplified aggregates from the olfactory mucosa of patients affected by distinct diseases.

The presented methodology is sensitive to structural differences in fibrils of the same protein obtained under different experimental conditions. NMR could be used to create disease fingerprints useful for an early-stage diagnosis.

Characterisation of molecular interactions of IGF2BP1 with its target RNA, single nucleotides, and possible inhibitors

Munz, Benedict; Ghazy, Ehab; Tripathee, Sunita; Preschel, Aleksander; Hüttelmaier, Stefan; Sippl, Wolfgang; Balbach, Jochen

IGF2BP1 (Insulin-like growth factor 2 mRNA-binding protein) is an oncofetal RNA-binding protein consisting of 2 RRM- and 4 KH-domains each organised in pairs.

Chemical shift perturbation (CSP) analyses of the KH12 and KH34 didomains with 60 nucleotide-long SRF-mRNA-fragment identified the binding site of this RNA in the KH1-domain. Comparison with RNA with one N⁶-methyl-modification at an adenine (m⁶A) showed the strongest differences to unmethylated RNA close to a GxxG-loop in the RNA-binding pocket.

Moreover, CSP analyses of KH12 and KH34 with single nucleotides identified the different binding sites of different nucleotides. Considering the comparably low specificity of IGF2BP1, this is especially relevant to understand “secondary” binding sites, especially of nucleotides not part of the consensus sequence.

STD-NMR of single nucleotides with the KH14-tetradomain showed that pyrimidine nucleotides (CMP and UMP) show weak STD-effects overall, especially in the bases while purine nucleotides (AMP, m⁶AMP and GMP) showed similarly strong STD-effects in both sugar and base region.

These results might also be interesting for future inhibitor design based on nucleotides.

CSPs analyses have shown that Val231 is part of the binding site for inhibitors in the KH12-domain. This residue is close to the RNA-binding pocket established in earlier measurements.

STD-NMR of inhibitors with the KH14 domain showed which parts of the inhibitors are important for binding and which are dispensable and could be further optimised.

Functional amyloid studies of the intrinsically disordered human parathyroid hormone (PTH)

Ilja Gordijenko, Shubhra Sachan, Manuel Weber, Bruno Voigt, Ulrich Weiniger, Jochen Balbach

Dissecting our understanding of amyloid formation, in the context of benign proteins and peptides have been overviewed by malign proteins involved in neurodegenerative diseases. The human parathyroid hormone (PTH), derived as amyloid fibrils from secretory granules of parathyroid glands could lead to improvement in this field. The main role of PTH is to regulate the homeostasis of blood calcium and phosphate levels by activating its cognate G-protein coupled receptor. While the 84-amino acid hormone forms cross- β fibrillar amyloid structure by its activating and drug-approved N-terminal region (1-34), its C-terminal, intrinsically disordered region (IDR) (35-84), remains largely elusive. Various fibrillation assays show that the flanking IDR of PTH84 impairs the lateral filament order and reduces the thermodynamic stability to an extent that monomer release from the fibrils reaches a physiological level. Additionally, those experiments revealed that the IDR increases the critical fibrillation, oligomer concentration, and screens secondary nucleation in respect to the IDR-truncated PTH34. Paramagnetic relaxation enhancement (PRE) NMR spectroscopy is used to elucidate possible intra- and intermolecular interactions within PTH84.

**Unfolding of Human Ileal Bile Acid-Binding Protein
Monitored by High Pressure NMR**

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Human ileal bile acid-binding protein (hI-BABP), a member of the family of intracellular lipid-binding proteins (iLBP), plays a key role in the transcellular trafficking and metabolic targeting of bile salts. Disorder-order transitions and sparsely populated hidden states in hI-BABP are thought to be key elements of ligand recognition, with implications for bile salt recycling and bile salt-mediated signaling events. To improve our understanding of the structural determinants of hI-BABP stability, high pressure NMR was used to probe local packing interactions and characterize the folding/unfolding process. Heterogeneity in pressure response, revealed by residue-specific analysis of chemical shift and intensity changes, indicates a deviation from two-state unfolding. Four specific protein regions such as the N-terminal β -strand, the helical cap, a hydrophobic cluster at the bottom of the β -barrel, and segments of the HIJ-region have been found to exhibit a pressure response differing significantly from the rest of the protein. Our analysis further shows that a low-populated higher-energy state, inferred by NMR relaxation dispersion measurements, becomes less distinct from the ground state at high pressure, corroborating the hypothesis that conformational exchange between a closed and a more open EFGH-region, which mediates ligand entry, is related to a partial unfolding of the protein.

DNP enhanced solid-state NMR investigation of the role of histidines in the inward directed proton-pump Xenorhodopsin

Mayer, Anne, Lentge, Stella, Weber, Yannick, Fischer, Julian, Becker-Baldus, Johanna, Glaubitz, Clemens

The regulation of ion gradients across cell membranes is essential for life. In archaea, bacteria, and unicellular eukaryotes, one mechanism of regulation involves microbial rhodopsins (MRs), photosensitive retinylidene membrane proteins. Xenorhodopsin from *Nanosalina* sp. (NsXeR) is a light-driven inward proton pump with an unusual transport direction that may protect against alkaline environments or regulate intracellular signalling.

Histidine has unique properties due to its versatile interaction capacity, tautomeric variability, and pH sensitivity of the imidazole group. In some MRs, it participates in hydrogen bonds or salt bridges near the retinal Schiff base (RSB), acts as part of proton donor/acceptor complexes and can function as a pH sensor or ion selector.

In NsXeR, H48 is part of the RSB surrounding and may function as its proton acceptor. H94 likely belongs to the proton release group at the cytoplasmic side, while H225 is also located towards the cytoplasmic side but with unclear function. We investigated the histidine residues in NsXeR using solid-state NMR at ambient temperatures as well as under frozen conditions to enable DNP enhanced data acquisition and cryo-trapping.

Our 1D and 2D NMR and DNP data, together with bioanalytical measurements and biochemical assays, reveal unexpected protonation behaviour of H48 and H225 across different pH conditions, as well as potential interactions between these histidine residues and functional impairments upon histidine mutagenesis. These findings support the hypothesis that histidine residues in NsXeR play a distinct role in modulating both protein function and structural organization.

NOE-Derived Thermodynamics of Ligand Binding in BRD4 Bromodomains

A deep understanding of protein–ligand recognition requires not only structure and dynamics characterization, but also a quantitative description of the thermodynamic contributions governing binding. While NMR spectroscopy provides atomic-resolution access to structure and dynamics, experimental determination of enthalpic and entropic contributions at this resolution remains rare.

The human bromodomains BRD4-BD1 and -BD2 exhibit highly similar structures and share approximately 80% binding-site identity, yet differ by three orders of magnitude in their affinity for the ligand GSK973. This system therefore represents an ideal model to investigate how subtle differences in intermolecular interactions translate into large affinity changes.

Here, we use NOEs as quantitative probes of protein–ligand interactions to establish an atomic-resolution structure–dynamics–thermodynamics relationship for GSK973 binding to BRD4-BD1 and -BD2. Structural ensembles of the free and bound states were characterized by NMR, and NOE build-up curves were acquired and converted into distance restraints to derive multi-state conformational ensembles. In addition, NOESY experiments recorded at multiple mixing times and temperatures enabled the extraction of interaction-specific population distributions, from which enthalpic and entropic contributions to binding were determined.

This approach provides calorimetry-like information at atomic resolution, allowing quantification of the energetic contributions of individual intermolecular interactions and elucidating the thermodynamic origins of the pronounced affinity difference between BRD4-BD1 and -BD2.

Cyclic EF-Hand peptides: Insights into lanthanide binding and structure

Yulia Perevedentseva, Nikolai Klishin, Frederik Kohls, Ruslan Nedielskov, Björn Drobot, Heiko M. Möller

The production of lanthanide-binding peptides has experienced a significant boost in recent years following the discovery of lanthanide-binding proteins such as lanmodulin, which exhibits picomolar affinity towards lanthanides. However, translating this exceptional affinity to short peptide sequences has remained challenging. Initial studies on linear peptides revealed high flexibility and only moderate binding affinities. Therefore, cyclic peptides represent a promising direction for further development, as the conformational constraints imposed by cyclization may alter their properties compared to linear analogues significantly. Here, we present a systematic NMR investigation of a series of lanthanide-binding cyclic peptides and their complexes with lanthanides. Affinity measurements alone are insufficient to fully describe their binding behavior, necessitating detailed structural characterization of the resulting complexes. Binding affinities were thus determined using NMR titrations, while structural insights were obtained through paramagnetic NMR methods, particularly pseudocontact shifts. Due to the small size and dynamic properties of these peptides, conventional NMR approaches relying on NOEs only, provided limited information about structure. However, the incorporation of paramagnetic restraints enabled the extraction of long-range structural information and the characterization of the metal-coordinating environments. Our results provide new insights into the structure-affinity relationships governing lanthanide binding in cyclic peptides. This work contributes to a deeper mechanistic understanding and supports the rational design of peptide-based systems for selective lanthanide recognition, separation, and future application in the circular economy.

Numerous of immunocompromised patients every year are affected by fungal candida albicans infections, which may lead to deadly conditions. Therefore, the need for selective anti-fungal drugs is greater than ever before. Inhibiting the first Bromodomain (BD1) of the Bromodomain-containing factor 1 (Bdf1), which consists of BD1 and BD2, is seen as a promising therapeutic strategy, being essential for the fungi's viability and virulence. Proteins of the BET-family, including Bdf1 are essential for gene regulation and epigenetic adjustments.

In the process, the DSi-poised fragment library, consisting of 896 fragments, was tested for binding against BD1. Three common screening techniques, namely thermal shift assay (TSA), Saturation Transfer Difference (STD) NMR and HSQC-based screening were used to determine potential pharmacophore structures that interact with Bdf1BD1.

The first filter of the screening process was mixing four ligands each and testing each mixture on the afore in BL-21 Escherichia coli expressed and afterwards purified protein. In accordance with previously set merits, positive hits were identified for each screening technique. Additionally, all positive fragments of TSA as well as STD-based screening were tested via HSQC-based screening. Afterwards, the ligands in the mixtures that showed positive signals were individually tested for interaction with BD1. Finally, merged feature and shared feature pharmacophore structures were identified.

These small molecules show consistent interactions with the target, making them suitable starting points for optimization. Although further improvement is needed, they provide useful insight into structure-activity relationship and establish a basis for future medicinal chemistry developments.

Solid-state NMR methods to study membrane-bound peptides

Strandberg, Erik; Wadhwani, Parvesh; Ulrich, Anne S.

Membrane-active peptides – such as antimicrobial agents, cell-penetrating carriers and fusogenic sequences – are involved in many biological functions and are also interesting as potential drugs or drug delivery systems. Solid-state NMR is a highly informative method to study these peptides in lipid bilayers under quasi-native conditions. We will present an overview of some straightforward static solid-state NMR methods, using several examples to illustrate advantages and disadvantages of the different approaches.

To study the effect that amphiphilic peptides exert on the membrane lipids, ^{31}P -NMR of the phospholipid headgroup can give useful information about lipid phase behaviour and bilayer perturbation.

To observe the structure, dynamics and oligomeric architecture of peptides in membranes, isotope labelling has to be used, and macroscopic alignment of the lipid bilayers on glass plates tends to be necessary. Labelling the peptide backbone within a folded segment (such as an α -helix) with a single ^{15}N -labelled amino acid can provide an estimate of the peptide tilt angle. By incorporating a stiff ^2H - or ^{19}F -labelled amino acid into several different positions along the peptide backbone, accurate information about peptide conformation, orientation and dynamics becomes available.

We will present results particularly from two model peptides, the 21-mer KIA21 and the shorter 11-mer BP100, which form amphipathic helices in membranes and are proposed to permeabilize membranes via different modes of action. A combination of NMR methods was used to scan a range of conditions in search of different stable states, such as an oligomeric transmembrane pore, for which the detailed structures were determined in each case. NMR was also combined with molecular dynamics simulations.

Improved detection of magnetic interactions in proteins based on long-lived coherences

Ianc, Octavian, Teleanu, Florin, Ciumeică, Andrei, Lupulescu, Adonis, Sadet, Aude, Vasos, Paul R

Living systems rely on molecular building blocks with low structural symmetry. Therefore, constituent amino acids and nucleotides yield short-lived nuclear magnetic responses to electromagnetic radiation. Magnetic signals are at the basis of molecular imaging, structure determination and interaction studies. In solution state, as the molecular weight of analytes increases, coherences with long lifetimes are needed to yield advantageous through-space magnetisation transfers.

Interactions between magnetic nuclei can only be detected provided the lifetimes of spin order are sufficient. In J-coupled pairs of nuclei, long-lived coherences (LLC's) connect states with different spin-permutation symmetry. We show sustained LLC's in protein Lysozyme, weighing 14.3 kDa, with lifetimes twice as long as those of classical magnetisation for the aliphatic protons of glycine residues. We found for the first time that, in a protein of significant molecular weight, LLC's yield substantial through-space magnetisation transfers: spin-order transfer stemming from LLC's overcame transfers from classical coherences by factors > 2 . Furthermore, in agreement with theory, the permutation symmetry of LLC-based transfers allows mapping interacting atoms in the protein structure with respect to the molecular plane of glycine residues in a stereospecific manner. These findings can extend the scope of liquid-state high-resolution biomolecular spectroscopy.

Quantifying Transmembrane Sodium Transport Rates in Living Cells Using ^{23}Na Relaxation-Exchange Spectroscopy

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Sodium ions crossing cell membranes play crucial roles in biological systems, including the maintenance of osmotic balance, transmission of neural signals, and involvement in metabolic processes, thereby supporting essential muscular and cognitive functions. Altered dynamics of sodium ions are associated with numerous common pathological conditions, such as chronic pain, Alzheimer's disease, and cardiovascular diseases. Traditional methods like patch-clamp and fluorescent probes, while useful, are invasive and unsuitable for in vivo or prolonged studies. ^{23}Na nuclear magnetic resonance (NMR) is a non-invasive technique directly targeting the sodium ions in cells, with relaxation properties (in particular the T_2 relaxation rates) proven effective to distinguish intracellular and extracellular sodium ions. However, quantifying transmembrane exchange of sodium ions by ^{23}Na NMR has not been achieved previously.

Based on multi-component ^{23}Na relaxation behavior in biological environments, we devised a relaxation-exchange (REXSY) strategy to examine sodium ion exchange across membranes. Application of this NMR-based exchange assay to yeast, HeLa, and U-87 cells showed that the sodium exchange rate correlates strongly with both cellular phenotype and the expression level of membrane channels. Intervention experiments using ouabain, mannitol and hypoxia confirmed that the REXSY measurements reliably detect and quantify variations in sodium transport rates, intra- versus extracellular ion concentrations, and intracellular protein-binding states. Thus, this transmembrane sodium monitoring approach allows non-invasive, overall assessment of cellular ion-channel behavior and presents a new instrument for functional cell research and drug-screening applications.

Cleavage by γ -secretase within the transmembrane domain (TMD) of amyloid precursor protein (APP) generates A β peptides that can accumulate in amyloid plaques in Alzheimer's disease pathogenesis. How γ -secretase selects its substrates has been an enigma as they do not have a consensus sequence. In our previous study, we discussed a dynamic signature that controls internal bending/kinking and orientation of the two halves of the TMD. The extent, however, had remained ambiguous due to limitations of NOE-based NMR structure determination. To solve this question, we measured residual dipolar couplings (RDCs) of APP-TMD, using a polyethylene glycol gel-based alignment medium with TFE/water mixtures. Using $^1\text{H}^{15}\text{N}$ RDCs, we analyzed the conformations of APP-TMD and two mutants, G38L and G38P, which previously had differed significantly in bend and orientation and cleavage efficiency by γ -secretase. By fitting snapshots from a molecular dynamics trajectory, we analyzed how well structures matched the measured RDC values. This indicated that G38L retained mainly its relatively straight conformation, whereas bent and—in the case of G38P—locally unfolded structures likewise contribute dynamically to the other two. The RDC selected structures thus reproduce the features of the NOE-derived structures, but show that all three TMDs retain some conformational adaptability.

^{19}F photo-CIDNP NMR as a tool for monitoring bacterial activity.

Dziubinska-Kuehn, Katarzyna Maria; Bragina, Olga; Ausmees, Kerti; Cooper, Callum; Spuul, Pirjo; Gray, Mark; Reile, Indrek.

Recent developments in NMR highlight a growing effort to combine hyperpolarization with medical diagnostic applications into a single, cohesive solution. The primary motivation for such investigations lies in the ability of hyperpolarized NMR to rapidly enhance the signal intensity, thereby substantially shortening acquisition times. Such capability could reduce diagnostic delays, particularly in acutely progressive, high-severity conditions, such as bacterial bloodstream infections or sepsis.

Among all hyperpolarization techniques, photochemically-induced dynamic nuclear polarization (photoCIDNP) remains one of the most robust, due to its ease of application and the significant signal enhancement achieved already in a single-scan experiment. Furthermore, it is highly compatible with both biomolecules and biological solution environments, such as human body fluids.

Our contribution demonstrates a successful application of ^{19}F photoCIDNP NMR for real-time monitoring of bacterial activity in vitro. The experimental design for the study included feeding bacteria with ^{19}F -labeled nutrients and collecting their growth medium at defined points during incubation, to subject it to photoCIDNP NMR as obtained. Based on a series of single-scan measurements, we could derive information on the nutrient depletion and the concurrent increase in metabolite concentration. Furthermore, selecting ^{19}F NMR enabled the signal acquisition to remain background-free, with only resonances from nutrients and metabolites present. This proof-of-concept study shows that photoCIDNP NMR could improve the speed of diagnosing bacterial bloodstream infections through quick single-scan experiments, an attractive alternative to current methods that require days of bacterial cultivation.

Phosphorylation dependent conformational equilibria for the human Aurora B:IN-box kinase complex exposed by ^{19}F NMR

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The human Aurora B kinase belongs to the Ser/Thr kinase family. Together with the C-terminal of the inner centromere protein (INCENP), the so-called IN-box, it forms an enzymatic complex that has key regulatory functions in mitotic events and is thus essential for cell division. This makes Aurora B an appealing anti-cancer target. Synergistic autophosphorylation is key for its activation, first on an activation loop residue followed by the IN-box TSS-motif.

Structural information on the human Aurora B:IN-box is currently very limited and experimental information on conformational dynamics almost absent. We thus set out to probe conformational equilibria and dynamics using ^{19}F NMR of both the fully phosphorylated and de-phosphorylated state of the complex, with and without presence of substrate. In conclusion the Aurora B:IN-box complex exists in a conformational equilibrium affected by the degree of phosphorylation as well as substrate availability.

**Advanced methods of amino-acid type recognition
for automatic resonance assignment of intrinsically disordered proteins**

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Intrinsically disordered proteins (IDPs) have been shown to play important roles in many biological processes for which protein chain mobility is crucial. Only NMR provides atomic-scale information on these proteins, thus enabling the determination of detailed structural propensities, dynamics, and interactions of proteins of this class. Nevertheless, the resonance assignment of IDPs can be very challenging. It is evident that the rapid dynamics of these molecules, in conjunction with high incidence of sequential repeats in the sequences of IDPs, result in the chemical shifts of these molecules occupying a narrow chemical shift range. This phenomenon gives rise to ambiguities during the sequential linking of the spin systems, which in turn results in typically short chains of sequentially-linked amino acid residues. Consequently, accurate amino-acid type recognition is essential for the accurate mapping of the chains on the protein sequence. The statistical values of chemical shifts are conventionally utilised for this purpose. As manual analysis of multidimensional chemical shift spaces is not very efficient, more advanced classification methods should be employed. Such methods include linear discriminant analysis and sliced-Wasserstein optimal transport. Despite the high efficiency of these methods, their incorporation into the automatic procedure of NMR resonance assignment is challenging. The poster presents these challenges, together with some proposed solutions.

Efficient Incorporation of Fluorinated Amino Acids in an *E. coli* Cell-Free Protein Synthesis System for ^{19}F NMR Applications

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Isotopic labeling has played a key role in overcoming size limitations in high-resolution biomolecular NMR spectroscopy. Due to its 100% natural abundance, high sensitivity, wide chemical shift dispersion, and negligible biological background, the ^{19}F nucleus is highly valuable for probing biomolecular structure, dynamics, and interactions. ^{19}F labeling improves spectral resolution and provides site-specific structural information, enabling studies of large and complex biomolecular systems. Compared with monofluorinated probes, trifluoromethyl (CF_3) groups offer higher sensitivity, favorable transverse relaxation properties, and can also enable high-resolution ^{19}F – ^{13}C TROSY experiments.

Monofluorine labeling of proteins can be achieved through recombinant expression, enzymatic reactions, chemical ligation, and solid-phase peptide synthesis. In contrast, biosynthetic incorporation of trifluoromethyl groups remains challenging because of cytotoxicity and poor compatibility with natural tRNA synthetases, resulting in low protein yields and high production costs. These limitations are further aggravated for complex, toxic, or membrane proteins. Cell-free protein synthesis (CFPS) systems provide an effective alternative by enabling rapid protein synthesis outside living cells and allowing direct control of transcription and translation. CFPS is particularly suitable for incorporating toxic non-canonical amino acids such as fluorinated methionine.

Previous studies demonstrated successful incorporation of trifluoromethyl methionine (tfm-Met) using continuous-exchange CFPS, although less than 1% of the supplied tfm-Met was incorporated into the target protein. In this study, an *E. coli* batch-mode CFPS system was optimized for expression of superfolder GFP (sfGFP) and applied for efficient incorporation of tfm-Met, as well as monofluorinated tryptophan and phenylalanine.

Investigations on the allosteric Ligand-Binding-Domain of PPAR γ

Höfurthner, Theresa, Nilsson, Daniel, Robertson, Angus J., Carlström, Göran, Takacs, Zoltan, Akke, Mikael

Allostery, the binding of a regulatory molecule to a remote site affecting the binding of another molecule at the active site, is a fundamental process for protein activity regulation and function. Although allostery has been recognized for decades, our understanding of the signal transmission between sites and the redistribution of the conformational ensembles remains limited. NMR Spectroscopy, together with other biophysical and computational methods, can advance our knowledge about allostery at an atomic level by linking structural rearrangement upon binding, dynamical changes in the micro- to millisecond time scale and thermodynamical parameters as affinity. Nuclear receptors are known to exhibit allosteric communication among three key sites: the ligand-binding site, the coregulator-binding site, and the transcriptional activation site. Furthermore, these proteins alternate between transcriptionally active and repressive conformation, which stabilizes upon ligand binding. In this work, we aim to describe the nuclear receptor PPAR γ in its open and active conformation by saturating with a known agonist, binding to the ligand binding site, and a coactivator peptide sequence. By utilizing ILV-methyl chemical shift information and methyl relaxation dispersion experiments, we aim to get a better understanding of how the intrinsic allosteric signal transmission works.

Calorimeter at Atomic Resolution: Application to protein stability

Tarang, Guneet Singh, Coudeville, Nicolas, Orts, Julien

Our current understanding of protein structural dynamics and associated thermodynamics limits progress in elucidating several biomolecular processes, including dynamic allostery, enthalpy–entropy compensation in drug-target interactions, protein dynamics-based rational drug design, and the discovery of cryptic binding sites. Solution-state NMR spectroscopy is uniquely suited to address these challenges by accessing the complete thermodynamic ensemble of biomolecules, provided that relevant thermodynamic parameters can be accurately extracted from experimental data. We propose the nuclear Overhauser effect (NOE) as a powerful approach: it is ensemble- and time-averaged, sensitive to molecular exchange, capable of detecting motions across a broad range of timescales and provides dense sampling of interactions involving both protein and ligand atoms. A theoretical treatment of the temperature dependence of NOE cross-relaxation rates, combined with precise measurements via exact NOE methodology, enables determination of changes in enthalpy, entropy, and heat capacity associated with fast timescale conformational transitions between specific atomic pairs. Using the WW domain, a well-characterized model for protein folding and stability, we are standardizing this methodology to extract residue-specific contributions to overall protein thermal stability. Systematic comparison of thermal stability data across multiple mutants will establish a quantitative benchmark, refining previous qualitative explanations. Ultimately, our goal is to validate predictions of protein stability and guide the design of mutants with tailored thermodynamic properties.

Dynamics and solvent exchange in the disordered N-terminal region of p53 in free form and in complex with S100P protein

Sebák, Fanni, Ecsédi, Péter, Nyitray, László, Bodor, Andrea

The N-terminal disordered transactivation domain (TAD) and the proline-rich domain (PRD) of tumoursuppressor p53 play important role in transcription, apoptosis and protein-protein interactions. The metastasis associated S100 family members are among the many targets that bind to these regions. Our goal was to characterize the structure of the p53¹⁻¹⁰⁰-homodimer S100P complex and describe the changes in dynamics. S100P binds Ca²⁺ ions in the loops between the helix 1-2 and 3-4, and this induces an open conformation. p53¹⁻¹⁰⁰ binds to the Ca²⁺ loaded dimer with the L14-T55 region, and TAD1 and TAD2 regions undergo a disorder-to-order transition

Backbone dynamics on multiple timescales were investigated by T_1 , T_2 , hetnoe, and cross-correlated relaxation experiments at different temperatures and B_0 magnetic fields. Upon interaction with S100P protein, low frequency motions become significant in p53¹⁻¹⁰⁰ TAD1 and TAD2 regions, while the PRD region remains highly flexible.

Further on, we show that solvent-protein interactions are of great importance for disordered proteins. To establish extent of solvent exchange at residue level CLEANEX experiments were performed. We found that in the free p53¹⁻¹⁰⁰, the N- and C-terminal regions are exposed most to the solvent, while TAD1 and TAD2 regions exchange poorly with the solvent protons. The proline-rich domain is partly solvent-exposed: E62-A76 region exchange poorly with the solvent protons, while T81-Q100 region have more solvent-accessibility. In the complex, p53 amide protons are less solvent exposed than in the free form.

Conformational plasticity of CTX-M-14 revealed by multitemperature relaxation dispersion NMR Spectroscopy

Ranit Kumar Mondal, Mikael Akke

Temperature is a fundamental thermodynamic parameter that modulates protein dynamics, allostery, ligand binding, enzymatic catalysis and other functions. A vast majority of the current knowledge on proteins is based on the static structures of the ground states. However, it is also well-known that proteins are not static structures and can exist in different conformational states that can spontaneously undergo transitions among themselves. Some of these states are lowly populated and can have short lifetimes and play a crucial role in protein functions. NMR spectroscopy has been widely used to characterize molecular structure, dynamics and interactions at an atomic resolution over several decades in a non-destructive manner. But the minor conformers are often not visible in the regular NMR spectra, owing to their low populations and transient lifetimes. Experiments like Car-Purcell-Meiboom-Gill (CPMG) relaxation dispersion have paved the way to study the conformational equilibria between the visible ground state and the invisible states undergoing exchange and have provided a comprehensive picture of the structural, kinetic and thermodynamic information of such elusive processes occurring in μ s-ms timescale. β -lactamases are functional enzymes that play a vital role in developing antibiotic resistance. CTX-M-14 is one of enzymes that belong to the class of Extended Spectrum Beta Lactamases (ESBL) and provides a model system to understand protein evolution. The antibiotic binding site is a buried “cryptic pocket” which is solvent-exposed upon displacement of a Ω -loop. In this study, we address the structure-activity interplay of the enzyme at three different temperatures. By combining multiple field ^{15}N NMR relaxation data for the free CTX-M-14 with the crystallographic data obtained for several antibiotic bound structures, we conclude that the wild-type variant adapts to a state reflecting real physiological activity at 37 °C, unseen at lower temperatures.

Model-free analysis of ^{15}N NMR relaxation data requires selection among alternative spectral density models. Conventional approaches select one optimal model per residue, but this hard choice can make fitted parameters noise-sensitive and underestimate uncertainty by ignoring model-selection ambiguity. Bootstrap aggregation, or bagging, offers a multimodel alternative by averaging parameter estimates over bootstrap-selected models.

Here, we benchmarked bagging using a realistic backcalculation approach. Synthetic model-free parameters were generated for 10,000 residues, including order parameters, internal correlation times, and exchange contributions. These were used to back-calculate R_1 , R_2 , and NOE relaxation data at four magnetic fields, after which realistic noise was added based on experimental signal-to-noise levels from Galectin-3C ligand complexes. The dataset was analysed using conventional AICc model selection and bagging, allowing direct comparison with known ground truth.

Bagging improved global recovery for most model-free parameters, including S^2 , S_f^2 , S_s^2 , τ_f , and τ_s , mainly by reducing large outliers. The benefit was most evident when AICc selected a model different from the ground truth; however, forcing the true model did not generally improve recovery, suggesting that parameter identifiability under realistic noise is a key limitation. Bagging also produced uncertainty estimates with substantially improved coverage, closer to the expected Gaussian behaviour than conventional Monte Carlo errors based on a fixed selected model.

These results support bagging as a stabilising multimodel approach for model-free analysis of multi-field relaxation data, particularly when robust uncertainty estimates are as important as point-estimate accuracy.

The EcSSB protein: from disorder to phase separation

Kovács, Benjámín, Szentpáli, Gergő, Sebák, Fanni, Ecsédi, Péter, Katran, Viktoria, Kovács, Mihály, Bodor, Andrea

The single-stranded DNA binding protein (SSB) plays a fundamental role in DNA metabolism, including replication, recombination, and repair. The *E.coli* SSB (EcSSB) investigated in this study is the prototype of tetrameric SSB-s. Its 177 residue sequence includes the structured oligonucleotide-binding (OB) domain (1–112) and the disordered C-domain (113–177) which acts as a flexible hub recruiting partner proteins and also establishes the propensity of EcSSB to undergo liquid-liquid phase separation (LLPS). In this work, we first assessed the level of disorder in the C-domain using the isolated (113–177) fragment based on secondary chemical shifts (SCS) combined with Student's t-distribution (SPIT) methods and dynamic NMR parameters. We found that the C-domain displays full disorder along its entire sequence without any structural propensity which substantiates its capacity to display multivalent interactions and drive LLPS propensity. The conformational landscape of the C-domain was further characterized by identifying minor (5–15%) *cis*-proline conformers.

Further on, we investigated the LLPS condensate of EcSSB in equilibrium with the dilute phase using spatially resolved NMR experiments. To best mimic physiological conditions no crowding agent was used, besides, the EcSSB condensate was prepared in a 3mm Shigemi tube and deuterated solvent was only present in a surrounding 5mm tube. Z-profiling revealed a significantly reduced (~55%) water content in the EcSSB condensate compared to the dilute phase and this composition is constant in the 5–45 °C temperature range. Notably, albeit significantly broadened, signals of the disordered C-domain remain detectable in the condensed state of the protein. Slice-selective ¹H experiments showed that in equilibrium the dilute phase contains negligible amounts of protein compared to the condensed phase. Based on our results the EcSSB condensate has a remarkable stability and density compared to those of human LLPS proteins such as FUS or tau.

NMR Investigation of Entropic Effects in Eremomycin–Ligand Interactions

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Glycopeptide antibiotics remain essential for the treatment of severe Gram-positive bacterial infections; however, the emergence of resistance increasingly compromises their clinical efficacy, highlighting the need for a deeper understanding of their molecular recognition mechanisms. We investigate the thermodynamic determinants of ligand binding to the glycopeptide antibiotic eremomycin using ^{15}N NMR relaxation measurements, with particular focus on conformational entropy changes accompanying complex formation.

Backbone order parameters were quantified for both the free and ligand-bound states, revealing that binding of the cell-wall analogue peptide NAc-D-Ala enhances local internal dynamics at the binding interface, resulting in an entropic gain for the host molecule. In parallel, reduced solvent accessibility was observed in the vicinity of the binding site. These findings provide experimental support for the hypothesis that entropic compensation associated with increased internal mobility contributes to the cooperativity between ligand binding and antibiotic dimerization.

In addition, cross-correlated CSA/DD relaxation experiments demonstrate that the *cis* configuration of the peptide bond at residue 6 is retained upon ligand binding in aqueous solution. Collectively, our results indicate that enhanced conformational entropy may represent an important factor governing glycopeptide antibiotic–ligand interactions.

Differential IDR Engagement Underlies Human ANP32B Preference in Influenza Adaptation

Chen, Yi-Hsin, Shukla, Shivangi, Yu, Lefan, Zarcho, Aldo, Schneider, Robert, Blackledge, Martin

ANP32 proteins are essential host factors for avian influenza A virus (IAV) replication, enabling assembly of influenza polymerase (FluPol) complexes and recruitment of nucleoproteins (NP). A 33-amino-acid deletion within the intrinsically disordered region (IDR) of human ANP32 proteins limits avian-to-human transmission by disrupting a key viral interaction site. Adaptive mutations in the FluPol subunits such as PB2 E627K overcome this barrier, promoting efficient use of human ANP32 proteins, with a marked preference for ANP32B over ANP32A. The molecular basis of this functional divergence, however, remains unclear. We are interested not only in the molecular role of this host protein in viral replication, a mechanism that exploits multivalent interactions to adapt to mammalian species, but also how we can use this information to conceive novel methods of viral inhibition. We use solution NMR spectroscopy, including paramagnetic relaxation enhancements using 8 different cysteine mutants of PB2 and extensive NMR exchange, to characterize interactions of human ANP32A and ANP32B with FluPol and NP, and probe the conformational organization formed in their dynamic ternary complexes. We show that wild-type FluPol interacts weakly with human ANP32 proteins, whereas the PB2 E627K mutation enables binding of the 627-NLS domain to both paralogs. Notably, ANP32B displays an additional transient interaction via a linear motif within its IDR, conferring enhanced multivalent engagement. Furthermore, we identify a conserved “EExD” motif mediating electrostatic interactions with NP in both paralogs, indicating shared NP recognition but distinct modes of FluPol engagement. These results suggest that subtle sequence variations within disordered regions of ANP32 proteins reshape their multivalent interaction networks. Overall, our findings provide a molecular perspective on ANP32 paralog preference during influenza adaptation, suggesting that PB2 E627K reshapes multivalent interactions with human ANP32 proteins to facilitate coordinated engagement of NP and FluPol.

Structural and Dynamic Characteristics of ISG15 and its Interaction to USP14

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Interferon Stimulated Gene 15 (ISG15) is a protein consisting of two Ubiquitin-like domains that is expressed as a response to interferon signaling in infections. It acts as a reversible protein tag through ISGylation and modifies both cellular and viral proteins as a mechanism of defense. Unlike ubiquitin that has been thoroughly studied in solution in terms of its structural and dynamical characteristics, ISG15 remains largely unstudied.

Here, we investigate the conformational behaviour of ISG15 in solution using NMR spectroscopy, examining both the wild-type protein and the commonly used C78S mutant. Both protein variants show similar dynamical properties that reveal the presence of multiple conformational states, providing new insights into millisecond-to-second timescale dynamics. Coupled with this, we investigated ps-ns timescale of dynamics and found that the linker connecting the two domains of ISG15 demonstrated relaxation profiles similar to those of the structured regions, indicating a relatively rigid interdomain arrangement. In addition, the C78S mutation, located in the linker, perturbs not only the linker itself but also the interdomain interface, further supporting a model in which the two domains remain in close contact rather than moving independently.

We also studied the interaction between ISG15 and USP14, a deubiquitinase with delISGylase activity, further revealing binding mechanisms of the ISG15-USP14 complex. This work gives novel insight into not only the structural details of ISG15 when free in solution, but also into its interaction mechanics with potential partners.

Comprehensive NMR Chemical Shift Assignments for 275 Dipeptides Common in Intrinsically Disordered Proteins

Underhaug, Jarl; Rindfleisch, Tobias; Taule, Emilie Fjeldberg; Miettinen, Markus S.

Accurate NMR chemical shift assignments are essential for studying proteins at atomic resolution, but remain difficult for intrinsically disordered proteins (IDPs), where spectral overlap limits complete side-chain assignments. A practical way to address this challenge is to study simpler systems: dipeptides serve as minimal models of IDPs and capture key features of their local chemical environment.

Here, we present a reference dataset with complete chemical shift assignments for 275 of the most frequent dipeptides in the IDP proteome, covering 93% of its dipeptides. Assignments include all NMR-accessible backbone and side-chain nuclei, yielding 11,571 validated chemical shifts. For dipeptides containing aspartic or glutamic acid, protonation-dependent shifts are additionally reported, providing insight into pH effects.

All spectra and assignments are provided in standardized, machine-readable formats, enabling direct use in automated assignment and method development. This dataset closes a major gap in side-chain chemical shift coverage for IDPs and establishes a consistent benchmark for experimental and computational studies of protein disorder.

NMR uncovers a hierarchy of backbone interactions in bulged dsRNA–PNA triplexes

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Non-coding RNAs frequently contain bulges that locally distort duplex geometry and create attractive recognition sites for triplex-forming peptide nucleic acids (PNAs). Despite their importance for RNA targeting, the structural factors governing PNA binding remain poorly understood. We combine multidimensional NMR spectroscopy, SHAPE-MaP, and thermodynamic analysis to investigate dsRNA–PNA triplexes spanning different bulge sequences and sizes.

We show that PNA binding actively remodels bulged RNA duplexes into binding-competent conformations. While PNAs complementary only to the RNA stem preserve looped-out bulge states, PNAs containing an additional nucleobase capable of recognising the bulged residue stabilise stacked-in conformations through Hoogsteen interactions. Surprisingly, triplex stability is not determined by the initial bulge geometry but instead by a cooperative interplay of bulge identity, nucleobase stacking, and backbone-mediated contacts. Adenine-containing bulges consistently support higher-affinity binding and exhibit spectroscopic signatures of enhanced stacking.

Most notably, NMR reveals a hierarchy of backbone-mediated interactions. Whereas many amide–phosphate hydrogen bonds outlast neighbouring base-pairing contacts, a subset exhibits exceptional protection from solvent exchange and thermal disruption, forming local stability hubs that reinforce adjacent Hoogsteen and Watson–Crick interactions. These observations suggest that the stability of dsRNA–PNA triplexes is encoded by a small number of exceptionally persistent backbone contacts rather than being distributed uniformly across the interaction network. By revealing how local RNA architecture reshapes this network, our work provides a structural basis for predicting and engineering recognition of dynamic RNA motifs.

The N-Myc MB0-MBI region interacts specifically and dynamically with the N-lobe of Aurora kinase A

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The intrinsically disordered MYC proteins are master regulators of cellular growth and function, but when deregulated they become cancer drivers. MYC-protein interactions are key to oncogenesis, and while disrupting such interactions would be of significant therapeutic benefit, the intrinsically disordered properties of MYC have dramatically hampered their characterization. Here, we apply an integrated structural biology approach to describe the structure and dynamics of the N-Myc–Aurora A complex, which is critical in neuroendocrine tumor progression. We reveal a functional interaction where multiple binding sites on N-Myc interact with the Aurora A N-lobe. The interaction is governed by aromatic clusters within the conserved MB0 and MBI motifs in N-Myc that interact with Aurora A in a dynamic binding mode that allosterically promotes kinase activation. We show that N-Myc binding to the Aurora A N-lobe can be inhibited by the small-molecule AurkinA, providing opportunity for therapeutical strategies to disrupt this interaction.

Emerging Technologies

ShimNetV2-RM: a neural network for post-acquisition lineshape correction in NMR reaction monitoring

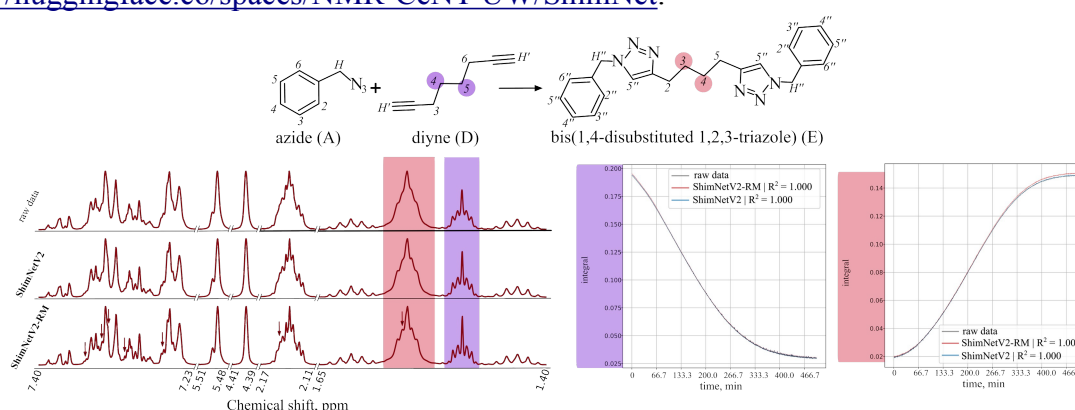
Jopa, Sylwia^a, Bukowicki, Marek^{a,b}, Kazimierczuk, Krzysztof^a

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NMR spectroscopy is widely used to monitor chemical reactions in situ by acquiring a series of spectra over time. However, such experiments are more demanding than measurements of stable samples, as repeated magnetic field optimization (shimming) before each acquisition is often impractical. As a result, gradual line-shape distortions may accumulate during the reaction, reducing spectral quality and resolution.

We propose ShimNetV2-RM, a neural-network-based post-acquisition shimming approach tailored for reaction monitoring. The method builds upon our previously developed ShimNet¹ framework and leverages well-shimmed reference spectra to constrain the correction of time-resolved data. This enables automatic and robust improvement of distorted spectra without manual parameter tuning. The approach was validated on representative copper-catalyzed azide–alkyne cycloaddition (CuAAC) reactions under realistic experimental conditions. By combining automation, speed, and improved reconstruction quality, ShimNetV2-RM provides a practical tool for laboratories performing routine reaction monitoring and large-scale NMR measurements, increasing spectral resolution and thereby facilitating component identification and reaction kinetics tracking. The source code is openly available at <https://huggingface.co/spaces/NMR-CeNT-UW/ShimNet>.



¹ Jopa, S., Bukowicki, M., Shchukina, A., & Kazimierczuk, K. (2025). ShimNet: A neural network for postacquisition improvement of NMR spectra distorted by magnetic-field inhomogeneity. *The Journal of Physical Chemistry B*, 129(24), 6078–6092. <https://doi.org/10.1021/acs.jpcc.5c02632>

Solid-state MAS NMR guides mechanochemical fluoropolymer recycling

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Fluoropolymers, such as PTFE, PVDF, ETFE, ECTFE, FEP and other related materials, are widely used in applications requiring exceptional chemical resistance: chemical processing, semiconductor manufacturing, energy technologies, aerospace. As of 2025, the global fluoropolymer market exceeded \$10 billion annually. However, their chemical resistance also means that they are difficult to recycle, and, as of today, incineration is the only viable strategy to degrade them. We have very recently discovered a strategy to degrade PTFE with a ~100% yield at room temperature by simply ball milling it with sodium metal, resulting in a mixture of carbon and NaF. We have now substantially improved this strategy to enable the use safer metals and a variety of other fluoropolymer feedstocks. Solid-state MAS NMR has been essential in guiding the discovery of these new, counterintuitive reactions: ^{19}F MAS NMR is a rapid way of quantifying conversion while ^{13}C MAS NMR reveals the atomic-level structure of the resulting carbons. We believe that this solvent-free, room temperature process is the future of sustainable fluoropolymer recycling.

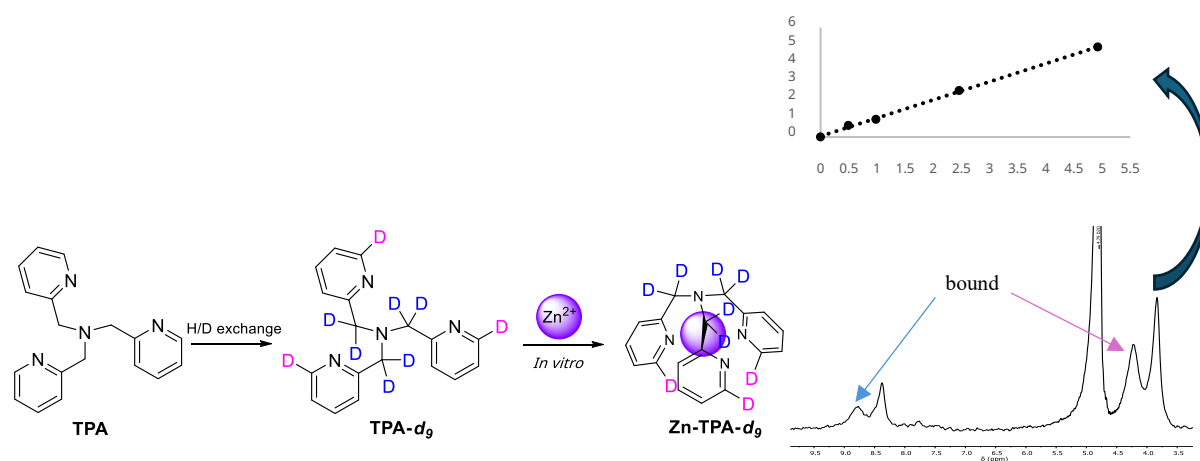
Deuterated molecular probe for zinc sensing

Grysette Daher, Lisa M. Fries, Stefan Göggler

Deuterium metabolic spectroscopy (DMS) and imaging (DMI) are arising as potent methodologies to map metabolism with high temporal and spatial resolution, accompanied by its simple and robust approach.

Another relevant field of research is detecting metal ions, in particular zinc, for its importance and involvement in many cellular processes. Herein we introduce the concept of deuterated metals probes for zinc sensing under the hypothesis that changes in chemical shift can be perceived in the protons adjacent to the binding pocket of TPA, therefore the substitutions of these for deuterons can be used to quantify zinc.

Selective deuteration of tris (2-pyridylmethyl) amine (TPA) in the positions α to central amino and 2-C position in the pyridine ring since it is expected to display the largest changes in chemical shift (determine by $^1\text{H-NMR}$). To the newly synthesised probe dissolved in aqueous HEPES buffer pH 7 (TPA- d_9 5mM), ZnCl_2 was added in increasing amounts. A linear response to Zn was detected down to 500 μM . Selectivity was evaluated challenging TPA- d_9 with Ca^{2+} and Cu^+ salts. In addition, longitudinal relaxation time were measure for TPA- d_9 an Zn-TPA- d_9 showing values between 34-41 ms in comparison to 366-427 ms for HDO signal.



Accelerating NMR by Reduction of Scan Accumulation Time

Mross, Sarah, Gizatullin, Bulat, Salgado, Billy, Casanova, Federico, Hasse, Hans, Münnemann, Kerstin

NMR spectroscopy is inherently time-inefficient, as most experimental time is spent on waiting for the nuclear spins to return to thermal equilibrium. This limitation is especially severe for low- γ nuclei such as ^{13}C , where long spin-lattice relaxation times (T_1) require long inter-scan delays and restrict sample throughput on costly instrumentation [1]. Paramagnetic relaxation enhancement (PRE) can drastically shorten T_1 times of all NMR-active nuclei [2], thereby reducing inter-scan delays. Typically, PRE is achieved by dissolving stable radicals or transition-metal complexes in the sample, which causes sample contamination, line broadening, and prevents the reuse of the PRE agents. The approach presented here circumvents these drawbacks by spatially decoupling the PRE agent from NMR detection by immobilization on a fixed bed [3]. After signal acquisition, the sample is shuttled from the NMR coil to a PRE agent chamber, where polarization is rapidly restored, and then returned for the next scan. This approach achieves a 9-fold reduction in acquisition time for quantitative ^{13}C NMR without compromising spectral quality or quantitative accuracy. Faster experiments result into higher throughput or increased signal averaging within the same time. It is broadly applicable to all kinds of NMR spectrometers, experiments, and nuclei as it targets T_1 relaxation rather than a specific pulse sequence.

We use a 90 MHz benchtop NMR spectrometer to demonstrate the acceleration of NMR by reducing the scan accumulation time. The accuracy of quantification was validated by ^1H and ^{13}C NMR of binary and ternary mixtures. Potential chromatographic bias from the PRE agent was excluded using complex samples such as wine and rapeseed oil. DOSY-NMR experiments confirmed the absence of shuttle-induced convection.

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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 (^2H) nuclei, a moderate-gamma quadrupolar nucleus, using shallow NV centre ensembles in diamond. Employing correlation spectroscopy protocols, we resolve ^2H 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.

Digital Twins Enable Rapid Experiment Characterization

Tobias Sjölander, Lorena Bianchet, Marco Rossignolo, Yousof Mardoukhi,
Manpreet Jattana, Thomas Lippert, Anurag Saha Roy, Shai Machness

Instrument calibration is a fundamental pre-requisite of experimental science. It is also time consuming, in emerging technologies like quantum technology or nano-imaging, the time required to carefully calibrate various control parameters are often a non trivial fraction of the total available experiment time.

The Qruise digital twin is our proposed solution to this problem. Based on a differentiable physics engine, the twin enables us to efficiently model the response of an instrument or experiment to arbitrarily chosen controls (voltages, signal generator frequencies...) By choosing the controls in an efficient manner the twin quickly updates its internal representation of the experiment, which in turn enables it to suggest the appropriate controls for a desired transformation - such as a pi pulse. In practice, this approach enables significant under-sampling during calibration thus saving both student and instrument time.

In collaboration with the modular super computing and quantum computing group, MSQC at Goethe University in Frankfurt and using an experimental platform of NV centers in diamond, we demonstrate a $>10x$ reduction in experiment time on common calibration and characterization tasks - such as pi pulse parameters, $T2^*$, and hyperfine coupling constants.

Originally developed with an eye towards scalable control software for industrial scale quantum computers, we also believe these techniques to be of use in methods development and quantum technology labs. Importantly, we see applications in sensing - especially in low SNR modalities.

Ultra-sensitive and spatially-resolved beta-NMR studies at CERN

Magdalena Kowalska for the VITO Collaboration at CERN

CERN and UNIGE, Geneva

At the VITO setup at ISOLDE-CERN, we perform beta-detected NMR on as few as 10^6 optically-pumped probe nuclei. Such ultra-high sensitivity is possible thanks to the combination of hyperpolarization with resonance detection by changes in asymmetric emission of beta or radiation. In addition, since we work with an ionic beam, we can control its implantation depth into the studied material, thus providing spatial resolution in sub- μm layers, without the need of magnetic field gradients. Recently, we have added to the already polarized isotopes: $^{26-28}\text{Na}$ ($I=3, 5/2, 1$) and $^{47,49}\text{K}$ ($I=1/2$), also ^{48}K ($I=1$) and ^8Li ($I=2$), and we attempted polarization of ^{11}Be ($I=1/2$).

For $^{47-49}\text{K}$, in combination with state-of-the-art coupled cluster and DFT calculations, we determined accurately their magnetic moments, which we then compared to combined nuclear and atomic physics calculations. In this way we were able to shed light on the distribution of magnetization in these nuclei.

In another study, we were able to investigate the source of slow Li diffusion in all-solid-state-battery (SSB) materials, which are promising future energy-storage devices, since they offer higher storage capacity and increased safety. However, in prototype batteries Li diffusion turns out to be extremely slow compared to that in bulk. We thus set out to determine the T_1 relaxation time of ^8Li implanted in different layers of the air-sensitive $\text{Li}_7\text{-xPS}_6\text{-xCl}_x$ ($x=1$ or 1.5) SSB materials at varying temperatures.

Finally, in an offline setup, we have worked on a transportable DNP system to be used for stable and long-lived nuclei known for nuclear medicine in PET diagnosis. For this, we commissioned a home-made 2.4 Tesla magnet that fits in a commercial LHe dewar and are now complementing it with a microwave system and sample holder, and cryogenic beta detectors.

MAS and Operando NMR Characterization of Anode Materials for Na-ion Batteries

de Sousa Amadeu, Nader; Asres, Nahom Enkubahri; Wu, Shu-Han; Appel, Paul Alexander; Fellingner, Tim-Patrick; Krug von Nidda, Jonas; Emmerling, Franziska.

Batteries undergo significant chemical and structural changes during operation.

Understanding these phenomena is crucial for improving battery capacity and long-term cyclability (lifetime). NMR spectroscopy can probe the local environments of atomic nuclei, providing unique insight into the chemical and physical changes occurring during battery charge and discharge.

The samples used in this study consisted of porous carbon materials (core) coated via chemical vapor deposition (CVD) with a carbon layer (shell), which is impermeable to electrolytes. This core-shell design separates the charge carriers (Na^+ ions in our case) from the electrolyte: ions are confined inside the shell structure, whereas the electrolyte remains outside. This separation drastically reduces electrolyte degradation during formation of the solid-electrolyte interphase (SEI) in the first cycles. As a result, Na-ion storage reversibility and thus the initial coulombic efficiency (ICE) are significantly improved.

By applying NMR spectroscopy, we can observe local differences in the environments surrounding ions and electrolytes under working conditions, thus allowing us to validate our hypothesis. Two major NMR methods were employed: operando NMR and *ex situ* magic-angle spinning (MAS) solid-state NMR. Operando NMR enables the measurement of static spectra during real-time charging and discharging, whereas *ex situ* MAS NMR provides higher spectral resolution for samples extracted at defined states of charge and packed into a MAS rotor under inert atmosphere.

Operando ^{23}Na NMR reveals the formation of Na clusters over repeated cycles. *Ex situ* MAS NMR provides detailed insight into the SEI region, allowing us to characterize the near-range environment of diamagnetic species with greater resolution.

PLIADES: A Data Space Platform for AI-Driven Metabolomics and NMR in Precision Medicine.

Larrinaga, Felix, Cuenca, Javier, Valencia, Xabier, Ayala, Unai, Legaristi, Jon, Iriarte, Asier, Alberdi, Ane, Roman, Ibai, Gil, Ruben, Ibañez de Opakua, Alain, Embade, Nieves, Millet, Oscar, Tsiakas, Kosmas, and Giakoumis, Dimitrios.

Introduction: Integrating multimodal health data, particularly NMR and metabolomics, is crucial for precision medicine. However, collaborative research is limited by privacy regulations (e.g., GDPR), data silos, and a lack of standardized sharing. We aim to develop a healthcare data space platform within the PLIADES project to enable the secure, traceable, and interoperable exchange of highly sensitive clinical metabolomic datasets.

Methods: PLIADES transitions from manual laboratory data transfers to a robust Data Space architecture. The platform connects research centers (e.g., CIC bioGUNE, MU-EPS) using data space connectors fully aligned with the latest International Data Spaces Reference Architecture Model (IDS-RAM). This infrastructure enables automated data harmonization, anonymization, and Health-DCAT metadata annotation to ensure discoverability and compliance.

Results: The architecture integrates advanced AI components to protect highly sensitive data. Privacy is drastically improved through two key capabilities: Federated Learning (FL) and a synthetic data generation pipeline. FL shifts focus from sharing raw NMR data to sharing predictive models, enabling collaborative diagnostic training across distributed datasets. Simultaneously, the synthetic data pipeline creates high-fidelity artificial metabolomic datasets that preserve statistical utility without exposing real patient information.

Conclusions: We outline the architectural evolution and deployed components of the PLIADES platform. Bridging NMR analytical data with secure Data Space technology provides an innovative, GDPR-compliant solution. Leveraging federated AI and synthetic data generation fosters secure collaboration and significantly enhances clinical prediction models in metabolomics.

OPTIMAL CONTROL OF TRANSMON DYNAMICS

Musselwhite, Callum; Kuprov, Ilya

The most promising physical platform for quantum device engineering is superconducting circuits, particularly transmons. From the mathematical modelling point of view, transmon systems are similar to spin systems, but they are much easier to design in scalable arrays and to control electromagnetically. However, due to the anharmonicity of transmons and the presence of decoherence, leakage, and control crosstalk, high-fidelity control requires robust pulse engineering techniques capable of operating under experimentally realistic conditions.

Using Gradient Ascent Pulse Engineering (GRAPE) routines implemented within SPINACH, microwave control pulses may be optimised in Liouville space to achieve robust operations while accounting for detuning errors, amplitude fluctuations, and open-system relaxation effects often found in real superconducting systems.

We present several cases where the SPINACH toolkit can reliably and accurately reproduce the dynamics of single and multi-transmon systems; notably the implementation of Frequency Robust Gates (FROG) to maintain high gate fidelity across an ensemble of transmon frequency offsets, and demonstration of highly efficient population transfer of a 3-level transmon using stimulated Raman adiabatic passage (STIRAP). By simulating superconducting systems of higher complexity, we aim to tackle notoriously challenging phenomena such as second-kind relaxation effects in transmon-photon coupled systems.

Synthetic T1 for Spinal Structural Lesions in Suspected Axial Spondyloarthritis: Comparison with T1SE

Authors: Qinxin Cai; Kelly Di Dier; Lieve Morbée; Nele Herregods; Elke Vereecke; Min Chen; Thomas Van Den Berghe; Eva Schiettecatte; Wei Li; Jacob Jaremko; Lennart Jans*

Background: Imaging assessment of structural lesions plays a crucial role in diagnosing axial spondyloarthritis (axSpA). Deep learning-based synthetic CT (sCT) derived from 3D-T1MGE has demonstrated strong performance for detecting sacroiliac structural lesions in patients with suspected sacroiliitis.

Objective : To evaluate the detection accuracy of synthetic T1 (sT1) generated from 3D-T1MGE using T1SE as the reference standard.

Materials and Methods : This retrospective study included patients who underwent spinal MRI for inflammatory back pain between May 2019 and September 2025 at Ghent University Hospital. Two readers independently assessed fatty corner lesions, facet joint ankylosis, Andersson lesions, discal ossifications, ankylosis, syndesmophytes, and erosions on T1SE and sT1. Detection performance of sT1 relative to T1SE was evaluated using SE, SP, PPV, and NPV.

Results : 135 patients (mean age, 40 ± 13.9 years; 65 males) were included. SE ranged from 0.86–0.97 for fatty corner lesions and 0.83–1.00 for other lesions, with SP of 0.97–1.00 and 0.98–1.00, respectively. PPV ranged from 0.80–1.00 and 0.73–1.00, while NPV ranged from 0.91–0.99 and 0.99–1.00.

Conclusion : Synthetic T1 demonstrated detection accuracy comparable to T1SE for assessing spinal structural lesions in axSpA, supporting its potential to replace routine T1-weighted sequences and derive both sT1 and sCT from a single 3D-T1MGE acquisition.

3D Printed Hyperpolarization Compatible NMR Tubes

Emil R. Mamleyev, Jakoba Wacker, Veronika Popova, Ali Ghotbi Varzaneh, Julia Schulte-Hermann, Jingyuan Xu, Marcel Utz, Sören Lehmkuhl

Digital light processing (DLP) 3D printing offers fast, low-cost fabrication of application-specific hardware for magnetic resonance experiments, but commercially available resins are rarely benchmarked for compatibility with liquid-state NMR and hyperpolarization. We systematically screened five widely used DLP resins for solvent stability in water, methanol, acetone, tetrahydrofuran, and chloroform, and classified them for mass and size changes, residual resin leaching from ^1H NMR of solvent extracts, ATR-FTIR, and thermogravimetric analysis. Among tested resins, Phrozen TR300 and Phrozen Ceramic White exhibited the lowest swelling and resin leaching across all solvents, as well as good thermal stability up to about 300°C , identifying them as promising candidates for in situ NMR applications. NMR tubes based on standard 5 mm geometries with circular and hexagonal inner cores of various diameters were then 3D-printed from TR300 and characterized using low-field (60 MHz, Magritek Spin Solve 60 Ultra) NMR experiments. The best-performing design, a circular core with 3.7 mm inner diameter, demonstrated a linewidth of 3.49 Hz with 3.29 Hz in the glass standard, and a residual resin background two orders of magnitude below the solvent signal. Using para-hydrogen-induced polarization via SABRE, we showed that printed tubes are compatible with hyperpolarization of pyrazine (60mM) and pyridine (60mM) through IrIMes-catalyst (3mM) without hydrogen leakage, which was delivered via the bubbling method and polarized in a transfer field of 6.5mT, and reached a signal enhancement of 1359 and hyperpolarization of 0.11%. Additionally, the RASER was successfully qualitatively tested under the same conditions and with an increased pyrazine concentration to 120mM.

These results establish 3D printed hyperpolarization-compatible NMR tubes as a robust, low-cost platform for SABRE and RASER studies in low-field benchtop experiments.

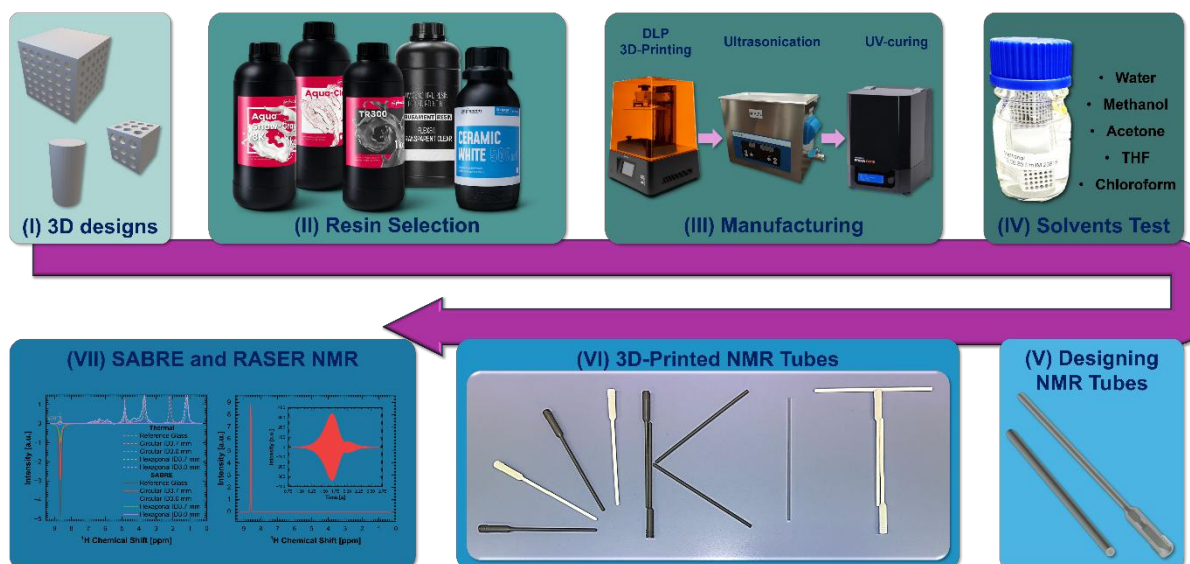


Fig.1. Schematic representation of the process flow: (I) designed test structures, (II) selection of best resins, (III) 3D printing and post-processing, (IV) exposure to the solvents and analysis from the extracts, (V) NMR tube designs, (VI) tube 3D printing and post-processing, (VII) NMR experiments with hyperpolarization.

Deep learning–based synthetic CT for spinal structural lesions in axial spondyloarthritis: comparison with CT

Authors: Qinxin Cai; Kelly Di Dier; Lieve Morbée; Nele Herregods; Elke Vereecke; Min Chen; Thomas Van Den Berghe; Eva Schiettecatte; Wei Li; Jacob Jaremko; Lennart Jans*

Background: Imaging assessment plays a crucial role in diagnosing axial spondyloarthritis (axSpA). However, routine MRI remains limited for evaluating osseous lesions, while additional CT increases workflow complexity and radiation exposure.

Purpose: To evaluate the detection accuracy of deep learning–generated synthetic CT (sCT) derived from 3D-T1MGE for spinal structural lesions in suspected axSpA, using CT as reference.

Methods: This retrospective study included patients with inflammatory back pain who underwent spinal CT and MRI within one month. Two readers independently assessed facet ankylosis, structural Andersson lesions, discal ossifications, ankylosis, syndesmophytes, and erosions on CT and sCT. Detection accuracy was evaluated using SE, SP, PPV, and NPV, with consensus readings as reference.

Results: 87 patients (mean age, 41 ± 14.2 years; 42 males) were included. SE of sCT was 1.00 for facet ankylosis, structural Andersson lesions, ankylosis, and erosions, 0.91 for syndesmophytes, and 0.67 for discal ossifications. SP ranged from 0.97–1.00, PPV from 0.80–1.00, and NPV from 0.97–1.00.

Conclusion: Synthetic CT demonstrated detection accuracy comparable to CT for assessing most spinal structural lesions in suspected axSpA, supporting reliable osseous assessment without additional CT, thereby simplifying workflow and avoiding radiation exposure.

Revealing Na⁺ Storage Mechanisms in Aqueous Sodium-Ion Batteries through Integrated EPR and Solid-State NMR

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The growing demand for sustainable and safe energy-storage technologies has intensified global interest in aqueous sodium-ion batteries (ASIBs), which offer inherent safety, low cost, and environmental compatibility.¹ Hierarchical porous carbons (HPCs) have emerged as promising anode materials due to their tunable porosity and electronic properties, and high surface area. However, understanding the mechanisms of sodium storage in such complex carbon frameworks remains limited.^{2,3}

In this work, solid-state magic-angle spinning nuclear magnetic resonance (MAS-NMR) spectroscopy is employed as a powerful tool to probe the local chemical environment of Na⁺ confined within the pores of HPCs. The observed chemical shifts reflect the interaction of Na⁺ with different carbon domains, pores, and defect sites. To complement these findings, electron paramagnetic resonance (EPR) is utilized to investigate the paramagnetic nature of the carbon matrix. The spin–spin relaxation time and linewidth variations are highly sensitive to interspin distances and local electronic environments, providing insight into the strength and nature of Na⁺–carbon interactions. This enables clear differentiation between weakly adsorbed sodium ions and strongly interacting species within confined carbon domains.

By integrating MAS-NMR, EPR, and electrochemical analyses, we uncover a potential-dependent and sequential Na⁺ storage pathway. The charge-storage process follows a stepwise mechanism: initial Na⁺ adsorption associated with SEI formation, subsequent insertion at graphene edges and nanopores, and finally intercalation into graphene interlayer spaces. These findings provide molecular-level insight into sodium storage in HPCs and offer rational design principles for advanced carbon anodes in ASIBs.

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Structural heterogeneity of serine-arginine-rich splicing factor 1 (SRSF1) studied by intermolecular hyperfine RIDME

Kuzin, Sergei, Galazzo, Laura, Yulikov, Maxim

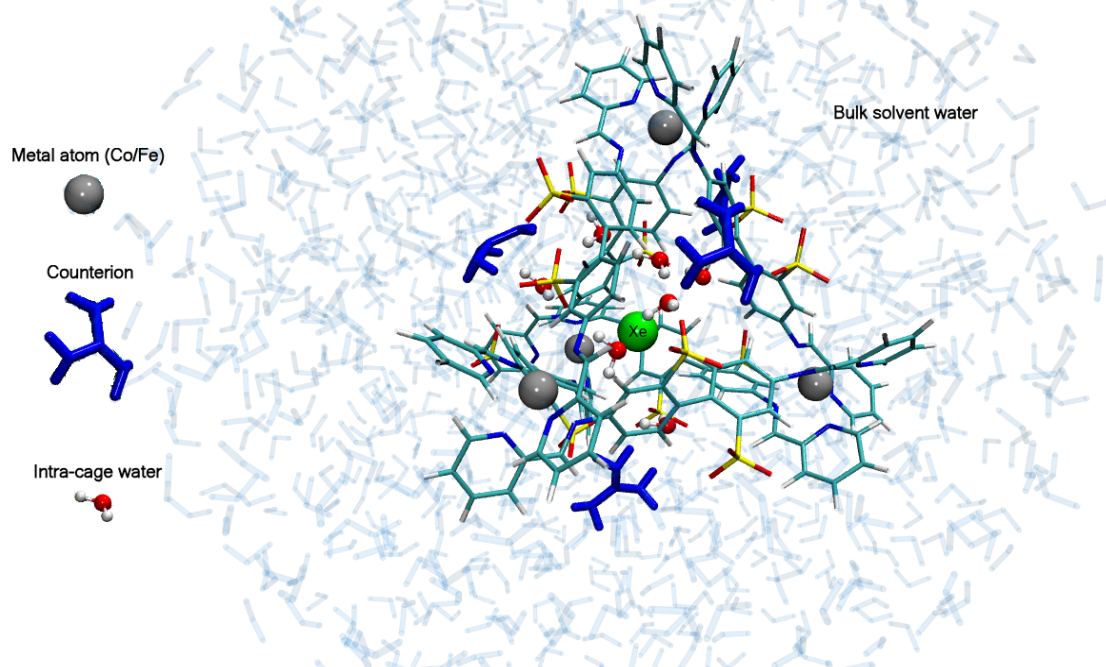
Structurally flexible macromolecules (proteins, polysaccharides, etc.) play an important role in biological functioning, but are challenging objects in structural biology. Electron paramagnetic resonance (EPR) is a powerful tool to characterise their conformational flexibility, e.g., via double electron-electron resonance techniques that provide site-to-site distance information. Previously, intermolecular hyperfine relaxation-induced dipolar modulation enhancement (ih-RIDME) was developed as a complementary structure-quantification method. The principle is to connect the proton-induced electron spin decoherence rate measured by the ih-RIDME with the local proton density in the vicinity (2-3 nm) of a spin label or a spin probe. Proton density is directly related to site-specific geometric information, e.g., backbone packing, intra- and intermolecular contacts, etc. The strength of ih-RIDME is that it can determine the full distribution of local proton densities in the sample under investigation rather than an average density. This makes the ih-RIDME a valuable technique for studying biomacromolecules.

In this contribution, we demonstrate the first application of ih-RIDME to a protein – the serine-arginine-rich splicing factor 1 (SRSF1). This is an RNA-binding protein whose tertiary structure features two rigid RNA recognition motifs and two intrinsically disordered regions. By engineering one and two nitroxide spin labels at different positions within the amino-acid sequence, we quantitatively characterised the global conformational flexibility of SRSF1, supported by *in silico* modelling. Moreover, we uniquely measured a correlation between inter-label distances and local proton density, indicating partial organisation of the SRSF1 conformational ensemble.

Multiscale Computational Spectroscopy of Hyperpolarized Xenon in Paramagnetic Metallo-Organic Biosensors

Gholami, Ehsan, Hilla, Perttu, Pikulová, Petra, Vaara, Juha

This work focuses on implementing a multiscale computational method to investigate hyperpolarized xenon as a sensitive, noninvasive probe in paramagnetic metallo-organic biosensors. The model includes a cage of the type $[\text{Co}_x\text{Fe}_{4-x}\text{L}_6]^{4-}$ ($x = 0-4$), surrounded by 4 counterions immersed in a water droplet model. The multiscale method involves Molecular Dynamics (MD) simulations alongside Quantum Chemical (QC) calculations of the paramagnetic ^{129}Xe NMR chemical shifts, to obtain dynamic and statistically reliable information that can be used for interpreting the experimental data. It has been experimentally observed that there is a non-additive proportionality between the number of cobalt corners and the chemical shift of the xenon in this system. This cannot be quantitatively modeled by considering a static, optimized geometry only. This motivated us to start investigating the system with different numbers of cobalt corners starting from 0 up to 4. Doing so, we can investigate the concomitant effects on the structure, dynamics, as well as the chemical shift of xenon. For the first part we need to generate statistically reliable configurations of the system, for later use in QC calculations, on top of the mechanistic investigation of the system. The questions include how the geometry of the cage is affected by changing the number of cobalt corners, how this affects the behavior of the water molecules and their interaction with the cage, and how all these affect the xenon position distribution and, consecutively, its pNMR shift. The simulations indicate that, with increasing number of the cobalt corners, the water has a growing tendency to enter the cage volume, which affects the position of the xenon atom inside the cage and influences its chemical shift.



Enhancing Magnetic Resonance Sensitivity via Engineered LOV Domains

Apet, Varvara, Qin, Ruonan, Krebs, Sebastian, Elgeti, Matthias, Matysik, Jörg, Coin, Irene

The inherently low polarization of magnetic resonance techniques limits the sensitivity of both EPR and NMR, despite their central role in structural biology. To address this challenge, we propose the development of a hypersensitive spin tag tailored for hyperpolarized EPR spectroscopy. Our approach exploits flavoprotein domains - such as Light-oxygen-voltage-sensing domain (LOV domain) - that generate spin-correlated radical pairs (SCPRs) upon photoactivation between a flavin cofactor and an aromatic residue. These SCPR-generating proteins will be employed as fusion tags for G-protein coupled receptors (GPCRs), enabling enhanced double electron-electron resonance (DEER) measurements. Sensitivity and performance will be further optimized through the site-specific incorporation of non-canonical amino acids (ncAAs) to modulate radical pair dynamics.

To complement hyperpolarized EPR, the same systems will be investigated using photoCIDNP to directly probe spin dynamics and electron transfer processes. The engineering strategy focuses on systematic mutagenesis of LOV domains to improve photostability and precisely control electron transfer pathways. Targeted modifications within the flavin-binding pocket and surrounding residues will enable tuning of radical pair formation and semiquinone stability, thereby enhancing spin polarization efficiency across both EPR and NMR readouts. By extending radical lifetimes and enhancing signal robustness under physiologically relevant conditions, the resulting optimized LOV-based spin tags will provide a versatile platform for in-cell studies at low concentrations and for elucidating the mechanistic basis of protein-mediated hyperpolarization.

Revealing Hidden Short-Range Order in Prussian Blue Analogues by Multinuclear Solid-State NMR Spectroscopy

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Prussian blue analogues (PBAs) are widely described as highly symmetric, vacancy-tolerant framework solids with a simple long-range structure. Yet, their electrochemical and magnetic properties are governed by short-range structural features that evade diffraction- and scattering-based characterisation. Here, we resolve the complete short-range structure of the diamagnetic Na-ion PBA, $\text{Na}_2\text{CdFe}(\text{CN})_6 \cdot \text{H}_2\text{O}$ using a comprehensive multi-nuclear, variable-temperature solid-state NMR spectroscopy approach. Direct ^1H — ^1H , ^1H — ^2H , ^1H — ^{23}Na and ^1H — ^{113}Cd dipolar correlations quantitatively define cation–water and framework–water proximities, enabling unambiguous assignment of all water and sodium environments. We reveal heterogeneous Na–water coordination motifs and defect environments that locally break the average crystallographic symmetry. Combined with first-principles calculations of the NMR shifts and defect formation energies, our results uncover a locally anisotropic and structurally differentiated network concealed within the long-range framework. By establishing an experimentally constrained short-range structural fingerprint in the absence of paramagnetic resonance broadening, we create a benchmark against which the spectroscopic signatures of technologically relevant paramagnetic PBAs are quantitatively interpreted. More broadly, we demonstrate that dipolar recoupling NMR can directly resolve hidden disorder in cyanometallate frameworks, redefining the structural description of PBAs from idealised periodic lattices to locally heterogeneous solids.

Next-Generation EPR: Combining High-Performance Q-Band Instrumentation with AI-Enhanced Spectral Processing

Zhiyu Sun, Yang Zhou, Zhuoyang Qin, Yue Zhang, Zhifu Richard Shi, Kebiao Xu

The advancement of Electron Paramagnetic Resonance (EPR) spectroscopy is increasingly defined by the convergence of robust hardware and intelligent software. While EPR remains a vital tool for probing paramagnetic centers, its broader adoption is often limited by instrument complexity and the steep learning curve of spectral analysis. Researchers at CIQTEK are addressing these barriers by developing high-performance hardware alongside a dedicated AI model for EPR, enhancing both accessibility and resolution.

On the hardware side, we developed a Q-band pulsed EPR system capable of 10 ns $\pi/2$ pulse wide-bandwidth excitation. Unlike conventional systems, the architecture employs a Solid-State Power Amplifier (SSPA), providing longer operational lifetime and significantly lower maintenance than traditional vacuum-tube technology. This platform bridges materials chemistry and structural biology by delivering substantial improvements in data quality. High sensitivity and spectral resolution enable analysis of complex metal hyperfine couplings while orientational constraints provide richer dipolar information for high-resolution DEER distance measurements. Complementing these advances is a three-layer AI EPR model designed to streamline the workflow from raw data to publication. Trained on more than 20,000 real and simulated datasets, it achieves test precision of 99.9% for simulations and 92% for complex real samples. The digital assistant automates spectral fitting, component characterization, and structured experimental reporting. It also provides predictive guidance by suggesting follow-up experiments to confirm sample certainty and validate results, making complex EPR analysis more accessible and efficient.

By combining high-bandwidth instrumentation with predictive analytics, EPR can evolve from a specialized technique into a more impactful high-throughput technology. This integrated approach improves data reliability while lowering the entry barrier for researchers in chemistry, biology, and materials science.

Photonic band gap resonators for high-sensitivity pulse EPR at 35 GHz and higher frequencies

Smirnov, Alex I., Milikisiyants, Sergey, Marek, Antonin, Schmidt, Hank, Nevzorov, Alexander A.

Pulse EPR spectrometers at X- and Q-band typically employ cavity resonators to achieve high sensitivity by confining microwave field within a small volume and enabling effective noise rejection. At W-band (95 GHz) and higher frequencies, non-resonant sample holders are more common. The latter designs simplify the operation and substantially increase bandwidth, which is essential for generating ns-scale pulses, but sacrifice resonant enhancement of the microwave field at the sample. Pulse double electron electron resonance (DEER) experiments are particularly challenging at any EPR frequencies because of the necessary compromise between the bandwidth needed for manipulating spins at two resonance frequencies and maintaining a sufficiently high Q-factor for adequate sensitivity. In this work, we summarize two major advances in developing a fundamentally different class of EPR resonators based on 1D photonic band gap (PBG) crystals. Specifically, we describe a PBGR architecture enabling a fully tunable external coupling: from under-coupled to critically coupled and then to over-coupled regimes. Secondly, we introduce a PBGR design in which the separation between the two high-Q resonant modes can be tuned – in some cases up to 1.1 GHz – to match the frequency requirements of DEER experiments. Our Q-band (34 GHz) oversized PBGR constructed from 2-inch sapphire discs achieved unloaded $Q \approx 27,300$ at room temperature under conditions close to critical coupling ($\beta \approx 1$). Integration of such resonators into a Bruker E580 Q-band EPR spectrometer demonstrated at least tenfold improvement in concentration sensitivity for echo-detected nitroxide signals. DEER experiments with dual-mode PBG demonstrated similar sensitivity improvements. Supported by NIH R01GM130821.

Mutagenesis-free assignment of a GPCR by PCS NMR Spectroscopy.

Häussinger, Daniel; Rieder, Pascal; Rößler, Philip; Wu, Fengjie; Grzesiek, Stephan; Gossert, Alvar.

G-protein-coupled receptors (GPCRs) are eukaryotic cell surface receptors that bind extra-cellular signalling molecules and trigger an intracellular response. They have vast importance as drug targets and, therefore, insight into their structures and signalling mechanisms is highly desired. We are reporting here on the assignment of the pharmaceutically particularly relevant human β_1 -adrenergic receptor (β_1 AR). The β_1 AR GPCRs form a difficult target, as they are large membrane proteins (> 100 kDa), that need to be expressed in eukaryotic cells, and their subtle structure does not tolerate even single-point mutations well. The standard assignment methods using 3D- and 4D-NMR experiments on doubly labelled constructs are therefore out of reach. We have recently introduced an assignment method for amino acid selectively labelled turkey β_1 AR by means of pseudo contact shift (PCS) NMR. As the β_1 AR is too sensitive to allow for single cysteine mutants as site-specific attachment points for our DOTA-based lanthanoid chelating tags, an indirect approach via tagging the small protein G mimics called nanobodies, proved to be successful. As a starting point, six valine assignments were known from tedious mutation experiments which were of great help. In the current work we demonstrate that the assignment can also be achieved *ab initio*, by using only the relative orientation of the paramagnetic susceptibility tensors from the tagged nanobodies as a starting point. In both constructs, the turkey and the human β_1 AR, also the methyl resonances of methionine were assigned, which is one of the most difficult to assign by PCS, due to the long and flexible side chain. In summary a new assignment method for large (membrane) proteins has been developed that should be universally applicable.

PELDOR spectroscopy of BtuB–TonB complex in the periplasm of *E. coli*

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One of the main goals of structural biology is to explore the structure and conformational dynamics of biomolecules in their native environment. As intracellular conditions are barely reproducible in vitro, the investigation of membrane proteins should be conducted inside the living cells. However, with the exception of a few works, most studies are still performed on purified and reconstituted membrane proteins, which may not fully reflect their functional states. Pulsed electron–electron double resonance spectroscopy (PELDOR/DEER) is a powerful technique for measuring nanometer-scale distance distributions and monitoring conformational changes in protein complexes. Here, we established a method to obtain structural information and directly observe conformational changes in the periplasm of the Gram-negative bacterium *E. coli* using PELDOR spectroscopy. Using this approach, we demonstrate the interaction between the outer membrane protein BtuB and the periplasmic protein TonB in *E. coli*. To selectively observe interactions in the bacterial periplasm, TonB labeled with Gd^{3+} spin tags was introduced into cells via electroporation. Selective labeling of BtuB on the extracellular side with a nitroxide label, combined with short cell recovery steps, enabled measurement of specific interprotein distances. The CW EPR and PELDOR data reveal differences in the conformational ensemble of the TonB–BtuB–cobalamin complex between in vivo and in vitro conditions, emphasizing the impact of the cellular environment. This approach provides high selectivity and enough sensitivity to study periplasmic processes directly in living bacteria.

Analysis of partial hyperfine decoupling during microwave spin locking

Lenjer, Marvin, Bennati, Marina

Systems of coupled electron and nuclear spins under microwave (MW) irradiation are frequently encountered in magnetic resonance spectroscopy. For example, the ability of MW spin lock pulses to decouple electron spins from their environment can be harnessed to reduce electron decoherence or to collapse hyperfine-split signals that would overlap in standard electron-nuclear double resonance (ENDOR) spectra. A similar situation is encountered in dynamic nuclear polarization (DNP), where the role of strongly hyperfine shifted nuclear spins in spin diffusion is still under investigation. Also here, continuous MW irradiation affects the effective couplings in the system.

In this work, we analyse the effect of MW spin locking with intermediate strength (i.e., with a Rabi frequency on the order of the hyperfine coupling) on the effective hyperfine coupling using ENDOR spectroscopy. We compare the performance of different pulse sequences using spin lock and phase-modulated MW pulses. The observed hyperfine decoupling is compared to theoretical predictions from analytical models and average Hamiltonian theory. Work to apply the experiments to more advanced spin locking or pulsed DNP sequences is still ongoing. With our results, we hope to contribute to the better understanding the effect of both constant and time-dependent MW locking fields on electron nuclear spin dynamics and the role that hyperfine decoupling plays in experiments like DNP.

A multifunctional lanthanoid tag for future application as PCS and FRET probe

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The investigation of biomacromolecules is of great interest for a variety of research fields, including biology, medicine and pharmacology.

Lanthanoid chelating tags are utilized to induce pseudocontact shifts (PCS), residual dipolar couplings and paramagnetic relaxation enhancement in NMR applications. These tags function as versatile instruments for elucidating biomacromolecular structures, dynamics, and interactions. In addition to their distinct magnetic properties, which are exploited for their PCS application, the unique electronic configuration of the lanthanoid ions renders them excellent probes for bioimaging techniques such as Förster resonance energy transfer (FRET) spectroscopy and fluorescence microscopy.

Herein, we report the design of a novel cyclen (1,4,7,10-tetrazacyclododecane)-based ligand intended for future applications as a PCS and FRET probe in the investigation of biomacromolecules.

The highly unsymmetrical ligand possesses an antenna residue with an absorption range up to 380 nm, as well as a binding site for covalent binding to cysteine residues. Consequently, the ligand offers the essential properties necessary for multifunctional usage.

Preliminary NMR and photophysical studies of different lanthanoid complexes have shown promising chemical shift ranges and successful energy transfer from the antenna moiety to the metal ion, resulting in the detection of intense and long-lived lanthanoid-centered emissions.

The investigations have culminated in the development of an adapted ligand structure, aimed at further refining the desired characteristics of the complex.

Deuterated lactic acid building blocks – A novel labeling strategy to determine intrinsic properties of lanthanoid chelating tags

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Lanthanoid chelating tags (LCTs) have emerged as an important tool for paramagnetic solution NMR studies of large biomacromolecules. By inducing large pseudocontact shifts (PCSs), they provide access to long-range structural information, enabling the analysis of protein structures, dynamics, and intermolecular interactions. The efficiency of an LCT strongly depends on its intrinsic properties, in particular anisotropy of the magnetic susceptibility tensor χ . Previous approaches for characterizing and determining the intrinsic magnetic susceptibility tensor χ have relied on complex site-specific isotopic labelling strategies and multistep syntheses. The associated synthetic effort significantly limits a rapid investigation and comparison between different linkers and tag designs, as well as their intrinsic properties.

Herein, we report an alternative labelling strategy of deuterating the lactic acid side-arm protons on our DOTA-M8 scaffolds. This significantly simplifies experimental access to intrinsic tensor parameters. The novel approach not only significantly reduces synthetic effort but also allows for a rapid estimation of intrinsic tensor properties on the free LCT. This method enables an efficient comparison of different linker designs and an accelerated development and optimization of possible future LCTs for improved paramagnetic properties.

W-band ENDOR reveals ^{19}F – Mn^{2+} interactions in fluorinated ATP complexes

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ATP is a central molecule in biological energy metabolism, and its hydrolysis drives many cellular processes. In ATP-dependent proteins, Mg^{2+} is the native cofactor; however, Mn^{2+} is frequently used as a paramagnetic substitute for structural investigations employing ESR spectroscopy. Previous studies have shown Mn^{2+} coordination to ATP involving the β - and γ -phosphates and the N7 position of adenine, with multiple coordination geometries complicating distance analysis [1]. This structural ambiguity motivates the use of techniques capable of probing Mn^{2+} -ligand distances in the Ångström-to-nanometer range, such as ^{19}F ENDOR, which has been applied to several paramagnetic centers, including trityl radicals, Gd^{3+} , and Cu^{2+} [2]. Despite these advances, ^{19}F – Mn^{2+} distance measurements remain largely unexplored in biologically relevant systems.

Here, we report, to our knowledge, the first ^{19}F – Mn^{2+} distance measurements in an ATP- γ -F–Mn complex using Q-band and W-band ENDOR, where ^{19}F is incorporated into the γ -phosphate group of ATP. Q-band Mims ENDOR shows strong overlap between the ^{19}F and ^1H hyperfine couplings, severely limiting resolution. In contrast, W-band ENDOR overcomes this overlap and fully resolves the two allowed ^{19}F ENDOR transitions.

These results establish a framework for ^{19}F – Mn^{2+} distance measurements in nucleotide systems and ATP-dependent proteins such as ABC transporters.

[1] A. Litvinov *et al.*, *J. Magn. Reson.* **2018**, 294, 143–152.

[2] A. Bogdanov *et al.*, *Phys. Chem. Chem. Phys.* **2024**, 26, 26921–26932.

Direct chiral discrimination in nuclear magnetic resonance (NMR) remains a significant challenge due to the indistinguishable behavior of enantiomers in conventional achiral environments. In this work, we investigate electro-nuclear magnetic shielding polarizabilities as a mechanism to induce chiral sensitivity in NMR measurements. Chiral tensors were computed using quantum chemical methods implemented in Gaussian and Dalton, providing detailed insight into the pseudoscalar chiral strength and its dependence on electronic structure, molecular geometry, and local chemical environment.

To experimentally probe these effects, we are developing an RLC-based resonant cell designed to generate controlled alternating (AC) electric fields within the sample during NMR experiments. This approach enables simultaneous coupling of electric and magnetic fields, which is essential for achieving direct enantiomer discrimination. Particular emphasis is placed on optimizing the resonator design, including maximizing the quality factor (Q), minimizing dielectric losses, and maintaining impedance matching to ensure efficient and stable field generation.

Current experimental efforts focus on overcoming practical limitations associated with field strength, sample loading, and resonator losses. Preliminary observations aim to establish a quantitative correlation between computed chiral tensors and experimentally measurable NMR responses. This work contributes toward bridging the gap between theoretical predictions and experimental realization, providing a viable pathway for enhancing sensitivity and expanding the applicability of chiral NMR spectroscopy.

Helium-3 magnetometers for high fields

Blümler, Peter, Fertl, Martin, Grafe, Joachim, Graf, Robert, Heil, Werner

Low magnetic fields ($< 10^{-2}$ T) can be measured with extreme precision using SQUID or SERF sensors, but at high fields nuclear magnetic resonance provides the greatest accuracy. Continuous frequency measurements require samples with long coherence times, which can be achieved with motionally averaged gas signals. This in turn demands hyperpolarized gases at only a few millibar pressure. ^3He is ideal because it can be hyperpolarized by metastability optical pumping, interacts only weakly with its environment, and has an independently determined gyromagnetic ratio. With suitable low-susceptibility containers, extremely long T_2^* times (100 - 200 s) and absolute field measurements are possible.

In addition to low-pressure hyperpolarized ^3He for ultra-precise magnetometry ($< 10^{-12}$), we have also produced high-pressure (up to 50 bar) thermally polarized ^3He for applications where optical polarization is impractical. These robust NMR magnetometers can operate from 1 - 300 K, and we describe methods to tune T_1 for rapid sampling across 5 - 300 K. Overall, ^3He magnetometers show strong potential as a new standard for high-precision magnetometry at high magnetic fields.

Instrumentation for Rapid Observation of Protein-Bound Ions

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Protein NMR has historically relied almost entirely on 3 isotopes – ^1H , ^{13}C , and ^{15}N – despite the fact that about half of all proteins bind metal ions. Metallothioneins (MT), for example, bind a wide range of different metal and non-metal ions (Fig. 1a, Messerle, et al. *J. Mol. Bio.*, 1990). These include $^{113}\text{Cd}^{2+}$, $^{199}\text{Hg}^{2+}$, $^{77}\text{Se}^{2-}$, and $^{207}\text{Pb}^{2+}$, which are more sensitive than ^{15}N . Their higher frequencies also mean stronger dipolar couplings to other spins (Fig. 1b). Though ^{113}Cd NMR spectra of MTII have been previously obtained, incorporation of other isotopes have not been widely reported. One of the main reasons for this is that most probes available to researchers are HCN or HXY probes. Though the HXY probes cover broad bands of available frequencies, they are usually still limited to relatively specific combinations of isotopes.

We are working to overcome this limitation with a “universal NMR probe kit” which will be capable of observing most combinations of isotopes of interest. The probe will be based on the Schaefer-McKay transmission line (TL) design (Fig. 1c, Scott et al. *JMR*, 2018). We will construct this probe out of short segments that screw together, so that a TL of any length can be rapidly constructed (Fig. 1d), allowing for the rapid adaptation of the probe to most necessary frequencies. We will use this ability to rapidly switch between frequencies to determine structural information about all four human MTs bound to a range of different ions.

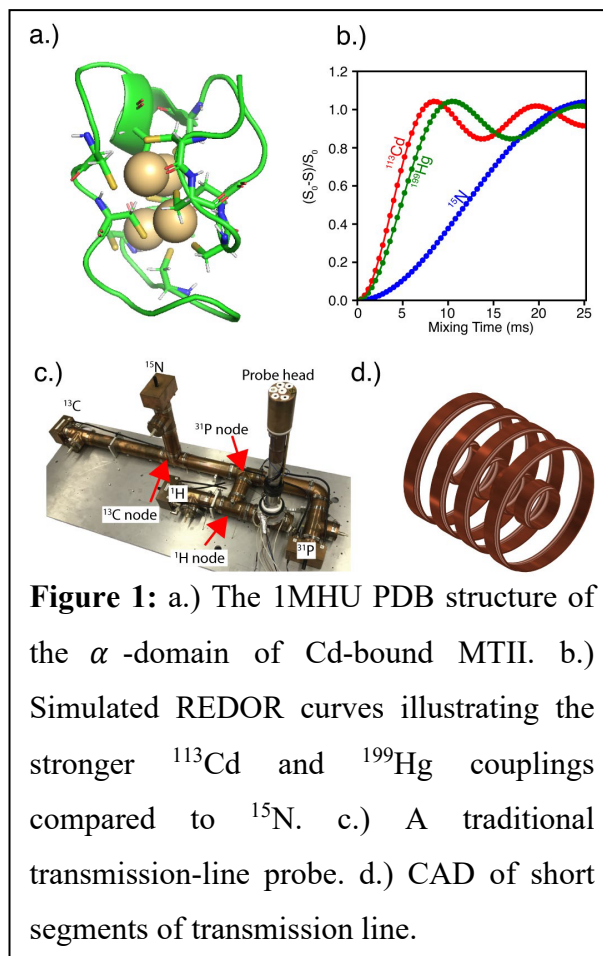


Figure 1: a.) The 1MHU PDB structure of the α -domain of Cd-bound MTII. b.) Simulated REDOR curves illustrating the stronger ^{113}Cd and ^{199}Hg couplings compared to ^{15}N . c.) A traditional transmission-line probe. d.) CAD of short segments of transmission line.

Bimodal Q-band Probehead with Improved Signal-to-Noise Ratio in Pulse EPR

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The concept of a probehead equipped with an additional low noise amplifier (LNA) is becoming increasingly popular to enhance the sensitivity of EPR spectrometers. Here we propose using an EPR probehead based on a Q-band bimodal cavity with strong isolation between the input and output ports, in combination with a low noise amplifier located outside the cryostat at room temperature. Pulsed EPR experiments demonstrate a 4-fold increase in the signal-to-noise ratio (SNR) of a bimodal probehead operating in transmission mode compared to its operation without an additional LNA. Performance of the bimodal probehead was also compared with a Bruker EN 5107D2 probe, which showed a 7-fold improvement that can be achieved by increasing the SNR by a factor of 2 due to the additional LNA and isolation of the detection channel from the input signal, and by factor of 3.3 due to a larger sample volume in the bimodal probe ($\sim 20 \mu\text{l}$) in the Q-band compared to the Bruker one ($\sim 6 \mu\text{l}$). The developed probehead can be used together with Bruker ELEXYS EPR spectrometers without modification of the microwave bridge.

Characterising multiphase flow using two dimensional T_2 – propagator correlation NMR

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Quantifying fluid flow in industrial settings and engineering contexts needs to be robust, safe and inexpensive. NMR and MRI is suitable for this task for both single and multiphase flows. For the latter, individual phases may easily be discriminated by their respective NMR relaxation times. In the meantime, fluid displacements can be obtained by a whole range of different NMR methodologies, such as MRI, propagator measurements, or NMR signal decays due to outflow.

Here, we suggest using the correlation of fluid displacement with T_2 relaxation. We identify the individual components by measuring the T_2 of the quasi-stationary fluid near the pipe wall. Furthermore, by determining the signal decay due to outflow, the average velocity of individual components can be obtained. We therefore measure the correlation between the fluid displacement and the corresponding relaxation times in a 2D Laplace-Fourier Transform experiment which extracts the T_2 values and the flow velocities simultaneously. The chief advantage of this method is to quantify liquid properties without stopping the flow.

One drawback of this technique is that it requires a long experimental time, a common difficulty for multidimensional (NMR) experiments. To mitigate this issue, we apply Poisson gap sparse sampling technique in which only a fraction of the full dataset is collected and then reconstructed by linear interpolation algorithms.

Current implementations (which will be presented) utilise high field wide bore spectroscopy NMR systems which allow us to develop the method under favourable conditions (homogeneous B_0 and B_1 fields, presence of 3D gradient system). The extension to low field NMR system and higher flow rates is subject to future research.

An Automated Titration Setup for NMR Spectroscopy

Kouřil, Karel, Narwal, Pooja, Zasukhin, Dmitrii, Safiullin, Kajum, Mager, Dario, Saliba, Georges, Korvink, Jan G., Meier, Benno

We present a fully automated titration system for NMR spectroscopy. It prepares arbitrary 2 component mixtures on demand and holds them in an NMR tube during observation. The system consists of three syringe pumps, a microfluidic mixer and a 3 mm flow-NMR tube connected by low-dead volume microfluidic tubing. Two syringes with the component solutions are connected to the inlet of the mixer, the output of the mixer is lead through a flow NMR tube to a third pump. The use of pumps on both inlet and outlet of the NMR tube allows for stop flow operation where the liquid is static during the NMR acquisition. Composition of the mixtures is controlled by adjusting the flow rates of the inlet pumps, the pump on the NMR tube outlet aspirates the total inlet flow.

We tested the operation of the system on Tryptophan — Bovine Serum Albumin (BSA) mixtures. We used it to prepare 9 mixtures with different tryptophan to BSA ratios and observed the binding by Saturation Transfer Difference (STD) experiments. We fitted the observed STD initial buildup rates with the exact formula for ligand bound fraction which yielded a dissociation constant of $57 \pm 11 \mu\text{M}$.

A Field-Tilt Implementation of Off-MAS Spectroscopy

Daniel Hottinger, Luca Theytaz, Matthias Ernst

Small magic angle offsets reintroduce a scaled part of the anisotropic interactions, e.g. dipolar coupling, which are averaged out to first order at the magic angle. To avoid the additional broadening caused by spinning off the magic angle during evolution and detection periods, a fast switching mechanism would make this method much more effective. One approach to generate magic angle offsets is to tilt the magnetic field. This can be achieved by adding a magnetic coil into the probe around the stator that generates a field in the x-direction.

From the feasible geometries, a Helmholtz coil with middle radius of 17.7 mm and 100 turns, operating at 9 A affords a 49.3 ± 0.2 mT field, which yields a magic angle offset of 0.14° at $B_0 = 20$ T. To ensure the stability, a backed coil with 0.45 mm diameter copper wire was chosen, with a ceramics holder. The experimentally determined relative homogeneity of the coil's magnetic field is $< 1\%$ within the volume of a 0.7 mm rotor sufficient for our purpose. Further measurements showed that the coil pair has a total resistance of 3.5Ω , giving a power of 350 W at 10 A. With a duty cycle of 100 ms/4 s, experimental measurements revealed a temperature increase of 24°C after 5 min of operation at 8 A, that can be significantly reduced with a cooling air flow of 2 bar, preventing excess heating of the probe and sample. The rise times for the manufactured coils are below 1ms, during which eddy currents might be generated in the superconducting magnet, with a longer decay than the rise time. To counteract these eddy currents, a set of three shielding coils was designed such that the outside magnetic field is significantly smaller, therefore lessening the magnitude (and decay time) of the eddy currents.

High-Field NMR Instrumentation and Cryogenic RF Detection for the Cosmic Axion Spin Precession Experiment

Unni, Malavika for the CASPER Collaboration

This poster presents the high-field NMR instrumentation and cryogenic RF detection methodology developed for the Cosmic Axion Spin Precession Experiment (CASPER) at the Helmholtz Institute Mainz. The platform combines a 14.1 T superconducting magnet with broadband tunable cryogenic LC resonators operating over the 70–600 MHz frequency range for ultra-sensitive magnetic resonance detection. A key feature of the setup is the cryogenic RF detection chain operating near 20 K, incorporating low-noise cryogenic preamplification to suppress thermal noise and enhance weak-signal detection. The detection platform is designed to approach the sensitivity requirements necessary for axion dark matter searches beyond the capabilities of conventional NMR instrumentation. Magnetic-field homogeneity is optimized using a cryogenic shim coil together with an external shim stack to improve linewidths, resonance stability, and long-term field performance. The RF detection chain and broadband tuning circuitry are characterized through detailed system noise analysis, including measurements of resonator noise temperature, RF gain, and amplifier noise figure to identify dominant noise contributions across the full detection chain. The poster further discusses measurements using hyperpolarized liquid-state ^{129}Xe samples, broadband resonance tuning strategies, and cryogenic probe performance for small sample volumes. These developments establish a versatile ultra-low-noise high-field NMR platform for axion searches, precision spin detection, and other applications requiring extreme magnetic sensitivity.

Hyperpolarization

Signal amplification by reversible exchange (SABRE) is a cost- and time-efficient hyperpolarisation method that enables signal enhancements of several orders of magnitude by transferring spin-order from parahydrogen to the substrate-to-be-polarised in a temporary iridium complex. The efficiency of SABRE depends on the applied magnetic field during the polarisation transfer, which is conventionally optimised using the level anticrossing (LAC) condition. For example, when targeting ^{19}F nuclei, highest polarisation levels are acquired with the magnetic field of *ca.* 5 μT .

In this work, the optimisation of the polarisation transfer to ^{19}F is taken one step further by applying an oscillating magnetic field during the polarisation transfer. Experiments were carried out using SpinSolve 43 MHz benchtop NMR spectrometer in combination with a custom-built SABRE system. The sample was prepared by dissolving 3-fluoropyridine (15 mM) and $\text{IrCl}(\text{COD})\text{IMes}$ (3 mM) into deuterated methanol (600 μl). Efficiency of ^{19}F SABRE was examined using both static and oscillating magnetic fields.

After optimising the polarisation transfer under static and oscillating fields, an improvement of 79% is observed when transitioning from the static to the oscillating magnetic field. The improved polarisation levels are also reproduced by comprehensive spin dynamics simulations that incorporate both coherent and incoherent spin physics as well as chemical exchange. We introduce a new *generalised LAC condition* that explains the efficient coherent spin dynamics under the oscillating magnetic fields. The new model also enables the optimisation of the field parameters to various SABRE systems, advancing the potential of SABRE to real-world applications.

Over four minutes of pyruvate T_1 using chemically and physically induced deceleration of relaxation

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The limited life-time of tracers remains the single greatest impediment for biomedical application of hyperpolarized MRI. Previously, this challenge was addressed for some ^{15}N compounds, like nicotinamide, using chemically induced deceleration of nuclear spin relaxation (CIDER) (doi: 10.1126/sciadv.adx2316).

Here, we use the insights to prolong the life time of the most prominent tracer in metabolic MRI, $[1\text{-}^{13}\text{C}]\text{pyruvate}$, to more than 4 min.

T_1 nuclear magnetic relaxation dispersion of thermally polarized pyruvate was significantly affected by buffer, EDTA, degassing, deuteration of solvent and substrate, and magnetic field, and studied using a custom magnetic-field-cycling setup (doi: 10.1021/acsmeasuresciau.5c00131). We validated the results *in vitro* using cervical cancer cells (HeLa) and DNP-hyperpolarized $[1\text{-}^{13}\text{C}]\text{pyruvate}$, polarized at 1.3 K / 6.7 T using a trityl radical, and measured at 1 T and 9.4 T 45 s after dissolution.

T_1 of pyruvate in H_2O was 29.3 ± 0.4 s at 31 μT (shortest value in study), while the longest T_1 was $[1\text{-}^{13}\text{C}]\text{pyruvate-d}_4$ in degassed D_2O , reaching 242.1 ± 9.4 s at 0.5 T. In samples for cell experiments, about 165 s were reached at 1 T. Modelling allowed us to identify the contributions of different relaxation mechanisms: inter- and intramolecular dipolar coupling, chemical shift anisotropy, paramagnetic relaxation from oxygen and metal impurities.

In metabolic cells experiments, testing of the optimized vs normal formulation yielded $38 \pm 4\%$ more pyruvate signal at the beginning of detection, $110 \pm 3\%$ higher T_1 of pyruvate at 1 T, and, most impressively, a $133 \pm 19\%$ increased SNR of metabolic product lactate – the essential metric to assess metabolic sensitivity.

Such a long T_1 time can therefore further boost the translational power of HP metabolic MRI.

Evaluation of a cryogen-consumption free and automated d-DNP setup for complex mixture analysis at natural ^{13}C abundance

Horbenko Yuliia, Adamski Wiktor, Charrier Benoît, Jannin Sami, Eshchenko Dmitry, Melzi Roberto, Kempf James, Giraudeau Patrick

Dissolution DNP (d-DNP) is a powerful tool for accessing information that is difficult or impossible to obtain using conventional thermal NMR spectroscopy. Numerous fields, such as drug screening and metabolomics, now benefit from the dramatically enhanced ($>30,000\times$) NMR signals from d-DNP. In particular, we recently applied this in clinical metabolomics studies of biofluids at natural ^{13}C abundance. We thus identified and quantified biomarkers that could not be detected by conventional ^1H NMR due to peak overlap.

However, that work relied on a d-DNP system that was not automated (thus impacting repeatability) and which incorporated a helium-consuming cryostat with corresponding high operating costs and environmental footprint. Here, we present the first evaluation of an automated and zero-cryogen-consuming d-DNP system. The system is fully automated in both cryogenic operation, and d-DNP workflow to the benefit of repeatability that is critical in analytical applications.

Key performance features assessed include the transfer time of dissolution output to injection into the NMR tube, as well as the quality of resulting hyperpolarized ^{13}C NMR lineshapes and overall repeatability. Results were obtained from model metabolite mixtures at natural abundance ^{13}C . Particular attention was paid to limit the impact of residual sample flow on spectral quality. Results show much-improved repeatability on liquid-state signals than previously obtained with the earlier-generation equipment, now revealing 5% coefficient of variation on absolute signal areas and 2% after normalization, importantly with a much-reduced operating cost and improved ease-of-use. This performance is very encouraging for the future of hyperpolarized metabolomics studies and beyond.

Single-chain nanoparticles (SCNPs) are formed by the collapse of individual polymer chains, generating entities comparable to proteins in size, internal structure, and function. Especially, the formation of hierarchies induced by complex folds of linear polymer chains can result in internalized compartments, reminiscent of pockets in enzymes. However, direct experimental access to their architecture or mode of contact remains a challenge. Here, the conformational organization of a prototypical amphiphilic SCNPs is dissected to reveal conformational details of its internally heterogeneous morphology driven by site-specific intramolecular compaction. Using a synergistic combination of unconventional paramagnetic NMR, hyperpolarized water-based dissolution dynamic nuclear polarization (d-DNP), and NMR-guided molecular dynamics simulations, intramolecular structures and solvent accessibility are mapped at atomistic resolution. These findings uncover distinct nanoscopic compartments formed via back-folding of PEG side chains toward the SCNP backbone. Furthermore, these compartments shield internal segments, mimicking hydrophobic pockets found in folded proteins. Thus, this work introduces a transferable methodology for probing functional compartmentalization in synthetic macromolecules. It provides a tool for rationally designing next-generation nanomaterials and enzyme mimetics with programmable internal order via residue-resolved structural information. At the same time, this method's prowess is evidenced through a high-resolution description of the local conformations found within hierarchically structured SCNPs.

Liquid-State ^{77}Se Overhauser Dynamic Nuclear Polarization of Organoselenium Compounds at 9.4 T

Rao, Yu, Yang, Luming, Bennati, Marina

Dynamic Nuclear Polarization (DNP) is now a robust tool for enhancing NMR sensitivity in the solid state, its translation to the liquid state presents unique opportunities. Among liquid-state hyperpolarization techniques, Overhauser Effect (OE) DNP is advantageous as it provides continuous and steady-state signal enhancement, contrasting with the one-shot techniques. OE-DNP at high fields has been pioneered through scalar-dominated mechanisms in nuclei such as ^{13}C , ^{19}F , and ^{31}P . To our knowledge, its application to some other important nuclei, such as ^{77}Se , has not yet been reported.

In this work, we present the first study of ^{77}Se OE-DNP. By optimizing the polarizing agent and sample geometry, we observed rapid polarization build-up on the order of seconds with enhancement factors (ϵ) ranging from 200 to 1000 at 9.4 T, corresponding to coupling factors up to -0.5 . This methodology was evaluated across a series of distinct selenium environments, including a drug molecule Ebselen. The mechanism was further investigated by DFT calculations, which suggest transient scalar interactions between the selenium center and the polarizing agent. The high ^{77}Se OE-DNP enhancement affords the sensitivity required for *in situ* monitoring of a diselenide metathesis reaction over 48 hours, providing sufficient temporal resolution for kinetic analysis. These results demonstrate the potential of OE-DNP for sensitivity enhancement in ^{77}Se NMR and suggest its broader applicability to other heavy elements.

Restoration of the hyperpolarized in vivo ^{13}C signal at a low magnetic field

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Low magnetic field acquisition is attractive due to its portability and lower cost^[1]. However, the inherently low signal-to-noise ratio (SNR) is a major limitation. Combining low-field detection with hyperpolarization can mitigate this by substantially increasing signal intensity. In this project, we aimed to observe the in vivo ^{13}C signal of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ using a self-constructed multinuclear low-field scanner operating at 66 mT^[2]. Initial in vivo measurements demonstrated that the longitudinal relaxation time (T_1) of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ is too short at this field strength to reliably detect the pyruvate signal.

Subsequent in vitro studies at 66 mT indicated that binding of pyruvate to albumin is one of the main causes for the pronounced T_1 reduction observed at low magnetic fields. Specifically, the T_1 of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ at 66 mT decreased from 47 ± 1 s in PBS buffer (H_2O) to 7 ± 1 s in an albumin solution (BSA, 11% in PBS-buffered H_2O). In comparison, measurements at 7 T magnetic field showed only a minor decrease in T_1 , from 41 ± 1 s (PBS buffer, H_2O) to 30 ± 3 s (BSA, 11% in PBS buffered H_2O).

To prevent albumin binding of pyruvate, displacement agents with high affinity for albumin were added to the pyruvate solution^[3]. The addition of ibuprofen, iophenoxate and n-octanoate increased the T_1 of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$ in albumin solution (BSA, 11% in PBS buffered H_2O) at 66 mT from 7 ± 1 s to 34 ± 2 s, 30 ± 5 s, and 37 ± 2 s, respectively.

In vivo experiments demonstrated that the addition of these displacement agents restored the detectable signal of hyperpolarized $[2-^{13}\text{C}]\text{pyruvate}$. Using a 90° flip angle, ^{13}C signals were measured 8 s after injection.

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Towards The In-Cell Detection of Therapeutical siRNAs: ^1H - ^{19}F CP MAS Experiments Using the New HFX Solid-State DNP Probe

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Solid-state NMR spectroscopy is a widely used method for the characterization of solid-state materials, which has its well-established place in pharmaceutical research. ^{19}F NMR is commonly used to study drug molecules, excipients, and polymers due to the abundance of fluorine in these materials and absence of fluorine in the biological background. However, biologically relevant concentrations of drugs are typically low and therefore the low sensitivity of ^{19}F NMR might become an issue. Dynamic nuclear polarization (DNP) NMR is a convenient method of choice to overcome this limitation. With DNP, NMR signals are enhanced by transferring polarization from electron spins to nuclear spins. In recent years, DNP-enhanced ^{19}F solid-state NMR has emerged as a promising approach for studying fluorine containing molecules. However, direct polarization of ^{19}F faces several disadvantages, among others the difficulty of developing a suitable radical solution optimized for direct ^{19}F hyperpolarization. The new HFX probe provides us with a possibility of performing ^1H - ^{19}F CP MAS experiments under DNP conditions, which represents a notable advantage in fluorine NMR and results in major sensitivity gain compared to the direct-detected fluorine experiments. This is mainly thanks to the possibility of transferring of the hyperpolarized proton magnetization to fluorine spins, but also since ^1H - ^{19}F CP MAS requires a shorter recycle delay for ^1H relaxation compared to ^{19}F . We demonstrated the performance of ^1H - ^{19}F CP MAS experiments on two siRNA tool compounds in radical solution, where we obtained DNP enhancement of and $\epsilon_{\text{on/off}}(^{19}\text{F}) \sim 150$. We also detect these compounds in-cell by the same method, which demonstrates the superiority of the ^1H - ^{19}F CP MAS DNP in fluorine NMR applications. We anticipate that the new HFX probe will find a broad range of applications including but not limited to pharmaceuticals, in-cell NMR and material development.

A stable SABRE catalyst for the hyperpolarization of [1-¹³C]pyruvate

Wacker, Jakoba, Lehmkuhl, Sören, Korvink, Jan G.

Signal amplification by reversible exchange (SABRE) can selectively enhance nuclear magnetic resonance (NMR) signals by multiple orders of magnitude. The transient coordination of parahydrogen and a substrate to an iridium-based polarization transfer catalyst enables the transfer of magnetization through the spin-spin coupling network of the resulting complex. The transfer of spin order from parahydrogen to the substrate is achieved without any modification to its chemical structure, making the hyperpolarization with SABRE regenerative.

SABRE in shield enables alignment transfer to heteronuclei (SABRE-SHEATH) is associated with long polarization lifetimes and background-free signals, making it particularly interesting for in vivo applications. Due to its biomedical relevance as a marker for metabolic diseases [1-¹³C]pyruvate is a key target molecule in SABRE-SHEATH.

The biocompatibility of SABRE can be increased by utilizing solvent mixtures of acetone and water (Ace-SABRE), which additionally facilitates the solubility of the catalyst and pyruvate even at high concentrations. However, Ace-SABRE is associated with a limited catalyst lifetime due to rapid catalyst degradation. This stands in contrast to the regenerative nature of SABRE and limits continuous as well as repeated (re)hyperpolarization. In this study, a structural variant of the standard IrIMes (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene) catalyst was developed that showed significantly increased stability in Ace-SABRE. IrIMes's robust counterpart exhibits a 17 times longer lifetime during the exponential catalyst activity decay under hydrogen atmosphere and a four times longer lifetime under exposure to ambient atmosphere.

This allows for reproducible and comparable measurements with the same sample over the course of many hours, making SABRE more streamlined and sustainable.

Xe host load optimisation in liposomes for ^{129}Xe HyperCEST NMR

Bayer, Viktoria, Schröder, Leif

^{129}Xe HyperCEST imaging using liposomal nanocarriers with molecular cages for reversible Xe binding represents a biocompatible and adaptable strategy for selective molecular imaging. Liposomes functionalised with cryptophane-A (CrA) cage structures embedded as lipopeptide-conjugates show excellent performance. However, the optimal concentration of CrA cages per liposome for HyperCEST remains unclear. To investigate this, liposomes containing various CrA concentrations were produced.

Liposome formulations were prepared with POPC as the main lipid component and fixed amounts of 10 mol% cholesterol, 4 mol% PEG2000 and 0.2 mol% Liss Rhod PE, and five contents of [0, 0.4, 1, 2, 10] mol% CrA-lipopeptides. The total CrA concentration was kept fixed at 2 μM (through variable liposome concentrations), and samples were dispersed with a gas mix containing 2 % hyperpolarised Xe at 3.5 bar overpressure. Z-spectra were acquired with saturation pulse parameters of $P_{\text{sat}} = 1 \text{ mW}$ and $t_{\text{sat}} = 8 \text{ s}$, and depolarisation experiments were performed at $P_{\text{sat}} = 40 \text{ mW}$ with t_{sat} varying from 0.1-15 s.

The experimental results demonstrate increasing efficiency with lower cage loads. Reducing the CrA content from 10 mol% to 1 mol% increased the normalised CEST response by $(28 \pm 2) \%$, while no significant improvement was observed below 2 mol%. These findings indicate that HyperCEST efficiency benefits from a more diluted cage, with an optimum between 0.4 and 2 mol% CrA. We hypothesize that depolarized Xe is produced instantaneously in the lipid environment and that the limiting rate factor is its release into the aqueous detection pool. This suggests that lipid content providing fast depolarization in the immediate cage vicinity should be prioritised over cage load when optimising future contrast agents.

Studying Hydrogen-Bound States of Hydrogenases under Turnover Conditions by Parahydrogen-Enhanced NMR

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Unraveling the mechanistic principles underlying fundamental natural processes such as hydrogen conversion may guide the design of more efficient and selective synthetic catalyst.^[1] Mechanistic pathways are often determined by low-concentrated transient species that evade direct observation and are intrinsically hard to study using mainstream analytical techniques.^[2] This is particularly pronounced for hydrogenase catalysis and the characterization of hydrogen-bound species of the active site as diamagnetic states cannot be detected by EPR-based methods. Instead, structural insights into these species relies on high-resolution diffraction data (XRD) or nuclear resonance vibration spectroscopy (NRVS) for obtaining information on the atomic level.^[3] In principle, ^1H NMR spectroscopy is ideal for the study of hydrogenase enzymes as both the product (H_2/H^+) and the substrate (H^+/H_2) can be detected. Recently, the methodology for the study of diamagnetic enzyme states was extended by overcoming the intrinsically low sensitivity of NMR through the use of parahydrogen.^[4] In combination with DFT calculations and spin dynamic simulations, this rendered the characterization of two formerly undetected on-cycle species of [Fe] hydrogenase catalysis possible. Additionally, we showed that parahydrogen can be used for the study of symmetric complexes resembling the reactivity of nitrogenase enzymes.^[5] Herein, we expand the application of this methodology to another class of hydrogenase enzymes and show that parahydrogen can be used for the study of hydrogen-bound intermediates with exceptional sensitivity under turnover conditions.

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PHIP-SAH Hyperpolarization of [1,5- $^{13}\text{C}_2$] OMPD

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Hyperpolarized [1,5- $^{13}\text{C}_2$] OMPD is a critical probe for in vivo filtration, perfusion, and pH imaging. While Parahydrogen-Induced Polarization via Side-Arm Hydrogenation (PHIP-SAH) is a cost-effective alternative to Dynamic Nuclear Polarization (DNP), polarizing this probe remains challenging. Achieving the high polarization on both ^{13}C sites required for pH imaging is hindered because the OMPD 5-membered ring exists in an equilibrium between two forms with distinct J-coupling networks, alongside weak direct coupling of the $^{13}\text{C}_5$ nucleus to parahydrogen. Consequently, initial tests with natural abundance ester yielded only $P(^{13}\text{C}_5) = 2\%$ vs. $P(^{13}\text{C}_1) = 20\%$.

Here, a PHIP-SAH-based hyperpolarization approach for [1,5- $^{13}\text{C}_2$] OMPD is presented, using a Boc-protected propargyl sidearm and PulsePol, a symmetry-based transfer sequence. Experiments with [1,5- $^{13}\text{C}_2$]-labeled OMPD elucidated the J-coupling networks of the open and closed ring forms. Successful polarization transfer relies on the closed form's coupling of $J(\text{C}_1\text{-C}_5) = 1.8 \text{ Hz}$, initially yielding $P(^{13}\text{C}_5) = 7\%$ and $P(^{13}\text{C}_1) = 7\%$. To effectively polarize both sites, the J-coupling network was tailored via selective ring deuteration. This simplifies the spin system and preserves the $^{13}\text{C}_5\text{-}^{13}\text{C}_1$ eigenbasis, drastically improving polarization from 7% to 26% on both sites.

Finally, ester cleavage and purification were explored. As this process requires highly basic conditions, it potentially compromises chemical stability. Optimizing the protocol minimized degradation, yielding up to 80% isolated OMPD in D_2O .

This methodology consistently provides high ^{13}C polarization post-purification, establishing PHIP-SAH as a robust platform to produce hyperpolarized [1,5- $^{13}\text{C}_2$] OMPD for molecular imaging.

Benchtop NMR Signal Enhancement of Metabolites in Urine Extract using SABRE

Fleischer, Simon, Yang, Jing, Ausmees, Kerti, Reile, Indrek, MacKinnon, Neil, Korvink, Jan Gerrit, Lehmkuhl, Sören

Rapid analysis of body fluids has become an important focus in health and metabolomics research. Urine is of particular interest, as it can be sampled non-invasively, and can give a comprehensive record of an individual's metabolic state.

Up until now, parahydrogen-enhanced NMR spectroscopy of urine has been performed by non-hydrogenous ParaHydrogen-Induced Polarization (nh-PHIP), allowing for the investigation of pharmacokinetics, as well as metabolite-based characterization of organism origin and characteristics. However, nh-PHIP is limited to indirect detection of metabolic markers by detecting hydride chemical shifts, which complicates the adaption of the technique to new workflows. We employ hyperpolarization through Signal Amplification By Reversible Exchange (SABRE). SABRE does not involve chemical modification of the target molecules, just as nh-PHIP. Furthermore, the SABRE process does not require a high magnetic field, which enables the use of benchtop NMR spectrometers for acquisition. At a modest magnetic field, we were able to detect several metabolites in a urine extract, and quantify the concentration of nicotinamide.

SABRE experiments were performed on a 60 MHz benchtop spectrometer and compared to spectra acquired using a 500 MHz and 800 MHz spectrometer. The concentration of nicotinamide in the extract was determined using standard addition, with high intra-sample reproducibility enabled by an automatic shuttling setup. This paves the way for further analyses using SABRE in complex matrices, with applications in metabolomics and other disciplines.

RASER images recorded on portable devices

Große-Tebbe, Ludwig, Simon, Fleischer, Sören, Lehmkuhl, Jan G., Korvink, Mazin, Jouda, Qing, Yang

Hyperpolarized contrast agents show great promise in medical diagnosis. However, widespread use of such molecular contrast agents is limited by the hardware requirements. For example, ^{13}C and ^{129}Xe contrast agents are clinically approved, but both require different frequencies for excitation and detection, which most clinical scanners simply don't have. Thus, both for cost reduction and wide distribution, retrofittability combining portable commercial hardware with existing scanners would be ideal.

Here we show an approach that addresses both challenges, excitation and detection. For excitation, we choose an excitation technique that does not require external rf-excitation. We employ RASER MRI (Radio amplification by spontaneous emission of radiation (RASER)). For detection, we split the signal to record images on both the Bruker hardware (Avance Neo 600) and the portable devices (a Zürich Instrument lock-in amplifier and a significantly less expensive redpitaya SDRlab 122-16 board). The experiment works in the following way: After a hyperpolarized sample is inserted into the magnet, RASER excitation flips the polarization until a set transverse magnetization threshold is reached and the lock-in amplifier triggers EPI encoding to record 128x128 images in 78 ms.

The Bruker device utilizes analog signal processing to detect on two channels (^1H and ^{13}C). In contrast, the Zürich Instrument lock-in amplifier with its digital data processing allows more flexibility, but is limited by the bit rate of the analog to digital converter (ADC) and the transfer rate to store the recorded signal. The redpitaya SDRlab 122-16 also uses digital data processing and can only record up to 122 MHz requiring demodulation since we record at 500 MHz, but allows for a higher bit rate (16 bit).

Reinterpreting the SABRE hyperpolarization of Pyruvate

Assaf, Charbel, Zhivonitko, Vladimir, Auer, Alexander, Duckett, Simon, Hövener, Jan-Bernd, Pravdivtsev, Andrey

Signal Amplification by Reversible Exchange (SABRE) is a hyperpolarization technique that enhances NMR signals by transferring spin order from parahydrogen (pH_2) to substrates via their simultaneous transient binding to an iridium catalyst. SABRE efficiency depends critically on hydride and ligand-exchange dynamics within a catalytic cycle. Hyperpolarized pyruvate is a particularly important target for SABRE due to its value for metabolic studies, where it enables the sensitive monitoring of biochemical processes relevant to cancer and inflammation.

We report here investigations into the mechanism of pyruvate hyperpolarization using spin-selected NMR experiments, combined with kinetic exchange modeling and density functional theory (DFT) calculations. By tracking the fate of hyperpolarized hydride and pyruvate signals, we found that intramolecular hydride exchange occurs faster than both pyruvate dissociation and H_2 loss. Furthermore, by optimizing chemical conditions, we successfully stabilized high concentrations of three key species in the catalytic cycle.

Subsequent characterization revealed that the minor isomer identified in the original studies was misidentified; it actually contains H_2 , pyruvate, Ir-IMes, and two dimethyl sulfoxide (DMSO) ligands rather than one. This discovery has led to the assembly of a revised mechanistic cycle that better supports the optimal SABRE hyperpolarisation of pyruvate. All these results were supported by DFT. Interestingly, while DMSO is present in all the detected complexes, pyruvate is not. We therefore hyperpolarized ^{13}C -DMSO by SABRE and used it as an additional probe to track the chemical fate of all species. Our measurements now provide a comprehensive map of the dynamic behaviour of DMSO, pyruvate, and H_2 during SABRE.

Ultimately, this study provides the groundwork for optimizing catalyst behavior and experimental parameters. By maximizing hyperpolarization efficiency, we pave the way for more robust SABRE applications in the field of metabolic NMR.

Efficient multi-site ^{13}C -hyperpolarization of pH sensor Z-OMPD via reversible exchange with parahydrogen

Ivan A. Trofimov, Juanita Ferreira, Martin Grashei, Adriana Sacristán, Robert Willing, Stefan Petersen, Maxim Zaitsev, Franz Schilling, Max von Delius, Andreas B. Schmidt

Z-OMPD (2-oxo-4-methyl-3-pentene-1,5-dioic acid) is a promising probe for non-invasive in vivo pH sensing, as was demonstrated by previous dissolution DNP study. In terms of NMR spectroscopy, its pH sensitivity is expressed in significant change of ^{13}C chemical shift values; this is particularly pronounced in biologically relevant pH range of 6–7.5. Z-[1,5- $^{13}\text{C}_2$]OMPD was previously polarized using SABRE technique (signal amplification by reversible exchange), however, ^{13}C polarization levels were <0.5%. The SABRE technique is based on exchange of parahydrogen and target substrate on a transient hydride complex, in which polarization is transferred via J -coupling network, typically in low magnetic fields.

Here, we report that careful optimization of temperature and pH in the SABRE solution, as well as implementation of SLIC (spin level-induced crossing) pulse enable efficient polarization of Z-[1,5- $^{13}\text{C}_2$]OMPD via SABRE achieving >10% on both C-1 and C-5 nuclei. Interestingly, if non-labeled Z-OMPD is used, ^{13}C polarization of up to 25% is achieved almost exclusively on the C-1 nuclei, which motivated further studies of the SABRE process. We found that the pH in SABRE solution does not merely affect the ^{13}C polarization levels but also changes the structures of the hydride complexes of Z-OMPD and their quantitative composition at different pH, guiding the direction of polarization transfer, as in OMPD^{1-} state significant (up to 14%) polarization is confined exclusively to the C-2 site.

Overall, this work highlights the relevance of influence of pH on the SABRE process that may be important for polarization of other substrates via SABRE. These investigations also mark an important step towards implementation parahydrogen-based hyperpolarized MRI for biomedical research.

Maximizing ^1H Hyperpolarized Signal Lifetimes through Long-Lived States in Dynamic Nuclear Polarization Experiments

Razanahoera, Aiky, Loutfi, Hadi, Minaei, Masoud, Kouřil, Karel, Sheberstov, Kirill, Meier, Benno, Bodenhausen, Geoffrey.

^1H Long-Lived States (LLS) in short aliphatic chains have lifetimes up to seven times longer than longitudinal relaxation times T_1 . Short aliphatic chains are present in a large variety of metabolites, amino-acids, neurotransmitters, drugs and other molecules. Their LLS are sensitive to their surrounding environment, which unfold new perspectives for high-throughput parallel drug screening powered by NMR.

However, ^1H LLS experiments at room temperature result in low signal-to-noise ratio and can be time-consuming. The combination of magnified contrast and high throughput is yet to be achieved.

We suggest the bullet Dynamic Nuclear Polarization (b-DNP) method to reach this objective, by virtue of its high-throughput transfer of frozen solids from a polarizer to an NMR spectrometer. After rapid dissolution, the resulting hyperpolarization comprises a population imbalance between combinations of singlet and triplet states of geminal protons, in other words a long-lived state. We have developed a tailor-made variant of the polychromatic Spin-Lock Induced Crossing pulse sequence to reconvert parts of these LLS into observable transverse magnetization. Within a single DNP experiment, we have determined on-the-fly the lifetimes T_{LLS} of several biomolecules simultaneously, lasting up to six times the duration of hyperpolarized longitudinal relaxation times T_1 . The enhancement factors of hyperpolarized LLS-derived signals obtained have exceeded two orders of magnitude compared to experiments carried out at room temperature.

Our future work will compare the lifetimes T_{LLS} in presence and absence of proteins. This method extends the applications of b-DNP to protons in molecules containing short aliphatic chains and may be valuable for drug screening.

Solid-State NMR measurement of Spin Diffusion and Relaxation for PHIP-Polarized Solids

Uluca-Yazgi, Boran, Singh Anushka, Heise, Henrike, Eills, James

Parahydrogen-induced polarization (PHIP) is an efficient method to generate nuclear hyperpolarization. However, its applicability is limited, since only molecules that directly participate in parahydrogen-based reactions can be polarized. Recently, approaches combining PHIP with solid-state spin diffusion (PHIP-SSD) have been introduced to transfer polarization from a PHIP-active precursor to other molecules in the solid state. This approach could provide an inexpensive route to hyperpolarize a wide range of molecules.

Here, we used solid-state NMR relaxation and spin diffusion measurements to explore how the solid-state environment influences polarization transport in PHIP-derived solids. Precursor systems were precipitated under controlled conditions, and we investigated different drying protocols, sample deuteration fractions, and temperatures. We observe pronounced differences in relaxation times depending on the sample preparation parameters. The ^1H T_1 values are largely independent of the precipitation acid (HCl vs H_2SO_4) and drying conditions, whereas the ^{13}C T_1 values differ strongly between protonated and deuterated samples, with protonated samples exhibiting much longer relaxation times (~ 200 s) compared to deuterated samples (~ 12 s).

Our results suggest that polarization transport in PHIP-derived solids is governed by an interplay between spin diffusion and relaxation processes. High-field solid-state NMR measurements provide insight into relaxation processes that influence spin diffusion in these systems. These findings highlight the importance of sample preparation conditions for optimizing polarization transfer in PHIP-SSD hyperpolarization experiments.

Exploration of ^{19}F - ^{13}C Cross-Relaxation and Investigation of a Partially Fluorinated Matrix for ^{19}F MAS DNP

Solid-state MAS NMR is a unique technique within the toolbox of analytical methods. In a multitude of instances, the implementation of hyperpolarization methods emerges as the only feasible option to study challenging systems. The most promising method for MAS NMR is dynamic nuclear polarization (DNP), which utilizes the transfer of the large electron polarization to nuclei during microwave irradiation. DNP has been successfully applied in many areas, enabling otherwise unfeasible experiments such as in-cell studies.

A notable development in recent years is the growing interest in ^{19}F NMR. Due to its absence from all biological building blocks, fluorine atoms can be utilized as highly specific spin-tags to gain structural insights in biological complexes. Furthermore, ^{19}F is a prevalent nucleus in active pharmaceutical ingredients, offering the distinctive ability to study them by ^{19}F NMR. Despite recent efforts, the advancement of ^{19}F DNP is currently constrained by the limited availability of dedicated MAS DNP probes capable of irradiating ^1H and ^{19}F resonances simultaneously.

In this study, we explore alternative approaches for making ^{19}F DNP accessible. In one direction, we have systematically investigated partially fluorinated glycerol/water/DFIP (1,3-difluoro-2-propanol) DNP matrices to facilitate relayed polarization transfer through a denser ^{19}F spin network. In this regard, we demonstrate, that with such an approach, a fourfold increase in the sensitivity of direct ^{19}F and ^{19}F - ^{13}C CP experiments on fluorinated model compounds can be attained. Furthermore, we have observed ^{19}F - ^{13}C cross relaxation (CR) under cryogenic DNP conditions, thereby establishing an alternative ^{19}F DNP pathway. We demonstrate that this CR pathway can be invoked for SCREAM-DNP (Specific Cross-Relaxation Enhancement by Active Motions under DNP) to site-specifically investigate ^{13}C nuclei that are in proximity to trifluoromethyl groups. In conjunction, our research contributes to the understanding regarding the mechanisms that govern the propagation of ^{19}F polarization during DNP.

Water soluble polarizing agents for high field DNP

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Dynamic nuclear polarization (DNP) overcomes the sensitivity limitation of magic angle spinning (MAS) NMR spectroscopy, enabling wider applications in many scientific fields. In DNP, a polarizing agent (PA) (generally biradicals) with unpaired electrons transfers polarization from electron spins to nearby nuclear spins under continuous microwave irradiation at low temperature, and the polarization spreads to bulk nuclei through spin diffusion.

Although bis-nitroxide biradicals (e.g. AMUPOL) show promising enhancements, their efficiency drops significantly at higher magnetic fields and MAS frequencies. Hetero-biradicals e.g. TEMTriPol and PyrroriPol were introduced to address this issue but are difficult to synthesize and often aggregate due to poor water solubility.

Here, four trityl-based biradicals (BR1–BR4) with different linkers and nitroxide moieties have been synthesized to improve performance. DNP performance at 14.1 T was evaluated using enhancement (I_{MW}^{ON}/I_{MW}^{OFF}) and buildup time. Higher enhancement and shorter buildup time indicate superior performance. BR3 showed the best performance with ~130 enhancement, most likely due to better water solubility.

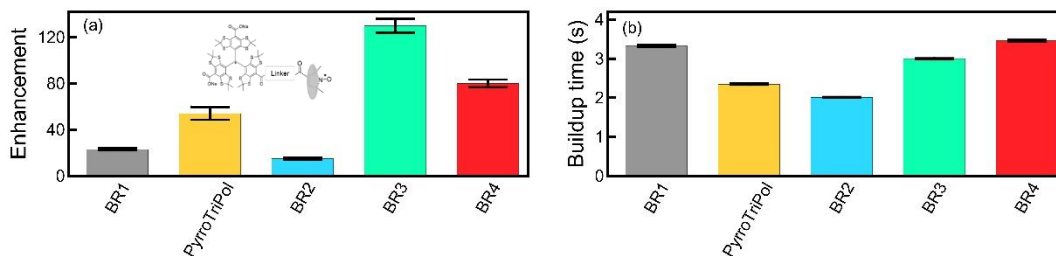


Fig 1. (a) Proton Enhancement and (b) Buildup time of the PAs. Inset of (a) represents the general structure of PAs. Solvent: d8-glycerol: D₂O: H₂O (60/30/10 v/v) containing 250 mM [U-¹³C, ¹⁵N] L- Proline. Experimental condition: sample temperature 95 K, MAS 8 kHz, [PA]=10 mM.

Overhauser Dynamic Nuclear Polarization for Multi-Nuclear Signal Enhancement in Continuous-Flow

Salgado, Billy, Mross, Sarah, Hasse, Hans and Münnemann, Kerstin

Benchtop NMR spectroscopy has high potential for reaction and process monitoring due to its compact size, low cost, and ease of integration into laboratory and industrial environments. These applications often require the analysis of flowing samples in order to provide sufficient temporal resolution, especially for fast reactions. However, benchtop NMR under continuous flow conditions is often limited by insufficient premagnetization and consequently low signal intensities. These limitations become particularly challenging when analyzing insensitive nuclei or highly diluted systems. Overhauser dynamic nuclear polarization (ODNP) provides a powerful approach to overcome these sensitivity constraints by significantly enhancing NMR signals [1,2]. Furthermore, Overhauser DNP is particularly suitable for analyzing fast flowing samples due to its rapid polarization buildup.

While ^1H ODNP is widely known, it suffers from limitations caused by overlapping signals, which can complicate spectral analysis. Here, we present hyperpolarization by ODNP for multiple nuclei under continuous flow, including ^{13}C , ^{15}N , ^{19}F , and ^{31}P . The ODNP signal enhancements were plotted against the flow velocity to determine the ideal flow rate for the corresponding nuclei. Moreover, in our approach the radicals necessary for ODNP are immobilized in a fixed bed, resulting in uncontaminated reaction mixtures. The use of heteronuclear detection reduces spectral overlap and expands the analytical capabilities of benchtop NMR. This strategy provides an alternative and versatile method for reaction and process monitoring.

[1] R. Kircher, H. Hasse, K. Münnemann, *Analytical Chemistry*, (2021), 93, 8897–8905.

[2] J. Phuong, B. Salgado, T. Labusch, H. Hasse, K. Münnemann, *Analytical Chemistry*, (2025), 97, 4308-4317.

Spin echo studies of heavily cobalt doped ZnO:Mn²⁺: the electron spectral diffusion under dynamic nuclear polarization

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Nonlinear effects were observed in some paramagnetic systems. The suitable system for maser applications might be Mn²⁺ in ZnO co-doped with the faster relaxer like Co²⁺.

In the present study we describe the measurements on Mn²⁺ system in hydrothermally grown ZnO. The applicability of ZnO:Mn²⁺ system for solid state optically pumped maser amplifiers is under consideration.

The optically pumped EPR spectrum of ZnO:Mn²⁺ system demonstrates the selectivity in the population of the manganese electron-nuclear spin levels. The cross-relaxation give rise to the process of dynamic nuclear polarization of ⁵⁵Mn in this system. The time dependent inversion of the populations for Mn²⁺ electron-nuclear states has been observed when applying the optical pumping below 3 K.

The thermal mixing DNP process taken in the high temperature approximation involves a spin system broken up into several heat reservoirs with different spin temperatures: the electron Zeeman, the nuclear Zeeman, and the spin-spin interactions reservoirs. The electron spectral diffusion is studied under dynamic nuclear polarization by using electron spin-echo-detected EPR spectra and Electron-Electron Double Resonance (ELDOR) technique in heavily cobalt doped ZnO:Mn²⁺ at the Co²⁺ exchange narrowing temperature range. It is established that the thermal mixing mechanism of DNP leads to the cooling of the common reservoir of electron dipole-dipole interactions of Mn²⁺ and Co²⁺ spins to the single spin temperature in the rotating frame. We show the variation of the polarization amplification of the common nuclear subsystem as a function of microwave irradiation frequency.

Rapid RASER MRI - Imaging without External RF-Excitation

Lehmkuhl, Sören; Yang, Jing; Fleischer, Simon; Chekmenev, Eduard Y.; Theis, Thomas; Appelt, Stephan; Korvink, Jan G.; Jouda, Mazin.

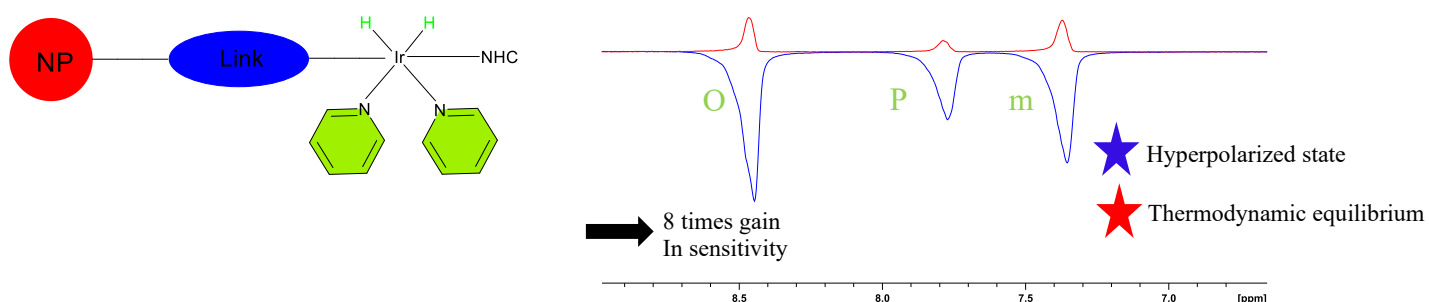
Conventional NMR and MRI rely on radio frequency (RF) pulses to excite nuclear spins and in turn generate NMR signals. These pulses require large high-power RF-amplifiers and cause heat deposition in the tissue, which must be minimized for safety, presenting a growing problem when moving toward ever-higher field MRI. An alternative to external rf-excitation is self-excitation using a RASER (Radiofrequency Amplification by Stimulated Emission of Radiation). In a RASER, the nuclear spins undergo a spontaneous transition, without RF excitation, from an over-populated state to a ground state. The required population inversion on the nuclear spin states can easily be provided by hyperpolarization techniques. In this work, we use the RASER approach instead of RF pulses for excitation before image acquisition with conventional EPI encoding.

We recorded ^1H RASER MRI images of SABRE-hyperpolarized pyrazine (120 mM) with a large matrix (128x128 pixels) in as little as 78 ms at 500 MHz. An EPI sequence is triggered once a set magnetization threshold is reached to ensure an identical signal level. This triggering is enabled by a lock-in amplifier, which also allows for signal acquisition. In this way, we also recorded a time-series of images using a single bolus hyperpolarized pyrazine highlighting the feasibility of dynamic tracking. The demonstrated approach allows recording MRI scans without transmit-receive electronics of the MRI scanner, which is highly desirable for portable MRI as well as the emerging field of hyperpolarized MRI using, e.g., ^{129}Xe gas or HP ^{13}C labelled biomolecules as molecular tracers and imaging agents.

Parahydrogen and Catalyst Grafted on Solid Supports for Enhanced NMR Sensitivity in Continuous Mode

YAZBEK, Mortada, HUBER, Gaspard, CAMPIDELLI, Stéphane

NMR spectroscopy is widely used to study molecular structure and dynamics, but its sensitivity is limited because only a small fraction of nuclear spins contributes to the detectable signal. Signal Amplification by Reversible Exchange (SABRE) is a hyperpolarization technique that overcomes this limitation by transferring spin polarization from parahydrogen to target molecular substrates without permanently altering their chemical structure. The present work focuses on the development of heterogeneous SABRE systems based on the immobilization of an iridium complex $[\text{Ir}(\text{IMes})(\text{H})_2(\text{sub})_3]$, which is typically usually used as a homogeneous catalyst. In order to achieve this, different solid supports (ss) were investigated, including silica and polystyrene materials, forming $[\text{Ir}(\text{ss})(\text{IMes})(\text{Cl})]$ moieties. Particular attention was given to the functionalization of these supports in order to facilitate the grafting of the iridium complex and improve its stability on the surface, and limits its leaching. Once immobilized, the supported catalysts were activated by introducing parahydrogen, enabling the formation of the active SABRE complex $[\text{Ir}(\text{ss})(\text{IMes})(\text{H})_2(\text{sub})_2]$ responsible for polarization transfer to an adequate substrate. The objective of this study is to explore the influence of the different components of the grafted catalyst on the hyperpolarization factor of the substrates. A clearer understanding is expected to contribute to the optimization of heterogeneous SABRE systems and to broaden their scope in analytical NMR and magnetic resonance imaging applications.



Schematic representation for Het-SABRE catalyst and corresponding pyridine hyperpolarized spectrum.

Site-specific distance measurements using heteronuclear cross-relaxation dynamics under MAS DNP

Taube, Florian, Eichwald, Clemens, Künze, Georg, Corzilius, Björn

Dynamic nuclear polarization (DNP) has routinely been used to enhance magic-angle spinning (MAS) NMR signals uniformly. In recent years, there has been an emerging interest in using DNP to also achieve site specific enhancement. One such approach is the Specific Cross Relaxation Enhancement by Active Motions under DNP (SCREAM-DNP), which exploits the fast reorientation dynamics of methyl groups, even at low temperatures. The scope of this application continues to broaden, particularly through the use of ring puckering dynamics besides methyl reorientation, and by combining it with techniques such as rotational resonance.

Typically, the polarization buildup in SCREAM-DNP experiments is analyzed by fitting to mono- or biexponential models. However, because the underlying spin dynamics is minimally described by the Solomon equations, exponential models are not fully able to describe the observed behavior. To address this limitation, we present a fitting approach based directly on the Solomon equations, offering a more precise description of such systems, and, most importantly, an informative physical interpretation of the obtained rate constants based on different processes in the multi-spin system. Besides detailed insights into the polarization transfer dynamics of systems containing methyl groups, this new approach allows a quantitative determination of the cross-relaxation rate constant between the methyl protons and a target nucleus. Based on the knowledge of this quantity, we were able to calculate site-specific, short range, effective interatomic distances to several heteronuclei (^{13}C , ^{31}P , ^{77}Se) using the well-known hydrogen-carbon distance within the methyl group as reference.

Recent progress in parahydrogen-induced polarization based on heterogeneous catalytic processes.

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Parahydrogen-induced polarization (PHIP) by means of heterogeneous catalysts has a number of essential advantages compared to the homogeneous counterpart of the technique. However, the nature of the mechanism of pairwise H₂ addition to unsaturated substrates on metal (nano)particles and other types of heterogeneous catalysts is still unclear, which poses a major obstacle to overcoming their main drawback of relatively low pairwise H₂ addition selectivity which results in the reduced PHIP levels.

A deeper insight into the underlying mechanisms is possible by exploring and comparing a wide variety of heterogeneous catalyst types in PHIP experiments as well as by performing dedicated mechanistic studies, both experimental and theoretical. The most recent results demonstrate that immobilized metal complexes can achieve pairwise selectivities of up to 40-50%, followed closely by supported liquid phase catalysts. A useful mechanistic insight into pairwise hydrogenation is provided by a comparison of the efficiency of S-T₀ mixing in the intermediates of homogeneous and heterogeneous hydrogenations, made possible by an appropriate choice of the unsaturated substrates. Another fruitful approach is the comparison of metal and metal carbide catalysts combined with DFT modeling.

All these achievements pave the way toward advanced practical applications. Heterogeneous version of PHIP allows one to readily produce continuous streams of catalyst-free hyperpolarized liquids and gases, with one notable example being the use of hyperpolarized hydrocarbon gases for lung ventilation imaging. In addition, advanced operando studies of industrially important heterogeneous catalytic processes are becoming feasible.

This work was financially supported by RSF (grant #25-13-00053).

Improved SABRE hyperpolarisation using pulse sequences to reduce effective coupling

Rodin Bogdan, Kozinenko Vilaiy, Eills James, Schwartz Ilai, Knecht Stephan, Dagys Laurynas

Signal Amplification By Reversible Exchange (SABRE) is a highly versatile hyperpolarization technique. Since parahydrogen polarization transfers without chemically altering the substrate, it allows for the continuous replenishment of the hyperpolarization source. However, optimizing pulse sequences remains challenging due to the interplay of coherent spin dynamics and chemical exchange. While an optimal transfer rate exists for a given exchange rate, a general method to controllably scale the transfer has been lacking. In this work, we show that pulse sequences designed to restore the strongly coupled regime and **precisely slow down** the transfer rate significantly outperform standard SLIC and SHEATH sequences for ^{15}N SABRE in nitrogen-containing molecules at low fields.

We introduce two approaches: a continuous-wave double-resonance frequency (DRF) SLIC variant, and PulsePol, a pulse sequence highly robust to B1 and B0 inhomogeneities. While operating through different mechanisms, both methods exploit the level anti-crossings and allow the transfer rate to be controllably scaled to match the exchange rate. This matching approach gives substantially higher polarization levels for slowly exchanging targets. For instance, with ^{15}N -acetonitrile, PulsePol and DRF-SLIC achieved **49% and 45% ^{15}N polarization**, respectively, drastically outperforming standard SLIC (21%) and SHEATH (12%). Furthermore, our simulations agree excellently with experiments, demonstrating DRF-SLIC outperforms standard low-field sequences across almost all exchange regimes, converging with SLIC only at very fast exchange.

We expect that these new methods and the overall framework for adjusting the speed transfer to match the exchange rate will be very useful for the whole SABRE community.

Efficient High-Field DNP of Biomolecules in Physiological Aqueous Solutions

Kuzhelev, Andrei

The solid effect (SE) in Dynamic Nuclear Polarization (DNP) has long been considered a phenomenon exclusive to solids or highly immobilized matrices. While recent studies have extended its reach to viscous fluids and lipid bilayers, achieving efficient hyperpolarization in the high-mobility regime of aqueous solutions at high magnetic fields has remained a significant challenge.

This study overcomes this limitation by demonstrating highly efficient high-field SE DNP in pure aqueous solutions at room temperature for the first time. By decoupling the physical influences of analyte size, concentration, and solvent viscosity, we have established a fundamental “size switch” for liquid-state DNP. Our findings reveal that small molecules (e.g., ATP) require a critical viscosity threshold of at least 6 cP for hyperpolarization, whereas macromolecules exceeding the 15-kDa threshold (e.g., 25-bp DNA duplexes) behave as “quasi-solids.” These larger systems exhibit efficient SE DNP in low-viscosity, biologically native environments, yielding signal enhancements of up to 20-fold.

Furthermore, we present the first demonstration of ^{31}P hyperpolarization in its pentavalent state under physiologically relevant conditions. This work shifts the paradigm of liquid-state DNP by offering a transformative, biocompatible tool for studying protein-ligand interactions, nucleic acid dynamics, and membranes in their native fluid states with high sensitivity.

This work was supported by the ERC Starting Grant (LiquidStateDNP, 101219713).

EPR and DNP-NMR of Sandstones

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Electron paramagnetic resonance (EPR) of natural rock samples has been proposed for geological dating based on radioactive dosage in the subsurface creating defects in quartz crystal structures. Quartz is the predominant component of sandstone (> 90 %) and can occupy a large fraction in granites (*ca.* 30 – 50 %); the high quantity of quartz makes the sensitive EPR of silica-centered radicals (E') well-suited for measuring these effects. However, it is well known from recent chemical literature that mechanical impact on quartz powders also forms E' centers (*e.g.*, ball milling, tumbling, grinding, etc.). Therefore, stable E' centers may form in quartz-rich geologic formations driven naturally by tectonic and tidal deformations, or induced by human activities like tunneling, mining, and geoenery applications. Herein, I will present results from laboratory-scale experiments of stress-induced microfracturing in Berea sandstones. Samples are subject to compressive and tensile loading or heating to induce microcracks, then analyzed by conventional CW X-band EPR. Results show the onset of additional E' signals on mechanically- or thermally-stimulated samples in addition to natural-occurring background E' signals.

Furthermore, we explore using Overhauser dynamic nuclear polarization (ODNP) for enhancing ¹H signals in H₂O-saturated Berea sandstones. ODNP has previously been used in rock samples for the purposes of spatially-resolved signal enhancement and signal discrimination. ODNP may be a powerful tool for signal enhancement and signal modulation in porous media, but it has found limited usage to date. The nuclear T_1 and T_2 relaxation times can be used as relative proxies of pore size in porous media. Further, the characteristic buildup of polarization in ODNP depends on the T_1 of both the electron and nuclear spins. Therefore, ODNP of fluids in porous

media should exhibit a polarization buildup that also depends on pore size. This phenomenon may enable a systematic modulation of signals corresponding to different pore sizes based on experimental ODNP parameters, which can be further exploited in imaging experiments. I will further present results of low-field ODNP-NMR experiments in radical-doped H₂O-saturated sandstone samples. A homebuilt magnet and DNP-NMR apparatus is used based on inexpensive ferrite permanent magnets and a simple birdcage-style DNP resonator, respectively (**Figure 1a**). Experiments are performed at approximately 50 mT, 2.1 MHz, and 1.4 GHz corresponding to the B_0 field, NMR frequency, and EPR/DNP frequency, respectively. Preliminary results show that the spin polarization is modulated according to DNP irradiation times and relative pore sizes (**Figure 1d**). This suggests that the characteristic DNP buildup time, T_{1B} , is also proportional to pore size. Consideration is given to using E' in natural rock samples as an endogenous source of radicals for ODNP. Finally, a unique mechanism is revealed showing how hyperfine couplings can be spatially encoded into NMR spectra when ODNP is performed in a constant gradient field.

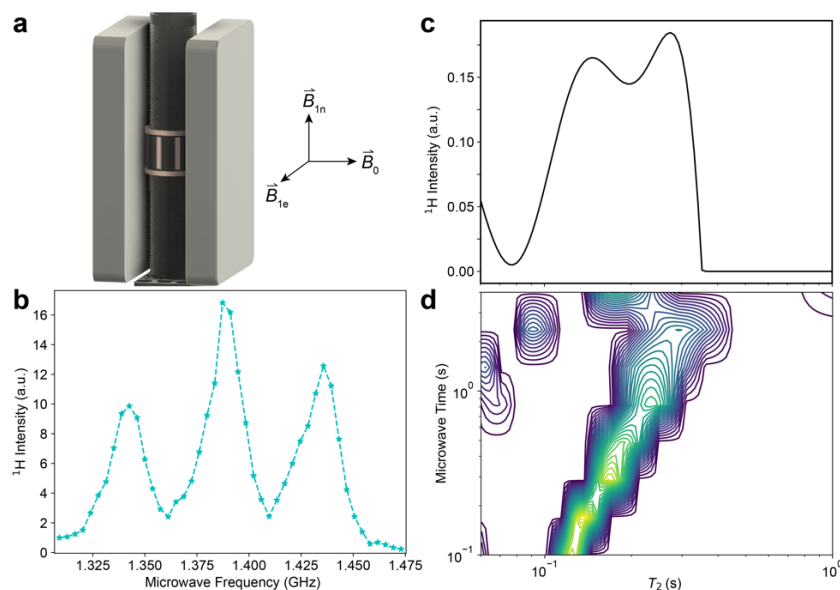


Figure 1. (a) 3D render of the experimental DNP-NMR apparatus using ceramic magnets, a solenoid for NMR, and a birdcage resonator for DNP, with the directions of their fields drawn as $\vec{B}_0$, $\vec{B}_{1n}$, and $\vec{B}_{1e}$, respectively. (b) The EPR spectrum of 2 mM 4-hydroxy-TEMPO in H₂O acquired with a frequency-stepped DNP-CPMG-NMR experiment on a saturated Berea sandstone sample. This entire spectrum is recorded without retuning the resonator due to its low- Q . (c) The T_2 distribution of 4-hydroxy-TEMPO in H₂O in Berea sandstone without DNP and (d) with DNP using variable microwave irradiation times.

Understanding the phenomena of EPR and DNP in rocks will be critical for understanding rock mechanics and leveraging large signal enhancements in NMR and MRI experiments. Our simple and low-cost DNP-NMR apparatus and experimental strategies are attractive options for researchers looking to implement these tools. These methods will find applications in geosciences, material sciences, chemistry, and physics.

Parahydrogen and proton exchange enabled hyperpolarization of steroidal and macrocyclic pharmaceuticals up to 1 kDa

Kozinenko, Vitaly, Rodin, Bogdan, Mamone, Salvatore, Knecht, Stephan, and Schwartz, Ilai

Parahydrogen induced polarization (PHIP) has established itself as an efficient method for overcoming the inherent sensitivity limitations of NMR and MRI. Originally feasible only for the homogeneous hydrogenation of unsaturated precursors, it now allows addressing a variety of molecular targets. One of the approaches enabling this transition is PHIP-relay. This method relies on the hydrogenation of an unsaturated precursor alcohol with hyperpolarization of the exchangeable proton, which then relays this hyperpolarization to the target via proton exchange. To date, PHIP-relay has been applied primarily to small alcohols, carboxylic acids, and carbohydrates.

We demonstrate that PHIP-relay is capable of providing high signal enhancement for sophisticated molecular systems relevant for pharmacology. The major improvement stems from the rational choice of the precursor molecule: utilizing BOC protected propargyl alcohol with a site-specific deuterium substitution in the CH₂ group enables both efficient hydrogenation and targeted polarization of the OH proton. We optimized both numerically and experimentally all relevant parameters, including the magnetic field strength for efficient polarization redistribution and the chemical composition of the sample. This allowed us to achieve 2% proton polarization (640-fold enhancement at 9.4 T) for both exchangeable and non-exchangeable protons for cholecalciferol (Vitamin D₃, MW 384) and cortisol (MW 362). For the macrocyclic immunosuppressant everolimus (MW 958), we attained 0.7% polarization. Noteworthy, the polarization level is almost independent of the concentration of the target molecule, allowing low concentration detection and quantification.

Our results establish PHIP-relay as a robust methodology for polarizing a broad class of steroidal and macrocyclic scaffolds. This work expands the scope of parahydrogen-based methods toward complex drug discovery, protein-ligand binding studies, and trace-level substance detection.

Boosting High Field DNP Performance Beyond 9.4 T

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Solid-state nuclear magnetic resonance (NMR) spectroscopy is a widely used analytical technique that can provide atomic-level structural information on a broad range of biomolecules and inorganic materials. Despite its extensive usage and versatility, NMR suffers from inherent low sensitivity due to low nuclear spin polarisation at thermal equilibrium. High-field dynamic nuclear polarisation (DNP) has emerged as an effective method for drastically enhancing NMR sensitivity by transferring polarisation from organic polarising agents to the nuclei of interest. The drive to reach theoretical enhancements of 658 relies on the efficiency of newly developed polarising agents (PAs), cryoMAS and hardware innovations. Verdazyl-based radicals offer promising alternatives to conventional nitroxide (bi)radicals, with a narrower electron paramagnetic resonance (EPR) signals, which is beneficial for enhancing polarisation transfer at higher magnetic fields beyond 400 MHz/263 GHz.

Recently, VerTEKoL, a novel Verdazyl-nitroxide-based biradical, has been identified as an efficient polarising agent for high-field DNP NMR spectroscopy. Given its promising performance at 600 MHz/395 GHz field strength, further optimisations are being explored to enhance DNP performance, particularly by refining sample preparation protocols, polarising agent concentrations, and temperatures, and by improving the ability to couple and transfer microwave energy throughout the solid at higher magnetic fields. A key objective in developing DNP as a reliable and routine analytical tool is to establish clear, standardised protocols to achieve optimal enhancements across various materials science applications. This presentation will cover our recent efforts to improve DNP performance above 400 MHz/263 GHz, including the effects of magnetic field strength, radical concentration, microwave power, magic-angle spinning, oxygen levels, etc. The performance of our new polarising agent, VerTEKoL, at different magnetic fields will be showcased. This study aims to demonstrate how high-field DNP can deliver transformative enhancements for both model and applied materials (**Figure 1**) and highlights the importance of considering a diverse space that can influence overall DNP

efficiency. Notable improvements can be achieved through simple considerations, while advanced hardware and synthetic chemistry will remain essential for further DNP development.

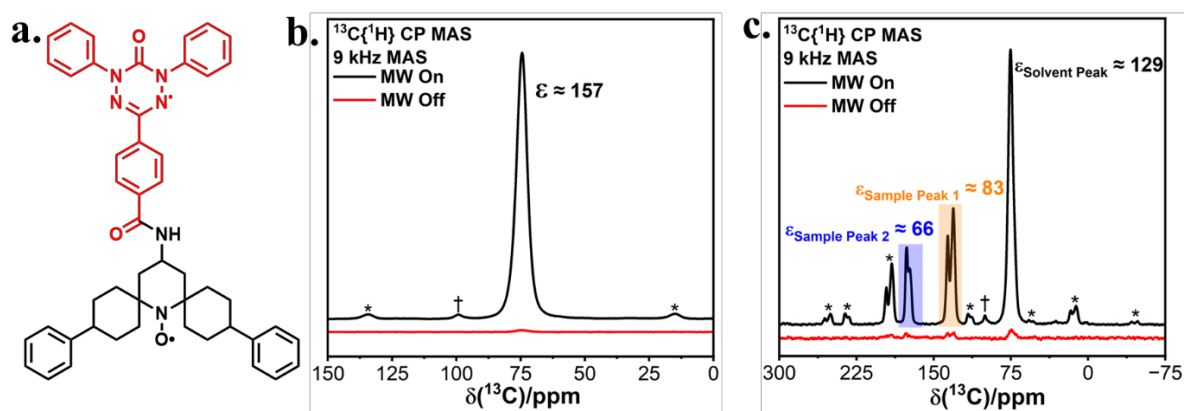


Figure 1. (a) Structure of polarising agent VerTEKOL. The $^{13}\text{C}\{^1\text{H}\}$ CP MAS DNP NMR spectra of glassing agent (b), and insulating hybrid material (c), recorded at 100 K and 600 MHz/395 GHz.

Parahydrogen-induced polarization via side-arm hydrogenation: expanding the agent portfolio beyond pyruvate

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Nuclear hyperpolarization (HP) techniques dramatically increase signal-to-noise ratio in magnetic resonance. The combination of heteronuclear MRSI with the administration of HP contrast agents enables detection and quantification of metabolic flux *in vivo*. One of the promising HP methods is parahydrogen induced polarization (PHIP), which exploits the singlet spin order of parahydrogen (pH₂) as a source of non-equilibrium polarization. Recently, PHIP by side-arm hydrogenation (PHIP-SAH) approach was proposed expanding the capabilities of PHIP for producing HP carboxylates in a biocompatible form. Here, we propose a PHIP-SAH-based procedure for hyperpolarizing several promising substrates: [¹³C]formate, [1,3-¹³C₂]acetoacetate, [1,3-¹³C₂]3-hydroxybutyrate and nitrate.

In general, the PHIP-SAH procedure includes three stages: 1) hydrogenation of the unsaturated alcoholic moiety of the ester precursor with pH₂; 2) transfer of polarization from the pH₂ nascent ¹H nuclei to the X-nucleus; 3) hydrolysis of the product to cleave off alcoholic moiety. In this study, each stage was optimized for every substrate. [¹³C]Formate was hyperpolarized with $P(^{13}\text{C}) \approx 0.4\%$ after complete hydrolysis. For nitrate, significant ¹⁵N polarization was achieved (2% after hydrolysis), exceeding values reported for other HP methods (1% for d-DNP, 0.1% for SABRE). In the case of acetoacetate, we showed that allyl [1,3-¹³C₂]acetoacetate can be efficiently polarized ($P(1-^{13}\text{C}) = 4.1\%$, $P(3-^{13}\text{C}) = 2.7\%$). However, optimization of hydrolysis revealed that HP [1,3-¹³C₂]acetoacetate is produced with $P(^{13}\text{C}) \approx 0.5\%$ only under harsh conditions, and in addition some HP ester remains unreacted and partial decomposition of acetoacetate into acetone and carbonate happens as well. By contrast, [1,3-¹³C₂]3-hydroxybutyrate undergoes hydrolysis readily while retaining appreciable ¹³C polarization ($P(1-^{13}\text{C}) = 1.1\%$, $P(3-^{13}\text{C}) = 0.07\%$).

This work was supported by the Russian Science Foundation (grant #24-73-10093).

^{19}F photo-CIDNP NMR as a tool for monitoring bacterial activity.

Dziubinska-Kuehn, Katarzyna Maria; Bragina, Olga; Ausmees, Kerti; Cooper, Callum; Spuul, Pirjo; Gray, Mark; Reile, Indrek.

Recent developments in NMR highlight a growing effort to combine hyperpolarization with medical diagnostic applications into a single, cohesive solution. The primary motivation for such investigations lies in the ability of hyperpolarized NMR to rapidly enhance the signal intensity, thereby substantially shortening acquisition times. Such capability could reduce diagnostic delays, particularly in acutely progressive, high-severity conditions, such as bacterial bloodstream infections or sepsis.

Among all hyperpolarization techniques, photochemically-induced dynamic nuclear polarization (photoCIDNP) remains one of the most robust, due to its ease of application and the significant signal enhancement achieved already in a single-scan experiment. Furthermore, it is highly compatible with both biomolecules and biological solution environments, such as human body fluids.

Our contribution demonstrates a successful application of ^{19}F photoCIDNP NMR for real-time monitoring of bacterial activity *in vitro*. The experimental design for the study included feeding bacteria with ^{19}F -labeled nutrients and collecting their growth medium at defined points during incubation, to subject it to photoCIDNP NMR as obtained. Based on a series of single-scan measurements, we could derive information on the nutrient depletion and the concurrent increase in metabolite concentration. Furthermore, selecting ^{19}F NMR enabled the signal acquisition to remain background-free, with only resonances from nutrients and metabolites present. This proof-of-concept study shows that photoCIDNP NMR could improve the speed of diagnosing bacterial bloodstream infections through quick single-scan experiments, an attractive alternative to current methods that require days of bacterial cultivation.

Removing Barriers to Hyperpolarization: Fluorinated SABRE Catalysts and Optical Routes to Nuclear Spin Polarization

Stevanato Gabriele*, Floreani Federico, De Biasi Federico, Faramawy Ahmed, Tubaro Cristina, Ruzzi Marco, Franco Lorenzo

Hyperpolarization methods such as Signal Amplification By Reversible Exchange (SABRE) and solution-state Dynamic Nuclear Polarization (DNP) dramatically enhance NMR sensitivity, yet their broader applicability remains limited by practical constraints. For SABRE biomedical applications, efficient catalysts for [1-¹³C]pyruvate hyperpolarization are required, yet their removal from the hyperpolarized solution often comes at the cost of reduced NMR signal intensity. In solution DNP, the need for high-power microwave sources can represent a significant technological and economic barrier.

Here we present two complementary strategies developed in our laboratory to address these limitations, thus broadening the scope of SABRE and DNP.

First, we report a new family of single-chain fluorinated Ir–NHC SABRE catalysts for [1-¹³C]pyruvate signal enhancement that retain in methanol-d₄ hyperpolarization efficiencies comparable to the benchmark IrIMes – of the order of ~4 % with 50% parahydrogen enrichment – while enabling efficient post-hyperpolarization removal through phase separation and filtration. Using a revised Redissolution SABRE protocol followed by aqueous extraction and fluorinated silica filtration, residual iridium concentrations below 100 ppb can be achieved, which is currently the lowest value reported. We can follow in this way the enzymatic in-vitro conversion from pyruvate to lactate in buffer using a cheap 50% parahydrogen setup. Most remarkably our results indicate that catalyst efficiency and removability need not represent competing design constraints.

Second, we demonstrate nuclear spin hyperpolarization in bulk room temperature solutions via optical excitation in a range of chromophore radical solutions, an approach that remains largely underexplored. Importantly, DNP enhancements of ~10 at ~0.3 T are achieved without the use of expensive microwave sources but using cheaper lasers and LEDs with <1 W optical power.

More broadly, these results illustrate how targeted molecular and experimental design can remove key technological barriers in solution-state hyperpolarization pointing toward the development of compact and accessible hyperpolarization platforms.

Statistical prediction of hydride chemical shifts in iridium complexes for parahydrogen-based hyperpolarization

Tessari, Marco, Dreisewerd, Lennart, Posthumus, Thom, Aspers, Ruud, Feiters, Martin, Likoza, Matej, Rutjes, Floris, Faganelli Pucer, Jana, Blaž, Huš

Iridium dihydride complexes play a crucial role in SABRE hyperpolarization, as they provide an efficient scaffold for converting singlet order from para-enriched hydrogen to enhanced magnetization of the metal ligands. The dihydrides NMR parameters (e.g. chemical shifts, coupling constants, linewidth, etc) have proven highly sensitive to the electronic structure, bonding pattern and association/dissociation processes at the metal center. They can, therefore, provide extensive information about the complex, its performance for parahydrogen-based hyperpolarization and ligand chemical structure. This has been used to detect and quantify dilute ligands in solution by measuring the hyperpolarized NMR signals of the corresponding iridium dihydrides. However, no accurate tool currently exists to identify metal ligands from dihydrides NMR parameters, representing a major limitation when investigating complex mixtures such as biofluids or natural extracts. Here, we report a study using multivariate statistical learning models to predict the chemical shifts of dihydride iridium complexes. For this purpose, ligands from two classes (e.g. pyridines and pyrazines) were systematically paired with three homologous 1,2,3 triazole-type co-ligands, yielding chemical shifts for 133 unique dihydride iridium complexes. After randomly splitting the data into training (80%) and test (20%) sets, several multivariate regression models were evaluated for supervised training. Bootstrap analysis shows that the best model significantly outperforms previously reported *ab initio* approaches, displaying a median mean absolute error of 0.0475 ppm and a coefficient of determination of 0.91.

Microwave-free ^{13}C hyperpolarization in randomly oriented diamond particles under magic angle spinning

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Among the different tools available for enhancing the sensitivity of NMR, hyperpolarization schemes have been widely explored because they can lead to signal enhancements of several orders of magnitude. Nuclear hyperpolarization protocols most often use electrons driven into a non-thermal spin state, from which polarization transfer to surrounding nuclei can occur. Although achieving a non-thermal electronic state can be accomplished using microwaves, methods based on the application of light are being explored.

Diamond nanoparticles containing Nitrogen-Vacancy centers (NVs) represent a promising platform for light-induced nuclear hyperpolarization, where the high surface-to-volume ratio could be exploited to polarize external analytes under ambient conditions. Hyperpolarization has been previously achieved in diamond micro- or nanoparticles at low (<0.4 T) magnetic field, using a combination of light excitation and microwave driving. However, the absence of demonstration compatible with magic-angle spinning (MAS) has so far precluded applications in the setting of solid-state NMR.

Here, we report the implementation of a protocol based on the physics of level anticrossings (LACs) involving NV, substitutional nitrogen (P1) and surrounding ^{13}C spins, working under MAS and illumination. In contrast to the earlier reports, no microwave is needed. Hyperpolarization was demonstrated on ^{13}C in the diamond lattice for two powder samples, with $\sim\mu\text{m}$ particle size and different ^{13}C content (1.1% and 20%), providing absolute ^{13}C polarization levels of $\sim 0.2\%$ -exceeding that obtained by the previous protocols for similar particle size. Besides the experimental results, we will detail the model involving level anticrossings in NV-P1- ^{13}C clusters to account for the observed behavior of the nuclear polarization and point to possible future applications for high-resolution sensitive NMR of substances outside the diamond.

Interplay between the exchange kinetics, molecular dynamics and polarisation transfer process in SABRE

Nazmul Hossain, Joni Eronen, Vladimir V. Zhivonitko, Juha Vaara, **Anu M. Kantola**

In the SABRE (signal amplification by reversible exchange) hyperpolarisation method, the polarisation of parahydrogen is transferred to substrate nuclei within a short-lived metal complex. Both coherent (through spin–spin couplings) and incoherent (through relaxation) processes contribute to the achievable enhancement. The optimal magnetic field for efficient polarisation transfer depends on the chemical shifts and spin–spin couplings of the relevant hydride and substrate nuclei. Transfer efficiency is further affected by the exchange kinetics and molecular dynamics, characterised by exchange rates and correlation times. These factors are influenced by parameters such as sample composition and temperature.

Because of this complexity, identifying the conditions to optimize SABRE hyperpolarisation is a multidimensional problem. By combining experimental NMR, quantum-chemical calculations, spin-dynamics simulations, and simplified models, we investigate the interplay between these processes. Relaxation and exchange NMR experiments for pyridine, pyrazine, 3-fluoropyridine, and 3,5-difluoropyridine provide insight into the intermediate complexes formed, as well as the relevant exchange and relaxation rates across temperatures.

Quantum-chemical calculations yield the quadrupolar, spin–spin, and dipolar couplings, together with chemical-shift tensors required for spin-dynamics simulations that model the polarisation transfer efficiency of pyridine under varied exchange and relaxation rates, at different magnetic fields. Finally, a simple model is used to describe the transfer efficiency and we compare its predictions with experimental and computational results to assess its usefulness in identifying optimal conditions.

Towards integrating hyperpolarized NMR into the chemical analysis workflow

Kerti Ausmees, Kärolin Kork, Claudia Kallaste, Maria Masik, Katarzyna Maria Dziubinska-Kuehn, Sören Lehmkuhl, **Indrek Reile**

While the concept of increasing NMR sensitivity by nuclear hyperpolarization has been known for decades, it has not been adopted in routine solution state chemical analysis environment yet. There are several reasons for this. Some hyperpolarization techniques require additional hardware that costs as much as a spectrometer. On top of that, no hyperpolarization method is a universal NMR sensitivity enhancer - all boost the signal of different analytes or analyte classes by different factors. Such limitations would be easier to tolerate, if hyperpolarization enabled valuable applications, and if hyperpolarized NMR analysis could be done with minimal changes to the routine chemical analysis workflow.

Herein we present our work towards achieving these goals: developing analytical applications for hyperpolarization and developing automated workflows. Our laboratory has implemented two hyperpolarization methods: parahydrogen hyperpolarization and photo-CIDNP hyperpolarization, both of which are among the more affordable hyperpolarization techniques. We will give an overview of some of the applications that we have developed for chemical analysis that would not be possible without hyperpolarization – for instance pharmacokinetic analysis with analyte concentrations that are three orders of magnitude below the limit of detection of regular NMR. We will then present our efforts towards adopting hyperpolarization in automated NMR analysis workflows. Parahydrogen hyperpolarization can be automated to run multiple NMR experiments and multiple scans on a single sample, while photo-CIDNP hyperpolarization can be performed on multiple samples, with the automation workflow starting to approximate the simplicity of regular NMR, but with up to several-hundred-fold enhanced sensitivity.

Coherent Control of ^{15}N Spin Relay in SABRE Enables over 5% ^{77}Se Polarization

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Recent advances in NMR and MRI have been driven by the development of hyperpolarization techniques that overcome intrinsic sensitivity limitations. Signal Amplification by Reversible Exchange (SABRE), a parahydrogen-based method relying on the reversible binding of substrates to the iridium complexes, has emerged as a versatile approach for enhancing nuclear spin polarization, most commonly for ^1H , ^{13}C , and ^{15}N nuclei. Here, we extend the scope of SABRE to ^{77}Se .

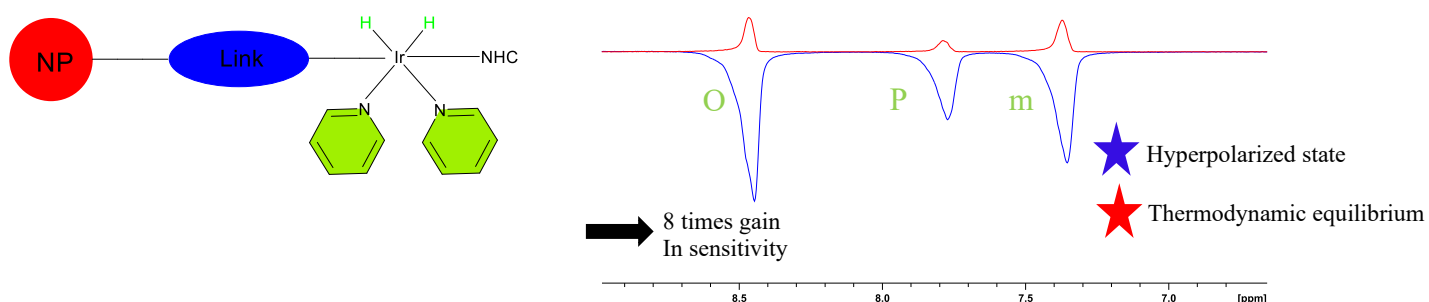
Using a selenium–nitrogen heterocycle, we investigate the mechanisms of ^{77}Se hyperpolarization. Direct polarization transfer from parahydrogen-derived hydrides to ^{77}Se proves inefficient; instead, hyperpolarization is achieved via a ^{15}N spin relay, enabling polarization levels exceeding 5%. This relay mechanism operates effectively at microtesla magnetic fields, even at natural isotopic abundance (0.028% of ^{15}N – ^{77}Se pairs), where spontaneous polarization transfer to both ^{15}N and ^{77}Se is governed by matching conditions tuned by the external magnetic field. Extending beyond microtesla fields to the more convenient Earth's-field range (50–100 μT), we employ resonant audio-frequency RF pulse sequences to actively control the ^{15}N spin relay, enabling highly selective polarization of ^{15}N and ^{77}Se spins without magnetic shielding. Demonstrating the broader utility of this approach, we apply the audio-frequency pulsing strategy to directly observe ^{13}C correlations in ^{15}N NMR spectra, highlighting its potential for probing heteronuclear networks beyond ^{15}N – ^{77}Se .

These results establish ^{15}N -mediated polarization transfer as a powerful and versatile strategy for ^{77}Se hyperpolarization and open new opportunities for extending SABRE to biologically relevant selenium-containing compounds.

Parahydrogen and Catalyst Grafted on Solid Supports for Enhanced NMR Sensitivity in Continuous Mode

YAZBEK, Mortada, HUBER, Gaspard, CAMPIDELLI, Stéphane

NMR spectroscopy is widely used to study molecular structure and dynamics, but its sensitivity is limited because only a small fraction of nuclear spins contributes to the detectable signal. Signal Amplification by Reversible Exchange (SABRE) is a hyperpolarization technique that overcomes this limitation by transferring spin polarization from parahydrogen to target molecular substrates without permanently altering their chemical structure. The present work focuses on the development of heterogeneous SABRE systems based on the immobilization of an iridium complex $[\text{Ir}(\text{IMes})(\text{H})_2(\text{sub})_3]$, which is typically usually used as a homogeneous catalyst. In order to achieve this, different solid supports (ss) were investigated, including silica and polystyrene materials, forming $[\text{Ir}(\text{ss})(\text{IMes})(\text{Cl})]$ moieties. Particular attention was given to the functionalization of these supports in order to facilitate the grafting of the iridium complex and improve its stability on the surface, and limits its leaching. Once immobilized, the supported catalysts were activated by introducing parahydrogen, enabling the formation of the active SABRE complex $[\text{Ir}(\text{ss})(\text{IMes})(\text{H})_2(\text{sub})_2]$ responsible for polarization transfer to an adequate substrate. The objective of this study is to explore the influence of the different components of the grafted catalyst on the hyperpolarization factor of the substrates. A clearer understanding is expected to contribute to the optimization of heterogeneous SABRE systems and to broaden their scope in analytical NMR and magnetic resonance imaging applications.



Schematic representation for Het-SABRE catalyst and corresponding pyridine hyperpolarized spectrum.

SABRE Hyperpolarization of Biologically Relevant LigandsA. Matuliukštytė¹, L. Dagys¹¹ Institute of Chemical Physics, Faculty of Physics, Vilnius University

Nuclear magnetic resonance (NMR) spectroscopy is widely used to study molecular structure and dynamics, but its sensitivity is limited by low nuclear spin polarization [1]. To improve sensitivity, stronger magnetic fields or hyperpolarization techniques are applied. One such technique is Signal Amplification by Reversible Exchange (SABRE), which uses parahydrogen as a source of spin order and enables fast signal enhancement without chemical modification of the target molecule. The efficiency of SABRE depends on reversible binding and chemical exchange at the metal catalyst center [1,2].

In this work, SABRE hyperpolarization of imidazole derivatives is studied. Imidazole is an aromatic heterocycle group often found in many antibiotic drugs and pharmaceutical compounds. In addition, the higher affinity to the iridium catalyst makes imidazole derivatives suitable SABRE target as it is demonstrated by the recent high spin-polarization achievements on antibiotic metronidazole [3]. This potentially enables fast NMR studies at millimolar concentrations for nitrogen-containing drugs, allowing single-scan NMR detection [1-3].

However, SABRE efficiency is strongly influenced by reversible ligand binding, exchange rates and by the selected method for polarization transfer from parahydrogen to the target [1–3]. In this work, SABRE was performed on a small variety of imidazole derivatives at room temperature by using a variety of different SABRE polarization protocols operating at low magnetic fields. The chemical dynamics were optimized by adjusting the co-ligand concentration in the solution. The SABRE efficiency was estimated by the achieved ¹⁵N signal enhancement compared to conventional NMR. The summarized SABRE performance will potentially serve as an important guide for future studies and lead towards novel pharmaceutical assays using hyperpolarized NMR.

Testing and Applying ESOTHERIC Pulse Sequences for Efficient ^1H to ^{13}C Polarization Transfer of PHIP

Yenal Gökpek¹, Martin Sandbrink¹, Jan-Bernd Hövener¹, Andrey Pravdivtsev¹

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Parahydrogen-induced polarization (PHIP) offers dramatic sensitivity enhancements in ^{13}C NMR and MRI. Still, efficient polarizers and the implementation of high-yield polarization transfer from ^1H to ^{13}C remain a challenge. Here, we present a systematic study of the ESOTHERIC pulse sequence for polarization transfer from ^1H to ^{13}C in ethyl acetate ($1\text{-}^{13}\text{C}$, deuterated and protonated), examining the effect of the number of refocusing pulses and the inter-pulse delay τ .

We found that only the phase of a single pulse can be adjusted in the ESOTHERIC sequence to make it compatible with the system's thermal polarization initial state (T-ESOTHERIC). Thermal measurements were performed on $[1\text{-}^{13}\text{C}]\text{ethyl acetate}$ to establish baseline transfer efficiencies and to calibrate sequence performance under well-defined conditions. A 2.51-fold signal enhancement has been measured for T-ESOTHERIC; 2.88 was predicted. The application of T-ESOTHERIC enabled convenient troubleshooting of sequence and RF parameters: useful properties for the in-house build polarizers. A correlation coefficient of 0.960 for T-ESOTHERIC was achieved between the simulations and measurements as a function of τ delays.

Hyperpolarization experiments were subsequently conducted using vinyl acetate precursor, enabling the evaluation of ESOTHERIC. Using an automated polarizer (Gökpek et al., ACS Meas Sci 2025), we varied temperature and hydrogenation time and achieved $(32.9 \pm 4.5)\%$ ^{13}C polarization for 1 mL of 50 mM of vinyl acetate- d_6 precursor and 2 mM of Rh-catalyst.

Our results demonstrate that careful optimization of the sequence and physical conditions is essential for maximizing ^{13}C polarization. These findings provide guidelines for implementing ESOTHERIC in PHIP settings, including its use in metabolic imaging.

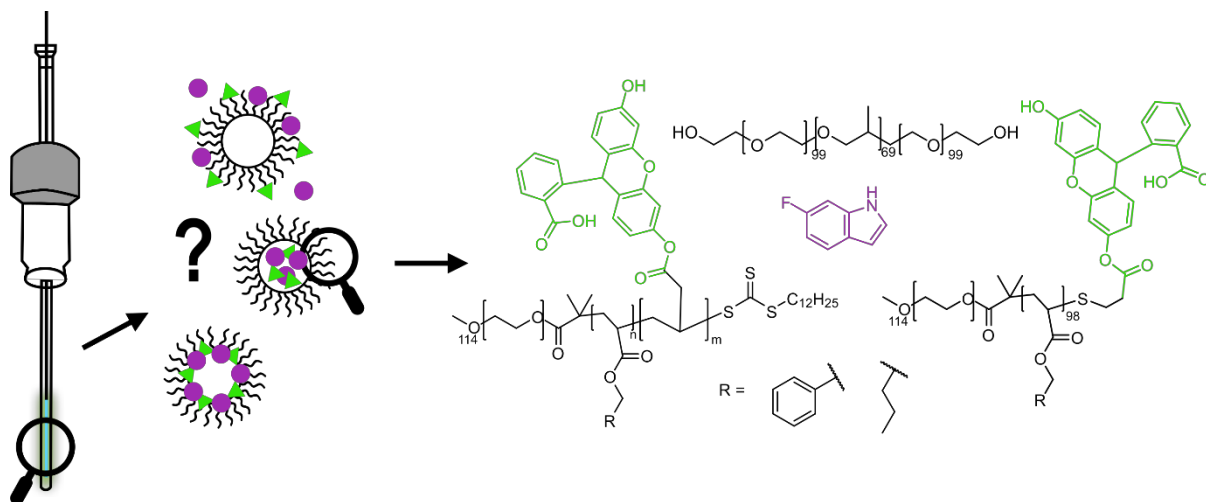
Exploring photo-CIDNP in polymeric micelles for signal enhancement of cargo molecules

Żmuda, Justyna, Stefańska, Marta, Pöppler, Ann-Christin

Polymeric micelles have long been investigated as carriers of small molecules, increasing their solubility and bioavailability. Self-assembled structures of amphiphilic copolymers offer a wide range of functionalization possibilities, giving them useful properties like response to stimuli, or targeted delivery. However, the drug loading efficiency is limited, often reaching a plateau at 10 wt%, while for very high drug loadings the micellar structure can be affected. Altered properties include size, stability and release kinetics, therefore understanding the structure-property relations is fundamental for the rational design of these systems. NMR spectroscopy is a versatile technique to study drug delivery systems, however, guest molecules at low concentrations are often difficult to observe next to the large polymer signals.

To tackle this problem, we decided to explore photo-chemically induced dynamic nuclear polarization (photo-CIDNP), a method of hyperpolarization that relies on light induced formation of radical pairs and enhances the signal through spin sorting mechanisms. The attractiveness of this method lies in a relatively simple setup and mild measurement conditions, that do not risk damaging the micellar structures.

Here, we used Pluronic F-127 as an established model system and methoxy poly(ethylene glycol)₁₁₄- *block*-poly(butyl acrylate/benzyl acrylate)₉₈ polymers with its fluorescein functionalized derivatives synthesized in-house to explore the effects of signal enhancement in aqueous micellar media. Our overarching goal is to probe the structure-property relations of encapsulated cargo as well as its precise location in micelles of ranging complexity, where this information is especially valuable.



Bubble-free *in situ* ^{13}C hyperpolarization inside MRI without external polarizer using parahydrogen pre-saturated solvents

Mohiuddin, Obaid, de Maissin, Henri, Zaitsev, Maxim, v. Elverfeldt, Dominik, Schmidt, Andreas B.

Hyperpolarization (HP) is a powerful technique for metabolic magnetic resonance imaging (MRI). Parahydrogen-Induced Polarization (PHIP) provides a cost-efficient high-throughput approach to produce HP probes. Current implementations require external polarizers that remain complex, costly, and prone to relaxation losses during sample transfer. Synthesis Amid the Magnet Bore Allows Dramatically Enhanced Nuclear Alignment (SAMBADENA), a PASADENA variant, was proposed to address these issues by performing hydrogenation, pulsed spin order transfer (SOT), signal detection, and administration directly inside the MRI bore. However, conventional hydrogenation requires gaseous pH_2 injection, where gas–liquid mixing introduces bubbles that degrade field homogeneity and compromise SOT.

We present a bubble-free method, where the solvent (acetone- d_6) is pre-saturated with pH_2 at high pressure (35 bar) and subsequently transferred to a custom-made spherical reactor preloaded with a sample held inside the MRI system. This novel two-stage reactor system eliminates gas-phase mixing prior to SOT. The spherical reactor geometry showed superior static field B_0 homogeneity and shimming compared to other reactor shapes. Using this method, 20 mM 2-Hydroxyethyl acrylate- d_3 (HEA in acetone- d_6 , 2.5 mM rhodium-based catalyst), forming 2-hydroxyethyl propionate- d_3 (HEP) after hydrogenation, yielded up to 16% ^{13}C (using pH-INEPT+ sequence) and 58% ^1H HP (using OPE sequence) at 7 T and at room temperature within 20 s. Lastly, we compare the two hydrogenation methods.

This study proves the feasibility of bubble-free *in situ* PHIP. While HP of biomedically-relevant metabolites still has to be demonstrated, the method holds potential to significantly reduce equipment demands for ^{13}C -HP MRI applications.

Low-Field Relaxation of Hyperpolarized ^{13}C Reporter for Sensitive Detection of Ligand-Binding

Narwal, Pooja, Lorz, Nils, Minaei, Masoud, Gossert, Alvar D., Meier, Benno

Ligand-protein interactions can be probed by ligand-observed NMR through binding-induced changes in relaxation and NOE transfers. However, sensitivity at high magnetic fields remains limited. Typically, high ligand concentrations of 200 μM and protein concentrations of $\sim 20 \mu\text{M}$ are required. The employed contrasts depend on fast exchange and may fail to detect high-affinity ligands. Competition-based approaches partially address this limitation but still suffer from sensitivity constraints. To overcome this sensitivity limitation, we employ bullet-DNP to hyperpolarize ^{13}C spins of the reporter ligand. This polarization is transferred to ^1H via ^{13}C - ^1H INEPT, enabling high-sensitivity ^1H detection at nanomolar concentrations.

To probe ligand-protein interactions, we exploit the sensitivity of the ^{13}C longitudinal relaxation rate (R_1) to changes in correlation time at low field. After bullet-DNP polarization, the sample is transferred through a magnetic tunnel to a low magnetic field (1.3 T), where the magnetization evolves for 10 s in the presence of protein. Under these conditions, the bound ligand relaxes faster due to its longer correlation time (τ_c) compared to the free ligand. This results in increased R_1 contrast between free and bound states. The remaining polarization is detected at high field (9.4 T) via ^1H detection, enabling indirect readout of low-field relaxation. This approach enables detection of ligand-protein interactions at concentrations as low as 2 μM protein and 14 μM ligand, representing a substantial reduction compared to conventional methods. Furthermore, the method extends to competition-binding experiments, enabling indirect detection of high-affinity ligands. Overall, this strategy combines hyperpolarization with low-field relaxation-based contrast to probe binding interactions at biologically relevant concentrations.

Deuteration of Substrates and Hydrogen Gas in SABRE-Active Systems.

Mushabbah Ashfaq, Joni Eronen, Pihla Jurvelin, Vladimir V. Zhivonitko, and Anu M. Kantola

NMR spectroscopy is a powerful technique for elucidating molecular structure; however, its inherently low sensitivity continues to limit its broader applicability. Hyperpolarization strategies, such as SABRE, have been developed to address this limitation, yet several aspects of SABRE chemistry remain to be understood. In this study, we systematically investigated proton–deuteron exchange processes in SABRE-active systems, a phenomenon previously reported but not comprehensively examined despite its potential to reduce hyperpolarization efficiency and compromise experiments requiring extended acquisition times. Deuteration was examined through multiple pathways, including isotopic exchange in the substrates, the hydrogen gas, the hydrides, and the deuterated solvent (methanol- d_4). A set of N-heterocyclic substrates—pyridine, pyrazine, s-triazine, 3-fluoropyridine, and 3,5-difluoropyridine was studied in systems containing activated $[\text{IrCl}(\text{COD})(\text{IMes})]$ precatalyst under SABRE-relevant conditions using hydrogen gas to mimic the SABRE system. Time-resolved NMR measurements over 7 days were acquired on Bruker Avance NEO 500 MHz instrument. The experimental results provide a systematic characterization of deuteration behaviour across different substrates, revealing distinct substrate-dependent exchange dynamics and deuteration pathways. These findings contribute to improving the understanding and reliability of SABRE-based methodologies and have broader implications for applications in materials science, biomedical imaging, and diagnostics, where enhanced sensitivity and reduced acquisition times are critical.

Parahydrogen-Induced Intermolecular Spin Polarization Transfer via Hydrogen Bonds.

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Nuclear spin hyperpolarization can increase NMR sensitivity by several orders of magnitude and enables many new applications in chemistry and biomedical imaging. Parahydrogen-based methods (PHIP/SABRE) generate polarization within seconds, but they often depend on specific substrate motifs or on proton-exchange steps that are hard to control and can suffer from rapid relaxation.

Here we explore an alternative mechanism: hydrogen-bond mediated PHIP (HBM-PHIP). In this concept, parahydrogen-derived spin order is relayed from a dedicated transfer agent to a target molecule through a stable hydrogen-bonded complex, using intermolecular J-couplings rather than chemical exchange.

We combine DFT (PBE0/QZ4P, COSMO chloroform) with spin-dynamics simulations to derive practical design rules. A microstate analysis predicts that, under relevant solution conditions, a single hydrogen-bonded transfer complex can dominate the ensemble (>99.9%), while solvent binding does not compete effectively. Spin-dynamics calculations show that transfer efficiency is mainly set by the ¹³C–¹³C coupling across the hydrogen bond: in an effective two-spin limit (e.g., decoupling or deuteration of a mediating proton), transfer to the target ¹³C can approach ~99% under suitable field-cycling conditions.

To make transfer faster, we screened 1,447 solvent additives placed near the hydrogen bond. Only a few markedly enhanced the key coupling—from ~0.014 Hz up to ~6.1 Hz—and simulations predict much faster and stronger polarization build-up.

These results position HBM-PHIP as a promising method to strongly hyperpolarize many different substrates including biomolecules.

Low-field proton relaxation and rapid resonant thermalization in hyperpolarized glasses

Singh, Pooja, Jurkutat, Michael, Safiullin, Kajum, Meier, Benno

Dynamic nuclear polarization (DNP) exploits the large thermal polarization of unpaired electron spins to enhance the relatively smaller nuclear spin polarization, thereby increasing NMR signal sensitivity by several orders of magnitude. This polarization transfer requires stable radicals as a source of unpaired electrons within the sample matrix. Techniques such as dissolution DNP and bullet-DNP continuously expand DNP applications. However, the same paramagnetic species essential for polarization transfer simultaneously accelerate nuclear spin-lattice relaxation, representing an inherent trade-off in DNP methodology. In dissolution DNP, hyperpolarized samples are transferred at low magnetic fields to the detection site, during which accelerated relaxation diminishes achievable polarization enhancements, particularly for ^1H DNP.

Here, we employ fast-field-cycling (FFC) NMR to systematically investigate proton spin-lattice relaxation in DNP-juice, the commonly used matrix in ^1H DNP, and its fully protonated analogue, varying the radical (TEMPOL) concentration (0–50 mM) and quantify relaxation across a field range of 20 mT to 2 T at temperatures between 5 K and 40 K. This comprehensive characterization reveals two distinct low-field relaxation pathways: **(I)** the expected TEMPOL-mediated proton relaxation ($B > \sim 0.25$ T), accurately described by a heuristic model consistent with first-principles calculations, and **(II)** an unexpected rapid resonant thermalization (RRT) phenomenon observed below ~ 0.25 T, which causes substantial proton polarization losses in less than a few ms. RRT is observed when either deuterium or radical is present. While the microscopic mechanism underlying RRT remains unresolved, it likely constitutes a fundamental limiting factor in practical proton DNP implementations.

Development of Computational Procedure to Evaluate Polarizing Agents for DNP in Solid State NMR

Bolognesi Enrico, Santioli Ginevra, Macchiagodena Marina, Niccoli Lorenzo, Perras Frédéric A. and Lelli Moreno.

Dynamic Nuclear Polarization (DNP) is one of the most versatile ways to enhance the sensitivity in solid state NMR. During the past decade, the focus has been put into polarizing agents (PA) that could reach high enhancement (up to 250 for AMUPol) at 100 K and 9.4 T, but at higher field there is a huge loss in enhancement, that makes necessary the development new biradicals optimized to work in this condition.

This presentation will focus on TinyPols, a recently introduced family of water soluble biradicals that have shown high enhancements (up to 210 at 18.8 T and 100 K) and sensitivity gains, especially the ones that contain antenna-like side arms that promote the PA's solvent interaction. To further push this limit, we combined the experimental approach with largescale DNP simulations that permits us to simulate the entire hyperpolarization process atomistically in a system that includes all spins involved in DNP and spin diffusion (biradical molecule and solvent bath) in their appropriate quantities and densities.

To do so, we developed a computational procedure that permits a fast preliminary computational analysis of new PAs, to obtain a complete evaluation of the newly designed PAs. Our approach combines the DNP simulations with Density Functional Theory (DFT) and Molecular Dynamics (MD) simulations in order to consider the conformational variability of the PA, the multiple intra- and intermolecular interactions with other PAs and the spin diffusion process across the solvent.

We will show this procedure applied, among others, to PAs of the TinyPol family, where we can predict the experimental trend and confirm the role played by the antenna side chains to increase the efficiency at high magnetic field.

Signal amplification by reversible exchange (SABRE) is a powerful parahydrogen-based hyperpolarization method that can enhance nuclear magnetic resonance signals without permanent chemical modification of the substrate. The efficiency of SABRE is commonly associated with the formation of the active iridium dihydride complex, in which parahydrogen-derived hydrides and the bound substrate form a transient spin-coupled network. However, SABRE samples may also contain short-lived, low-concentration intermediates produced through substrate loss, chloride coordination, or solvent coordination. Although these species are often difficult or impossible to observe directly by conventional one-dimensional NMR spectroscopy, they can strongly influence polarization transfer by changing the hydride chemical environments, exchange rates, and singlet-triplet leakage pathways.

In this work, chemical exchange saturation transfer (CEST) NMR was used to investigate hidden hydride-containing intermediates in SABRE samples prepared with the IMes-based iridium catalyst and four nitrogen-containing substrates: pyridine, pyrazine, 3-fluoropyridine, and 3,5-difluoropyridine in methanol- d_4 . Saturation was applied across the hydride region, and attenuation of the free hydrogen signal was monitored to reveal exchanging hydride resonances. Similarly saturation was also performed in the substrate region and free substrate signal was monitored. Measurements at 270 K and 300 K showed that, in addition to the commonly observed tris-substrate SABRE complex, several low-population intermediates are present. These include chloride-bound complexes with inequivalent hydride ligands and, solvent-coordinated species. The relative abundance and exchange behavior of these intermediates depended strongly on the substrate. Pyrazine and 3,5-difluoropyridine showed detectable chloride-bound species with measurable hydride exchange rates, while pyridine and 3-fluoropyridine displayed weaker intermediate signals and slower exchange.

The results demonstrate that SABRE polarization behavior cannot be fully described by considering only the dominant tris-substrate complex. Short-lived intermediates provide additional exchange and relaxation pathways that may reduce or modify the available parahydrogen spin order. Identifying these species is therefore essential for understanding SABRE mechanisms and for optimizing catalyst, substrate, and temperature conditions for efficient hyperpolarization.

Achieving uniform and strong *in situ* NMR sample illumination in cryogenic probes

Gorman, Daniel, Nilsson, Mathias, Golovanov, Alexander P.

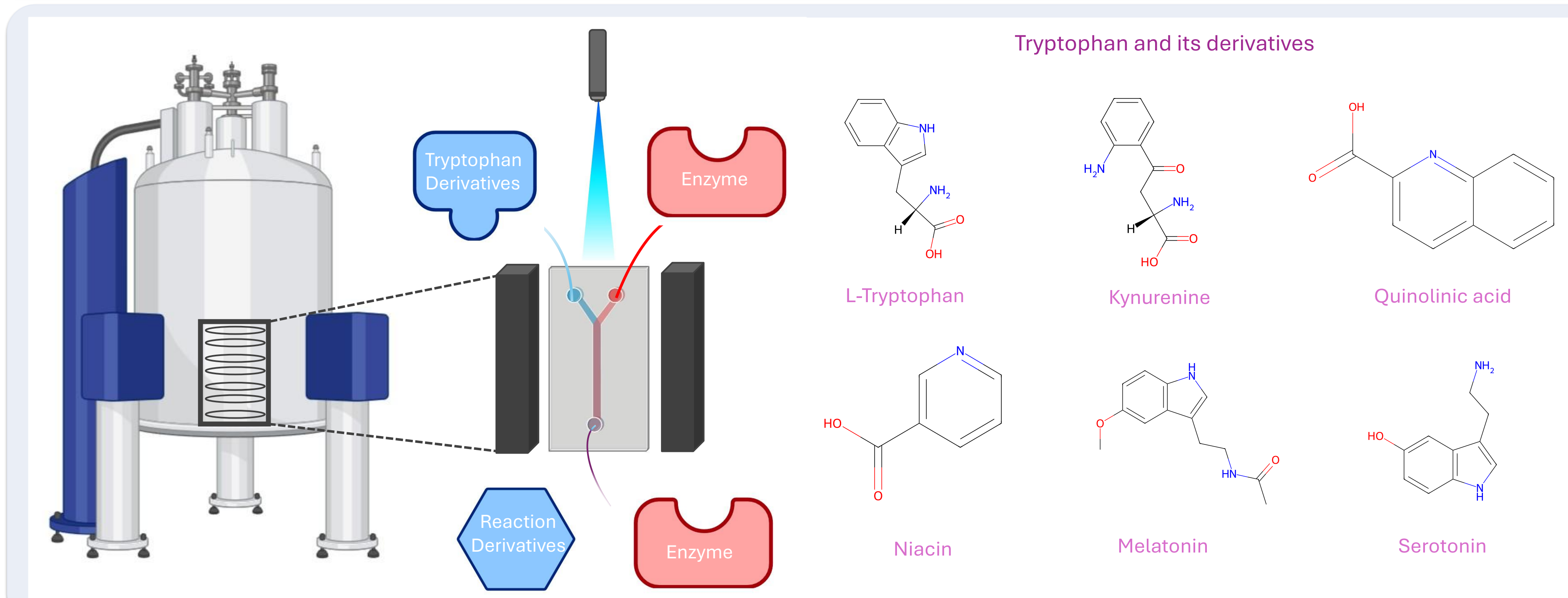
Photochemical and photocatalytic reactions studied *in situ* by NMR spectroscopy require efficient, uniform sample illumination that is compatible with high optical density samples and high-throughput workflows. Previously it had been suggested by Wüster et al. (Magn. Reson., 2024, 5:61) that routing optical fibre through a flow accessory port of a cryoprobe allows to illuminate samples from the bottom, enabling automation. While this approach works with samples with low optical density, for highly optically absorbing samples light penetration is severely limited by the large optical path along the sample. Here, we systematically investigate bottom-illumination strategies for light-coupled NMR experiments, examining how light propagation pathways, sample tube geometry, surface treatments, and fibre positioning influence illumination intensity and spatial uniformity.

Using photo-chemically induced dynamic nuclear polarization (photo-CIDNP) as a quantitative, spatially resolved measure of light intensity, we identify three dominant illumination pathways: direct axial transmission through the sample, propagation within tube walls with side scattering, and light transport within the annular gap between the sample tube and probehead bore. Direct axial illumination leads to markedly non-uniform light in optically absorbing samples, whereas redistributing light around the sample and scattering it laterally, using the NMRtorch principle, as earlier suggested by us, significantly improves uniformity. By combining flat-bottom sample tubes, engineered wall etching, and optimised fibre–tube spacing, we achieve near-uniform illumination across the NMR-active volume while maintaining high overall light intensity for a sample with an optical absorbance of 1.7. These results establish practical design principles for robust, automation-compatible illumination setups for light-coupled NMR studies.

Real-time monitoring of tryptophan oxidation in microfluidic flow using photo-CIDNP and PRESS localization

Hatice Nur Baser, Dr. Neil MacKinnon
Karlsruhe Institute of Technology

Introduction and Setup



Introduction

- ❖ Compact NMR systems require new sample handling strategies for reliable analysis at small scales.
- ❖ Microfluidic platforms offer precise control over mixing, reaction timing, and detection, enabling real-time process monitoring.
- ❖ This study integrates microfluidics with Photo-CIDNP-enhanced NMR for efficient detection of tryptophan derivatives and enzymatic reactions.

Motivation

- ❖ Reduced sample volumes in microfluidics lead to lower NMR signal-to-noise ratio (SNR).
- ❖ Enhancing signals with Photo-CIDNP allows for faster, more sensitive measurements.
- ❖ Monitoring tryptophan derivative formation provides insights into metabolic pathways and enzyme activity.
- ❖ Combining microfluidics and NMR supports miniaturized, real-time chemical analysis.

Results

Signal Enhancement for Process Monitoring: Photo-chemically induced DNP

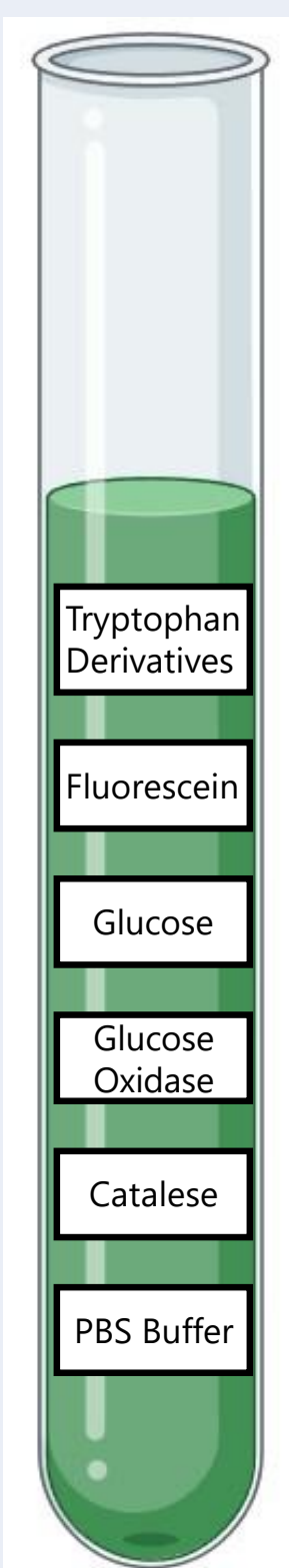


Photo-CIDNP uses a photosensitizer to enhance nuclear polarization via optical excitation.

- ❖ Improves SNR of molecules participating in the radical pair transfer mechanism
- ❖ Requirements: dye molecule, light source
- ❖ Targets: primarily aromatic molecules (selective)
- ❖ Challenges: photodegradation and short lifetime of the dye

Absorbance Experiments

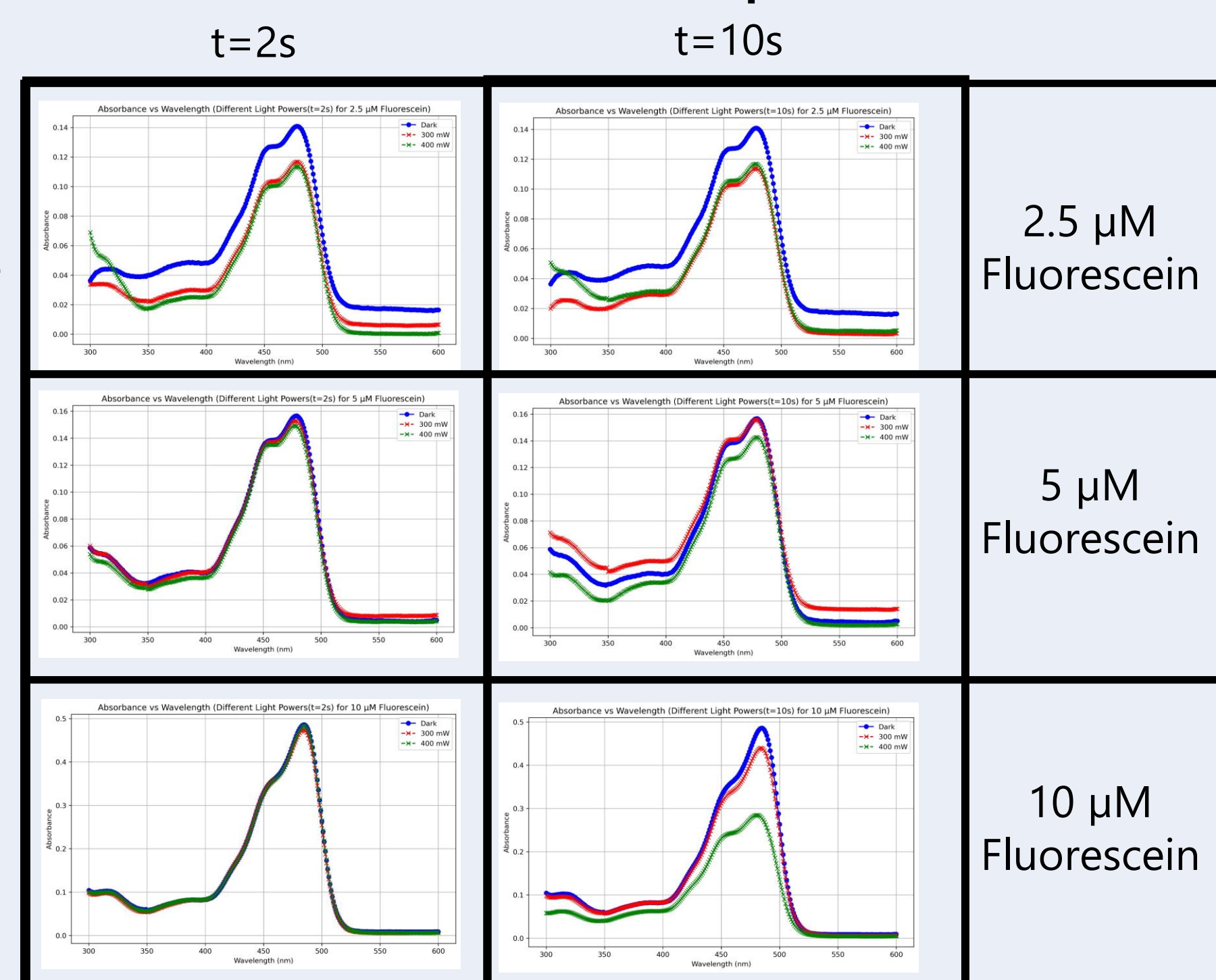
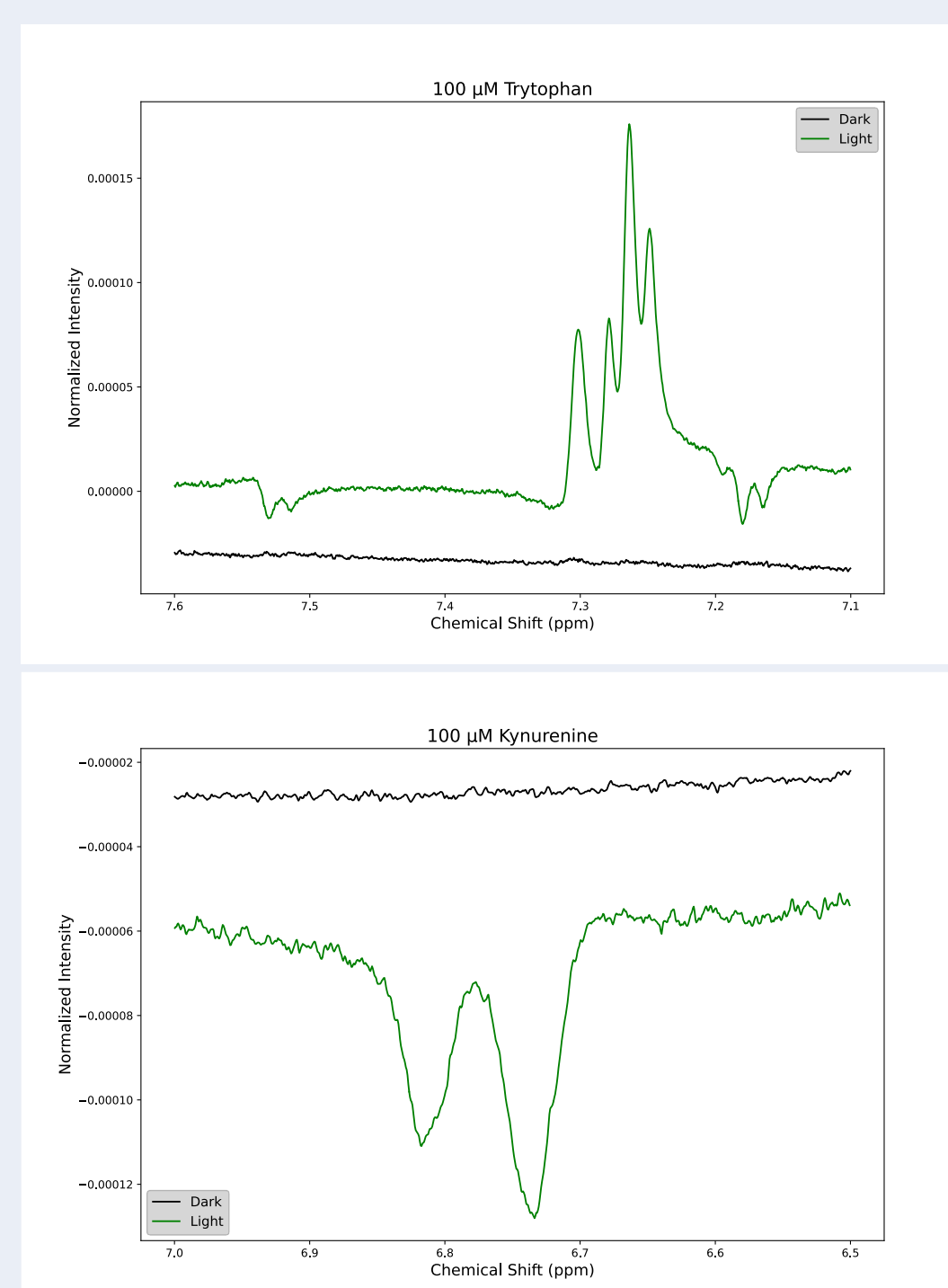
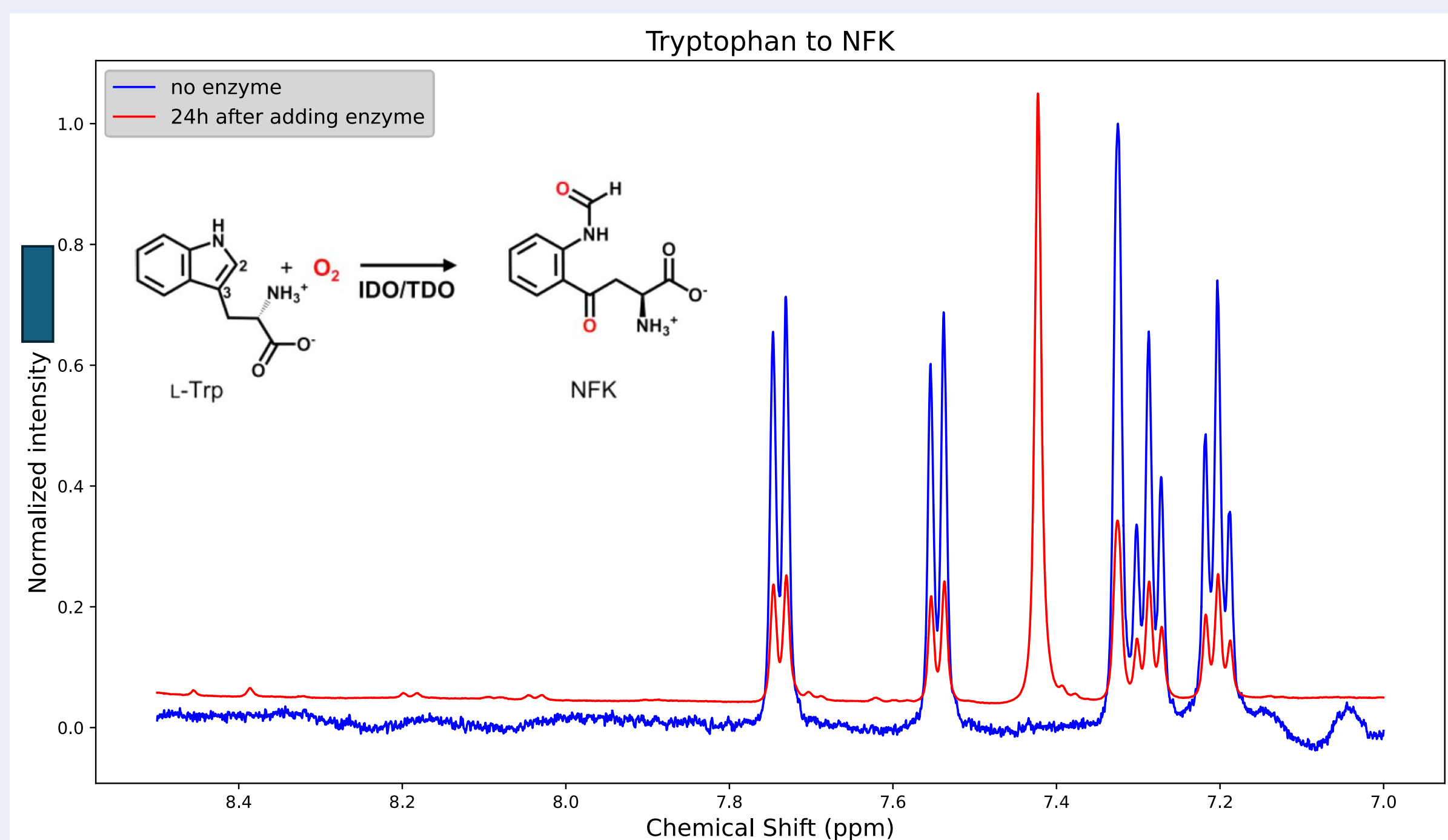


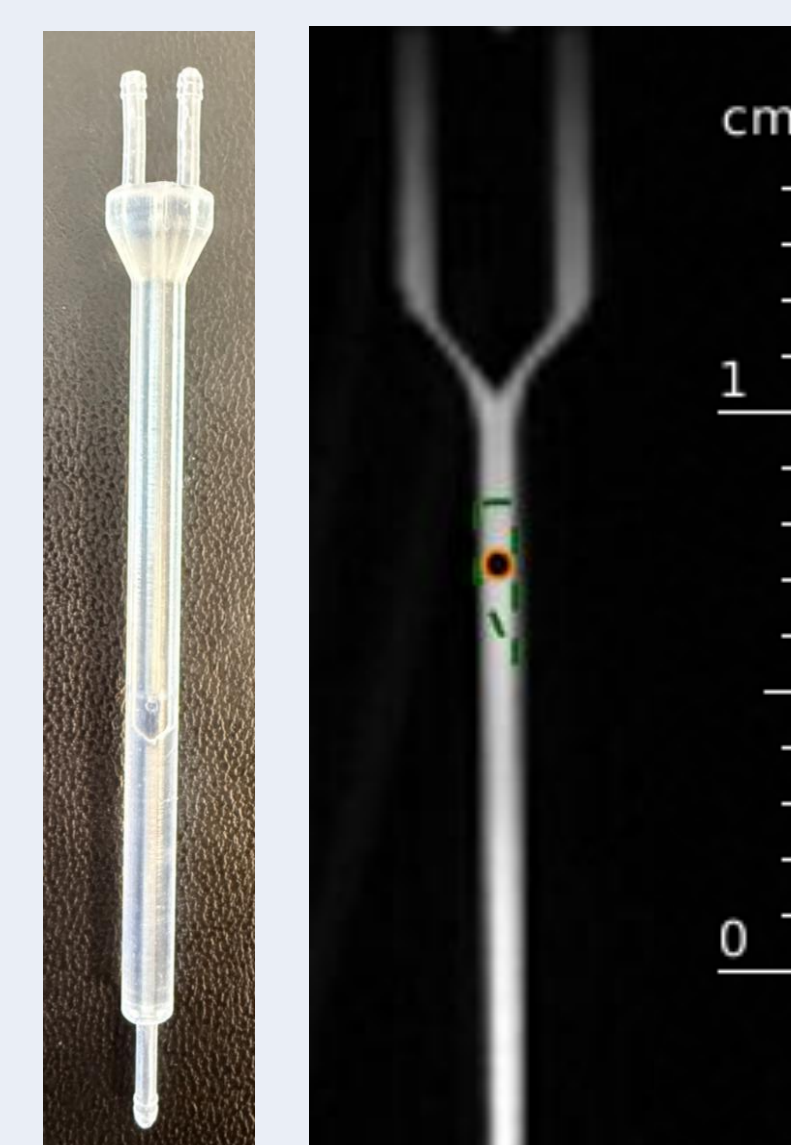
Photo-CIDNP Experiments

- ❖ Irradiation source: 450 nm laser diode at 300 mW power
- ❖ Irradiation time: 1s

Tryptophan Pathway



Spatially resolved monitoring of tryptophan-to-NFK conversion



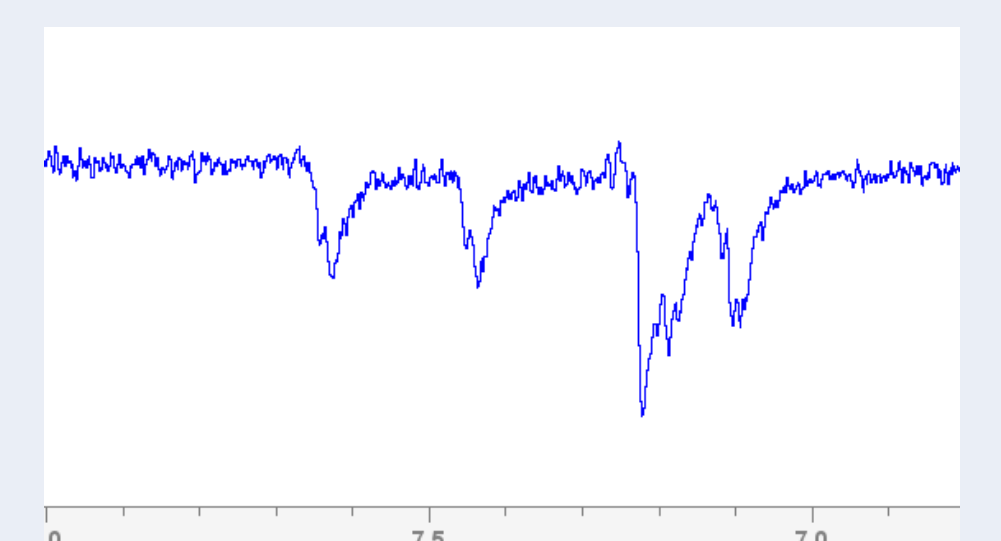
3D printed micromixer device

PRESS (Point-Resolved Spectroscopy) is a localization technique that selectively probes a defined sample volume

- ❖ Enables spatially selective observation inside the microfluidic channel
- ❖ Allows localized monitoring under continuous flow
- ❖ PRESS selectively probes the illuminated reaction region
- ❖ Combining PRESS with photo-CIDNP improves localized detection sensitivity

- ❖ Pulse sequence: PRESS (Point RESolved Spectroscopy) with 650 MHz Magnet

- ❖ Solution: 30 mM Tryptophan + 200 μM Fluorescein in 10 mM PBS



- ❖ 4096 scans 2 hours(dark)

- ❖ Voxel size: 0.7 mm * 0.7 mm * 10 mm = 3.8 μL

Outlook

- ❖ Can photo-CIDNP enable real-time monitoring of Trp-to-NFK conversion under continuous flow?
- ❖ How can PRESS localization improve spatially selective detection inside microfluidic channels?
- ❖ What are the optimal light-delivery conditions for enhancing photo-CIDNP signals in small-volume NMR systems?

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Dr. Neil MacKinnon

DNP-enhanced solid-state NMR for the compartment-selective analysis of polymer micelles

Ulrich, Kersten, Bensons, Edvards, Sjalsjö, César, Corzilius, Björn, Pöppler, Ann-Christin

NMR spectroscopy is a powerful tool for the structural analysis of drug-loaded polymer micelles but lacks sensitivity for the detection of molecular moieties only present in minor fractions. Yet, in advanced drug delivery systems - incorporating multidrug loading, stimuli-responsiveness, and active targeting - these low-concentration species often dictate the overall performance. To facilitate the analysis of such complex systems, we use dynamic nuclear polarization (DNP) enhanced solid-state NMR.

By covalently attaching nitroxide polarizing agents (PA) to polymer end-groups, it becomes possible to place the PA at defined positions within these micelle-containing samples. This enables localized signal enhancement through direct DNP, longer-range enhancement as polarization is transported throughout the sample by spin diffusion, and selective signal attenuation of nearby nuclei due to paramagnetic relaxation effects. Micelles of different sizes are compared, as the interplay of bleaching and enhancement strongly depends on the relevant length scales. Moreover, experimental results are compared with simulations of diffusive polarization transport within the sample.

This approach enables the detection of drugs within the polymer matrix at low loading, the determination of their spatial localization within the micelles, and an improved understanding of the micellar geometry in the solid-state. Together, these results demonstrate the potential of DNP-enhanced NMR for the compartment-selective analysis of nanostructured drug delivery systems.

Towards cryogenic preservation and centralized distribution of PHIP-hyperpolarized ^{13}C fumarate crystals

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Parahydrogen-Induced Polarization (PHIP) is a hyperpolarization technique that has been used to produce ^{13}C -hyperpolarized metabolic imaging probes. Although only a limited number of molecules can currently be hyperpolarized by PHIP, fewer than with dynamic nuclear polarization (DNP), the cost of a PHIP polarizer is roughly an order of magnitude lower. $[1-^{13}\text{C}]$ Fumarate is one such molecule that can be hyperpolarized by PHIP, and several studies have successfully precipitated PHIP-hyperpolarized fumarate crystals, producing a purified solid that can be re-dissolved for preclinical imaging. We see considerable potential from this approach, as a way of storing polarization in the solid fumarate crystals, since solid-state samples regularly exhibit longer T_1 times than molecules in solution.

In this work, we have constructed a polarizer to produce hyperpolarized $[1-^{13}\text{C}]$ fumarate in the solution state at around 50% ^{13}C polarization. After performing an acid-based precipitation to purify the fumarate, and subsequently redissolving the hyperpolarized crystals, we measured up to 26% ^{13}C polarization in the solution-state. The solution-state ^{13}C T_1 of fumarate at Earth's field produced by our polarizer is around 100 s, whereas the solid-state T_1 measured at 14.4 T under 14 kHz MAS is around 200 s.

A ^{13}C T_1 of 200 s in the solid sample is still insufficient for extended storage and transportation, so we are investigating the use of a cryogenic vessel for sample storage in liquid nitrogen, as lower temperature can extend T_1 . Our preliminary data show that freezing the hyperpolarized $[1-^{13}\text{C}]$ fumarate in liquid-nitrogen in a 300 mT field can extend the ^{13}C T_1 relaxation time significantly. We observed a biexponential polarization decay with time constants of 7 and 45 min. We are still in the process of optimizing our experimental procedure, but this extended lifetime suggests a strategy in which hyperpolarized $[1-^{13}\text{C}]$ fumarate crystals could be produced centrally and distributed on demand to clinical sites, facilitating broader use of PHIP in metabolic imaging.

Wavelength-Resolved Photoinduced Nuclear Spin Polarization across a Broad Optical Range (350–650 nm)

Photo-chemically induced dynamic nuclear polarization (photo-CIDNP) is commonly studied at fixed excitation wavelengths selected according to the absorption maximum of the photosensitizer or laser availability. This approach assumes that optical absorption determines the efficiency of nuclear spin polarization. Here we show that this assumption is not generally valid.

We report wavelength-resolved photo-CIDNP measurements for a tetraphenylporphyrin–1,4-benzoquinone donor–acceptor system across the 350–650 nm spectral range. NMR signal enhancements by CIDNP were measured using two excitation schemes: a tunable laser source and a broadband lamp combined with narrow band-pass optical filters. The CIDNP amplitudes were normalized to the incident photon flux, enabling direct comparison of polarization efficiencies across wavelengths and between excitation sources.

The resulting CIDNP action spectrum is strongly non-monotonic and differs substantially from the optical absorption spectrum. Strong absorption does not necessarily produce the largest NMR signal enhancement, while efficient CIDNP can be observed at wavelengths with weaker absorption. These results show that the effective excitation wavelength depends not only on photon absorption, but also on excited-state relaxation, radical-pair formation, luminescence, and spin-selective recombination.

The main wavelength-dependent trends observed with laser excitation are reproduced using filtered broadband lamp illumination after photon-flux normalization. Thus, the excitation wavelength is an independent control parameter for liquid-state photo-CIDNP, and wavelength-resolved measurements can be implemented without a tunable laser source. This provides a simple approach for optimizing light-induced nuclear spin hyperpolarization.

Observation of the 1-¹³C-Pyruvate Metabolism in Lung Cancer Cells using Hyperpolarization.

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Introduction

Hyperpolarized metabolic imaging in NMR is a powerful technique, which allows for the direct observation and investigation of metabolic processes. The investigation of tumor metabolism is of particular interest, because an altered metabolic profile can be detected much earlier than any potential pathological manifestation, allowing for earlier treatment when used in medical diagnostics. In this work, we investigated the pyruvate-to-lactate metabolism of a lung cancer cell line (H460) and the impact of the cell surface protein Cluster of Differentiation 109 (CD109) comparing the wild-type cell line with CRISPR-Cas9 knock-out (KO) clones. CD109 is known to be expressed in lung cancer cells and higher concentrations are associated with increased mortality. Therefore, it can serve as possible biomarker for detection of early recurrence or treatment option.

Method

H460 cells are resuspended in 200 µl Phosphate-Buffered Saline (PBS) for starvation to allow for a quick take-up of hyperpolarized 1-¹³C-pyruvate. These cells are subsequently transferred into a Shigemi NMR tube, positioned in a 400 MHz NMR spectrometer at 310 K shortly before the experiment. Hyperpolarized 1-¹³C-pyruvate is generated using dissolution dynamic nuclear polarization (dDNP) as an aqueous solution of which 260 µl are injected into the NMR tube by means of a syringe-tube-system within 25 s after hyperpolarization. The acquisition of a ¹³C-NMR spectrum is started immediately. This experiment is executed with H460 wild-type cells and with its CD109 KO clones.

Results and Outlook

We found the spin-lattice relaxation of 1-¹³C-pyruvate in our sample to be $T_1 = 80$ s for ¹³C. Moreover, the metabolism of 1-¹³C-pyruvate to 1-¹³C-lactate can be observed. The integral of the 1-¹³C-lactate signal increases in the first 60 s of acquisition before it starts to decay. This shows the pyruvate-to-lactate metabolism of the tumor cells in real time.

Future approaches include comparing H460 and its KO clones in their metabolism rates as these are higher in tumor cells.

The controversy regarding partially negative lines of orthohydrogen molecules

Pietrzak, Mariusz; Czarnota, Marek; Mames, Adam; Ratajczyk, Tomasz

Hydrogen gas at room temperature consists of 75% orthohydrogen (o-H₂) and 25% parahydrogen (p-H₂) at thermal equilibrium. Enrichment in p-H₂ can be achieved by cooling the gas in the presence of a catalyst, which enables para–ortho conversion. After reheating to room temperature without the catalyst, the non-equilibrium composition is preserved, creating a reservoir of latent nuclear spin hyperpolarization.

When a p-H₂-enriched mixture is introduced into an NMR sample containing a dissolved catalyst, equilibration begins, and the conversion of p-H₂ into o-H₂ may generate enhanced NMR signals. During this process, hyperpolarized o-H₂ with non-equilibrium energy-level populations can be formed. Although these populations may arise through different mechanisms, only a single ¹H NMR signal is observed, which can exhibit positive, negative, or vanishing amplitude. Remarkably, it was only recently discovered that o-H₂ formed during equilibration can display a dispersive character, called a partially negative line (PNL), in ¹H NMR spectra.

Here, we show that the PNL effect can be observed using various iridium compounds, not only the Ir-NHC catalysts commonly used in signal amplification by reversible exchange (SABRE). The existence, amplitude, and phase of the PNL effect depend strongly on the catalyst, ligand, and solvent. For example, the PNL showed a plus-minus phase in benzene (C₆D₆), whereas perfluorinated benzene (C₆F₆) exhibited a minus-plus pattern. Therefore, a series of experiments was conducted in solvent mixtures with different ratios of C₆D₆ to C₆F₆. For the 55:45 ratio, a single positive resonance of o-H₂ was observed, but the PNL effect vanished. The question now arises as to what determines the phase of the PNL signal, and why it depends on the solvent and changes within the solvent mixture. We will speculate on these issues.

Li-metal DNP for solid-electrolyte interphase characterisation in anode-free batteries: impact of field distortions

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Dynamic Nuclear Polarization (DNP) enhances NMR sensitivity at Li metal surfaces and provides selective access to interfacial species in battery systems. Here, Li-metal DNP is applied to investigate the solid–electrolyte interphase (SEI) in anode-free lithium batteries through hyperpolarisation transfer from metallic lithium to nearby nuclei at the buried metal/SEI interface.

Ex situ MAS experiments were first performed to establish the interfacial selectivity of the method. $\{^7\text{Li}\}^{19}\text{F}$ cross-polarisation measurements confirm that LiF signals originate from regions close to the Li metal surface. DNP performance was then evaluated in static, battery-like configurations relevant to in situ experiments. In these systems, metallic cell components perturb the microwave field, while cathode materials introduce additional magnetic field inhomogeneities that affect DNP efficiency. In particular, efficient enhancement of both metallic lithium and SEI signals is observed for Cu mesh current collectors, whereas Cu foil and cathode-containing configurations significantly suppress SEI enhancement. These results highlight the role of cell architecture in determining microwave field distributions and, consequently, DNP performance. Understanding these effects is essential for the design and interpretation of in situ DNP experiments in battery systems.

Towards higher molar polarization with lower compound concentration

Laurynas Dagys, Izabelė Navickaitė, Nojus Radzevičius, Edvinas Orentas

ParaHydrogen-Induced Polarization (PHIP) is an efficient route to achieve hyperpolarization and to overcome the challenges imposed by the limited sensitivity of NMR spectroscopy. PHIP process involves a chemical reaction between a target molecule and parahydrogen gas containing high nuclear singlet spin order which can be transformed into magnetization of a targeted nucleus. Recently, PHIP has advanced its methodology to not only reach high polarization but product concentration as well, leading to high so-called molar polarization. Exploration of these extremes enabled the detection of usually feeble effects like intermolecular NOE (iNOE) and to a better understanding of non-linear regimes of spin dynamics. Recently, we demonstrated high ^1H molar polarization and iNOE using the PHIP precursor 1- $^{13}\text{C},\text{D}_6$ -dimethyl acetylenedicarboxylate (DMAD), which contains a single unsaturated alkyne group. In the present study, we investigated a PHIP precursor based on a benzene base functionalized with three alkyne groups, which could potentially provide high molar polarization at lower concentrations. Our preliminary analysis suggests that, although this precursor exhibits lower solubility and reactivity compared with DMAD, it still produces appreciable polarization levels and may represent an attractive candidate for further studies.

Monodentate Ir–Pyruvate Complex Indicates Effects of Ir–Carboxyl Binding on ^1H – ^{13}C Transfer

Schleker, Philipp; Singh, Anushka; Eills, James

Parahydrogen-based hyperpolarization of pyruvate has emerged as a highly promising approach for enhancing NMR sensitivity and enabling metabolic MRI applications for cancer diagnostics. Over the past years, several powerful methodologies, including SABRE, SABRE-SHEATH, SLIC-SABRE, and PHIP-SAH, have demonstrated efficient hyperpolarization of ^{13}C -labelled pyruvate and significantly advanced the field. Despite these achievements, the mechanistic role and quantitative contribution of individual catalytic intermediates during the polarization transfer process remains challenging to understand, limiting the rational optimization of direct ^{13}C hyperpolarization.

In this contribution, we investigate the impact of the Ir–carboxyl interaction on ^1H – ^{13}C polarization transfer efficiency by focusing on the equilibrium between covalent and dative coordination of pyruvate to the iridium center. To this end, an activated monodentate iridium–pyruvate complex was isolated in the aprotic solvent acetone and characterized under parahydrogen conditions. While the complex exhibits hyperpolarized hydride resonances, no detectable polarization transfer to the ^{13}C nuclei of pyruvate was observed.

Experimental conditions are presented and discussed with respect to their mechanistic implications for SABRE-type polarization transfer pathways. The results provide new insight into the structural requirements for efficient ^{13}C hyperpolarization and highlight the potentially critical role of substrate binding character in catalytic spin-order transfer processes.

**Dissolution DNP instrumentation at the
Swedish NMR Centre for hyperpolarized liquid-state NMR**César Själssjö¹, Ulrika Brath¹, Vladimir Denisov²¹ Swedish NMR Centre, University of Gothenburg, Gothenburg 40530, Sweden² Biomedical Engineering Division, Department of Clinical Sciences-Lund, Faculty of Medicine, Lund University, 22100 Lund, Sweden

Dissolution dynamic nuclear polarization (dDNP) enables transient signal enhancements of several orders of magnitude by generating high nuclear spin polarization in a frozen solid and rapidly converting it into a hyperpolarized liquid for NMR detection. The Swedish NMR Centre has installed a dDNP polarizer operating at 5 T and approximately 1 K next to an 800 MHz NMR spectrometer, providing access to this methodology for applications in hyperpolarized NMR and MRI.

Here, we present the implementation and characterization of the dDNP work using sodium [1-¹³C]pyruvate as a model substrate. The solid-state sample contained 1.5 M sodium [1-¹³C]pyruvate in a glycerol/water glassing matrix, with trityl OX063 as the polarizing radical and gadoteric acid as an electron-relaxation agent. Microwave irradiation was optimized by frequency sweep to maximize the ¹³C signal, followed by monitoring of the solid-state polarization build-up. After polarization, the sample was rapidly dissolved with hot pressurized D₂O and transferred by helium gas to the NMR spectrometer for liquid-state acquisition.

The system produced a maximum ¹³C polarization of $27.1 \pm 0.2\%$ for [1-¹³C]pyruvate, corresponding to an enhancement of approximately 1.5×10^4 , with a liquid-state T_1 of 27.2 s. These results demonstrate efficient solid-state polarization transfer, rapid dissolution, and successful detection of hyperpolarized pyruvate in solution. The platform enables studies of fast chemical and biochemical processes, low-concentration metabolites, hyperpolarized probe development, and future metabolic MRI applications.

Imaging

Abstract

Objective: The hypothalamus, consisting of histologically and functionally distinct subunits, plays a crucial role in pathology in adolescents with major depressive disorder (aMDD), but the neuroanatomical alterations of hypothalamus in aMDD are still poorly understood. This study aimed to explore the subunit-level volumetric alterations in the hypothalamus in aMDD and their associations with depressive symptoms and sleep quality.

Methods: High-resolution 3D T1-weighted images were obtained from 83 drug-naïve patients with aMDD and 59 age-, sex-, education years-, and handedness-matched healthy controls (HCs). The MRI data were preprocessed and segmented into five bilateral hypothalamic subunits using FreeSurfer software. We investigated volumetric alterations in hypothalamic subunits and examined whether these structural changes mediated the relationship between depressive severity and sleep quality.

Results: Patients with aMDD showed significantly smaller volumes in the left anterior-inferior subunit (a-iHyp) and larger volumes in the right inferior tubular subunit. The volumes of the left a-iHyp were significantly negatively correlated with both sleep quality and depressive severity in the patients with aMDD. Furthermore, the volumes of the left a-iHyp partially mediated the relationship between depressive severity and sleep quality.

Conclusion: These findings provide new insights into distinct hypothalamic subunit alterations in patients with aMDD. A smaller volume in the left a-iHyp may serve as a neural substrate for the association between depressive severity and poor sleep quality, suggesting a potential neural mechanism underlying both sleep disturbances and depressive symptoms in patients with aMDD.

Dynamic Mastication Imaging using Upright MRI: Visualising Oral Biomechanics and Potential BMI Associations

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Mastication is a complex biomechanical process involving coordinated motion of the teeth, jaw, tongue and soft tissues during food processing and swallowing. However, most imaging studies of oral biomechanics have been performed in supine MRI scanners, limiting the ability to observe chewing behaviour under natural physiological conditions.

This work presents the development of a dynamic upright MRI protocol for visualising mastication in a seated posture using a 0.5 T ASG Paramed open MRI system. Thirty-five healthy adult volunteers with contrasting BMI histories participated in the study. Participants performed controlled chewing cycles using a standardised banana sample while dynamic image series were acquired with a rapid 2D gradient-echo sequence (TR/TE = 5.7/1.2 ms, 200 frames, 2.6 Hz temporal resolution, 77 s acquisition).

Both head and neck RF coils were evaluated for imaging performance, with the neck coil providing improved coverage and contrast. The resulting image series were reconstructed into dynamic videos enabling visualisation of successive stages of mastication, including bite progression, occlusal contact, tongue–palate interaction, bolus formation and swallow initiation.

Preliminary observations suggest differences in craniovertebral posture and bolus progression between participants with different BMI histories, indicating potential biomechanical variation in chewing behaviour. These results demonstrate the feasibility of upright dynamic MRI for investigating the anatomy and biomechanics of mastication in vivo.

The proposed protocol provides a platform for future quantitative analysis using motion tracking and deep learning–based image analysis to characterise masticatory patterns and explore their associations with anthropometric and metabolic factors.

MRI methodologies to follow liquid transport in fiber-based packaging

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The packaging industry is undergoing a major transformation, with increasing emphasis on fiber-based materials. As single-use plastics remain a significant environmental threat, there is growing demand for renewable and recyclable alternatives that provide comparable performance. One promising alternative is the dry-forming technology, at Varberg based company Yangi, Sweden, which has the potential to replace plastic at scale. In this process, fluff cellulose pulp combined with chemical binders is shaped into thin, rigid products through hot pressing. Compared with conventional wet-formed processes, this method significantly reduces water consumption while producing high-rigidity packaging. Aligned with circular economy principles, the technology aims to minimize waste and support closed-loop lifecycles. However, several factors influence the properties of the final product and liquid transport in dry-formed packaging, including the source and type of cellulose pulp, the addition and application of chemical binders, and fiber alignment or deformation during hot pressing. Optimizing the dry-forming process therefore requires in-depth material characterization and a deeper understanding of liquid transport mechanisms within cellulose fiber networks.

In this work, MRI is used to investigate liquid and moisture transport in fiber-based materials. One part of the study focuses on flat dry-formed packaging. A methodology for *in-situ* MRI measurements was developed, enabling one-dimensional signal intensity profiling to estimate the rate and penetration depth of liquid transport under different dry-forming conditions. In addition, one-dimensional diffusion measurements were performed to follow, separately, liquids in complex mixtures, such as a mixture of oil and vinegar, relevant to food packaging applications.

Another part of the study focuses on dry-formed packaging with complex three-dimensional geometries. To investigate the influence of fiber alignment and deformation on liquid transport, a miniature radiofrequency (RF) coil was designed and adapted to the shape of the packaging, enabling localized MRI measurements with high sensitivity.

These methodologies provide deeper insight into liquid transport in fiber-based packaging and contribute to the development of higher-performance, sustainable packaging, that support the transition to a more sustainable, biobased economy.

Optimal control-based RF pulse design for velocity encoding in Magnetic Resonance Velocimetry

Péan Honorin, Morlot Christophe, Perrin Jean-Christophe

Magnetic Resonance Velocimetry (MRV) is a non-invasive imaging technique enabling precise measurement of velocity fields in complex flow systems. Current MRV methods rely on phase-contrast (PC) sequences using bipolar magnetic field gradients to encode flow velocity, which inevitably leads to quite long echo times (TE). For high-velocity flows or higher-order motion, this can result in important measurement errors.

This work proposes to apply an optimal control-based approach to MRV, aiming at reducing acquisition time for flow characterization in engineering and biomedical applications. Using the GRAPE algorithm, a radiofrequency (RF) pulse is computed to encode a velocity-dependent phase under a constant field gradient, eliminating the need for a bipolar gradient and reducing TE. The cost function is defined such that a linear relationship between spin phase and velocity is established. The method is validated on a Hagen-Poiseuille flow model, where spins distributed across a cylindrical tube follow a parabolic velocity profile.

Numerical simulations demonstrate successful convergence of the optimization and accurate velocity-dependent phase encoding across the full frequency bandwidth. A full numerical simulation of the conventional PC-MRI is also implemented as a reference, into which the optimized RF pulse can be directly substituted, enabling a rigorous comparison between both approaches. The RF control field is further integrated into a gradient echo sequence on a 3T MRI scanner, and an experimental framework is presented toward the reconstruction of velocity maps, paving the way for validation of the proposed approach.

The proposed approach will subsequently be extended to other reference benchmark geometries, such as the FDA nozzle, exhibiting more complex flow patterns than the Poiseuille flow.

MRI Evaluation of Brain Changes During Experimental Therapies for Neurodegenerative Diseases

Zalewski, Tomasz, Kozłowska, Urszula,

Alzheimer's disease (AD) is characterized by progressive neurodegeneration, systemic inflammation, metabolic dysregulation, and cognitive decline. Increasing evidence indicates that disruption of the blood–brain barrier (BBB) contributes to disease progression. Impairments in tight junction assembly between brain endothelial cells, together with neuroinflammation, lead to increased BBB permeability and neurovascular dysfunction. Therefore, monitoring BBB integrity may provide an important indicator of therapeutic efficacy.

In this study, we investigated the effects of long-term psilocybin administration on neurovascular function in the 3xTg-AD mouse model. Mice received weekly psilocybin treatment, and outcomes were compared with untreated 3xTg-AD and wild-type control groups. Chronic treatment effects were assessed using preclinical magnetic resonance imaging (MRI) at 9.4 T. Dynamic contrast-enhanced (DCE) MRI with a gadolinium-based contrast agent (Gadovist) was used to evaluate blood–brain contrast agent kinetics and BBB permeability. Psilocybin treatment normalized contrast-agent kinetics in 3xTg-AD mice and reduced Gadovist accumulation in the brain parenchyma, suggesting improved neurovascular function or enhanced clearance mechanisms. MRI-based morphometric analysis revealed no significant changes in hippocampal or white matter volumes following treatment.

Complementary molecular and cellular analyses were conducted to further characterize treatment effects. Together, these findings demonstrate the utility of MRI for evaluating novel therapeutic strategies and identify psilocybin as a potential systemic and central immunomodulatory agent capable of mitigating AD-like pathology by modulating neuroinflammation and neurovascular dynamics.

Prediction of Post-Medication Stroke Recurrence in Intracranial Atherosclerotic Plaques Based on a Hierarchical Dual-Sequence Fusion Network (HFusionNet)**Abstract**

Objective: This study proposes HFusionNet, a hierarchical dual-sequence fusion framework designed to jointly model habitat-derived subregion features and whole-plaque global representations from T1 and T1Gd MRI for predicting stroke recurrence after standard medical treatment in intracranial atherosclerotic plaque.

Methods: For each sequence, the network constructs a dual-branch representation consisting of a micro-scale stream encoding fine-grained habitat subregion descriptors and a macro-scale stream capturing the overall intraplaque characteristics, thereby forming a complementary micro-macro feature structure. A sequence-specific hierarchical encoder is used to learn modality-dependent embeddings separately from T1 and T1Gd, after which subregion-level and whole-region features within each sequence are projected into a shared latent space and adaptively integrated through a gated hierarchical fusion block to preserve detailed habitat patterns while maintaining global structural context. To enhance inter-sequence information exchange, HFusionNet introduces a bidirectional cross-attention module between the T1 and T1Gd branches, where features from one modality attend to those of the other, explicitly modeling cross-sequence dependencies and contrast-enhancement-related complementarities instead of relying on direct concatenation. The cross-attention-refined representations are then delivered to a Transformer-based fusion head that performs global token-level modeling across all modality-specific and scale-specific embeddings, capturing higher-order dependencies and coordinated response patterns through multi-head self-attention. This end-to-end hierarchical fusion strategy enables structured cross-sequence and multi-scale representation learning.

Results: Experimental results demonstrate that HFusionNet achieves superior predictive performance compared with conventional radiomics pipelines and single-sequence habitat-based models. In internal validation and external test cohorts, HFusionNet attained AUCs of 0.851 and 0.834, respectively, outperforming conventional radiomics models (0.767 and 0.746) and traditional habitat models (0.833

and 0.767). After integrating clinical variables, the combined model further improved performance, achieving an AUC of 0.890 in the external test cohort.

Conclusion: HFusionNet effectively integrates micro-habitat and macro-plaque features through hierarchical dual-sequence fusion, and captures complementary information across sequences via cross-modal attention mechanisms, significantly enhancing the accuracy and generalizability of stroke recurrence prediction after standard medical treatment, providing a new paradigm for multi-sequence MRI radiomics analysis.

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Introduction: To establish Dynamic Glucose-Enhanced (DGE) MRI kinetic features as mechanistically interpretable, pathology-aligned biomarkers that noninvasively distinguish immune-hot from immune-cold gliomas.

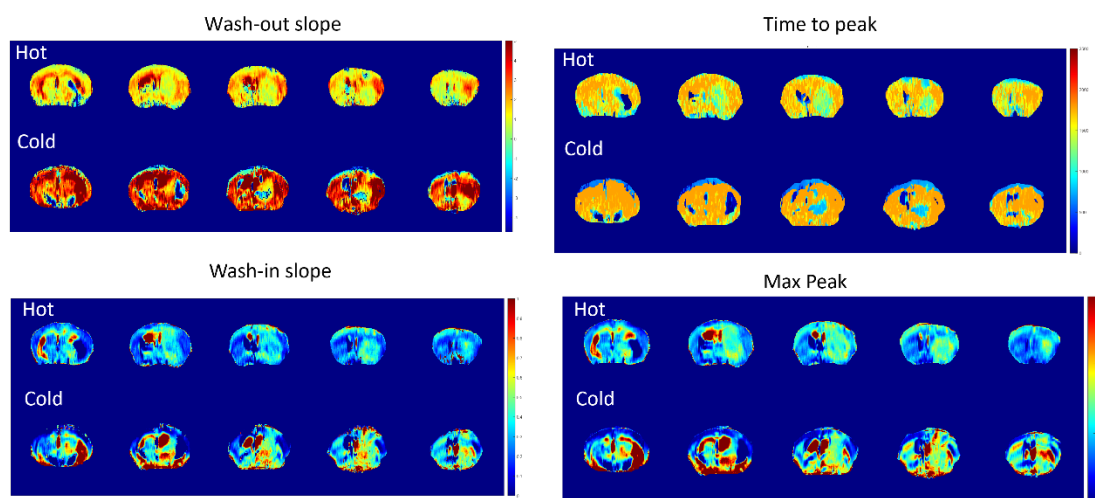
Methods: Glioma immune states were modeled via the CXCL12–CXCR4/CXCR7 axis (cold: intact signaling; hot: tamoxifen-induced Cxcl12 knockout). DGE-MRI time series were fitted with LSTM and segmented by change-point detection to extract wash-in, time-to-peak ($-\ln\text{TTP}$), maximum, and wash-out slope (Tail). Features were mapped using $\log(\text{intra/peri})$ core-vs-rim ratios. Pathology provided quantitative references (CD8 penetration, CD68 burden; $\pm\text{CD31}$). Mechanistic β -weights summarized feature importance and formed an imaging PredHot score.

Results: DGE kinetics separated immune states: Tail and $-\ln\text{TTP}$ were the top-ranked features (β -weights), while Max carried limited information. Trend-only correspondence showed Tail and $-\ln\text{TTP}$ tracking with CD8 penetration (directional agreement). Parametric maps visualized intratumoral heterogeneity—hot tumors exhibited stronger retention patterns and altered time-to-peak relative to cold.

Conclusions: DGE-MRI yields noninvasive, mechanistically interpretable readouts of the tumor immune microenvironment. Wash-out slope (Tail) and time-to-peak ($-\ln\text{TTP}$) are robust candidates to differentiate immune-hot vs immune-cold gliomas and to localize functional heterogeneity beyond anatomy.

Keywords

DGE-MRI; CEST; glioma; immune hot tumor; immune cold tumor; wash-out slope; time-to-peak; CD8;; noninvasive biomarker.



Representative DGE-MRI parametric maps in glioma. Top row: immune-hot; Bottom row: immune-cold. Columns show (left→right): Wash-out slope (Tail), Time-to-peak ($-\ln\text{TTP}$), Wash-in slope, and Maximum. Colors encode feature values (blue = negative/clearance; red = positive/accumulation). Hot tumors display stronger retention patterns and distinct time-to-peak compared with cold tumors, consistent with pathology-derived immune differences (e.g., increased **CD8** penetration). Abbreviations: DGE, Dynamic Glucose-Enhanced; TTP, time-to-peak.

Rapid chemical composition mapping using MRI via model-based reconstruction

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Chemical transformations are fundamental to process engineering, making a comprehensive understanding of *in-situ* reactor dynamics crucial for system optimization. In this context, Magnetic Resonance Imaging (MRI) has emerged as a powerful technique to investigate important process parameters such as phase distributions, flow, and temperature. Moreover, magnetic resonance enables the resolution of distinct chemical species via their chemical shifts. Yet, mapping these shifts across reactor environments remains scarce, as current techniques typically compromise on either chemical or temporal resolution. Recently, model-based reconstruction has enabled the quantification of chemical species from relatively standard acquisitions. Because the participating components in a target reaction are typically known *a priori*, these methods offer a promising framework for monitoring chemical reactions with high temporal resolution. Herein, model-based chemical composition mapping of baseline mixtures, progressing from systems with singular resonance peaks to those with more complex chemical signatures, is presented. Data were acquired on a 40 cm bore vertical MRI scanner using standard multi gradient echo sequences. Crucially, each composition map can be acquired in under a minute using a standard acquisition scheme while preserving sufficient spatial resolution and improving upon the temporal resolution of previous studies such as those by von Harbou *et al.*. Furthermore, magnetic field inhomogeneities arising from the large bore and suboptimal shimming can be accounted for directly within the reconstruction model, making the technique more robust. This technique represents a vital step toward the real-time monitoring of chemical reactions in dynamic engineering systems.

Modulated gradient NMR method for detecting circular motion

Topgaard, Daniel, Clarke, Daniel

What does the solar system and a tornado have in common with a fluidized beds in the chemical industry or blood in the microcapillary network? One common feature is the partially circular motion of their constituents in the sense that a particle after a certain time tends to move towards, say, left instead of right, forwards, or backwards compared to its original direction of motion. While the circular motion in the solar system and a tornado can be tracked in detail by noting down the positions of the planets or test balloons as a function of time, it is considerably more challenging to follow the motion of fluids inside opaque objects such as an *in operando* chemical reactor or the head of a living human being. In the same way as detailed characterization of circular motion is important for understanding the inner workings of planetary systems or predicting the weather, it is equally important for rationalizing mixing efficiencies and reaction rates in chemical engineering applications or assessing blood capillary geometry and function in pathological tissues such as angiogenic tumors or arteriovenous malformations, having implications for disease prognosis and treatment decisions. Here we propose to expand the theoretical description of translational motion to pinpoint features corresponding to circular motion and, correspondingly, augment the theory for NMR motion encoding to enable specific detection of these features.

Liquid Methods

A multiplexed pulse sequence to reveal ^1H - ^{13}C correlations of hyperpolarized metabolite mixtures at natural abundance

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One of the ways to achieve enhancement of inherently low sensitive liquid state Nuclear Magnetic Resonance (NMR) methods is via dissolution Dynamic Nuclear Polarization (d-DNP). The latter allows to hyperpolarize solid vitrified matrices at cryogenic temperatures, that after quick dissolution with hot solvent and transfer to the NMR detection volume may reveal signal enhancements on the order of tens of thousands. As the hyperpolarized magnetization decays irreversibly in liquid state, it necessitates a single-shot strategy. For many years, the family of Ultra-Fast (UF) methods has made it possible to observe ^{13}C - ^1H correlations of hyperpolarized small molecule mixtures, but with significant penalty on the signal-to-noise ratios (SNR), and the need to compromise between resolution and spectral widths, due to the spatially encoded nature of UF experiments. In this work, we propose an alternative to single-scan UF-Heteronuclear Correlation (UF-HetCor) method, named *Selective Multiplexed Acquisition of Sequentially Harnessed* (SMASH) NMR spectra, which in this example, is based on selective excitation of hyperpolarized ^{13}C magnetization followed by reversed refocused *Insensitive Nuclei Enhanced by Polarization Transfer* to ^1H , where it is detected. The multiplexed character of the experiment allows to achieve single-scan (compatible with d-DNP) 2D HetCor spectra with 10-20 times greater SNR, and similar or better resolution in F_2 and F_1 , to the ones obtained using the UF method. We illustrate the potential of SMASH to analyse hyperpolarized complex metabolite mixtures at natural ^{13}C abundance. We anticipate that the proposed method will further our insights in studies concerning small molecule mixtures, in the fields of metabolomics, environmental, food or drug sciences.

Effects of proton exchange on the lifetimes of long-lived states in aliphatic chains

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In an aliphatic chain that contains an AA'XX' structure, a long-lived state (LLS) can be created based on the singlet-triplet imbalance. When the spin states involved are immune to dipole-dipole relaxation, they are long-lived. These long-lived states (LLS) have a relaxation time T_{LLS} much longer than the longitudinal relaxation time T_1 . However, these T_{LLS} are strongly dependent on the spin system's additional coupling with surrounding spins with which they may interact, leading to relaxation leakage. Thus, even small perturbations of the environment can lead to a significant decrease of the T_{LLS} . In this study, we found that for some chemicals containing functional groups that can undergo proton exchange, such as $-NH_2$ or $-COOH$, their T_{LLS} can be strongly pH dependent, and their variation obeys a titration curve. Thus, different relaxation times are assigned depending on the protonation form of the molecule. Qualitative interpretations of these findings are discussed.

Ultra-selective NMR experiments in continuous flow

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The understanding of chemical reaction mechanisms is essential for optimizing processes and discovering new reaction pathways. Reaction monitoring with NMR is usually performed through in situ experiments, carried out directly inside an NMR tube. However, this approach may not accurately reflect the true evolution of the system.

Flow NMR has recently emerged as a powerful alternative enabling continuous, real-time analysis under controlled reaction conditions. In this approach, the reactor is connected to the spectrometer through a flow tube, allowing a fraction of the reaction mixture to be continuously drawn to the NMR probe. For such applications, the selected NMR experiment must be rapid, sensitive, capable of efficient signal discrimination, and robust to flow-induced effects. GEMSTONE^{1,2} is an ultra-selective pulse sequence designed to isolate a single multiplet, thereby overcoming spectral overlap and enabling targeted signal observation. Its high selectivity, short duration, and good sensitivity make it particularly useful for reaction monitoring. However, significant sensitivity losses are observed when GEMSTONE is applied directly to a flowing sample.

In this work, we introduce flow-compatible versions of GEMSTONE suitable for monitoring applications, incorporating flow compensating strategies such as transverse encoding and gradient moment nulling. Then, it will be integrated as an ultra-selective excitation block in correlation experiments, such as 1D TOCSY or NOESY, and used for flow monitoring.

We established two flow-compatible experiments: similar to the initial GEMSTONE with transverse encoding, and another named fo-GEMSTONE (“flow-optimized”), based on a modified gradient pattern. In contrast to the previously observed severe signal attenuation under flow, these pulse sequences significantly recover signal intensity while maintaining selectivity.

These developments open new perspectives for reaction monitoring and offer clear advantages for studying dynamic systems, including short-lived intermediates and out-of-equilibrium species.

[1] Kiraly, et al. *Angew. Chem. Int. Ed.* **2021**, 60 (2), 666–669.

[2] Bazzoni, et al. *Angew. Chem. Int. Ed.* **2023**, 62 (50).

NMR methods for studying liquid biphasic samples: application to SABRE hyperpolarisation

Verhaeghe Guillaume, Huber Gaspard, Berthault Patrick

As it is well-known now, NMR suffers from an intrinsic low sensitivity due to low polarisation at thermal equilibrium. A usual way to circumvent this issue is to use hyperpolarisation techniques that allow the weak NMR signal to be transiently but significantly enhanced. SABRE is such a technique that uses transfer of polarisation from para-enriched hydrogen to substrates via reversible links on a catalyst. Nevertheless, the most common used catalysts are mainly efficient in organic solvents, whereas some applications must be carried out in aqueous medium. One way to get hyperpolarised species in aqueous solvents is then to extract them from the organic phase using biphasic samples.

To prove the transfer, a fast NMR sequence has been developed to spatially monitor the behaviour of solutes in two immiscible solvents. Named Radsl-CSI, for Radial-slice Chemical Shift Imaging, it is based on a clever selection of slices (as shown in Figure 1), coupled with imaging techniques to image the sample.

On one hand, derivative versions of the sequence have shown efficiency to monitor spatially and temporally the migration of a compound in a biphasic sample (here, acetonitrile in D_2O/CD_2Cl_2), and to measure its self-diffusion through the sample. On the other hand, the sequence has made it possible to highlight the transfer of hyperpolarised pyridine from the dichloromethane phase, where SABRE occurs, to the aqueous phase.

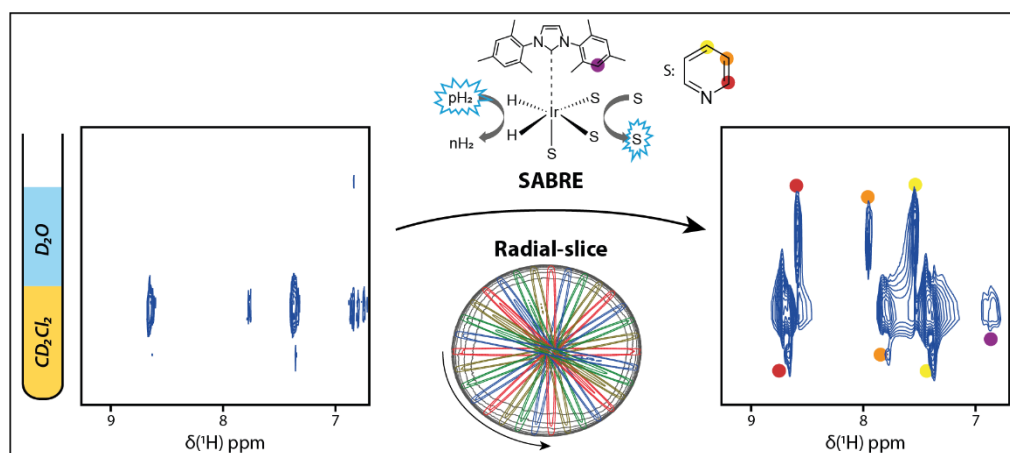


Figure 1 – CSI 1H contour plots, using the Radsl-CSI sequence, of the spectral aromatic region of a D_2O/CD_2Cl_2 biphasic sample, evidencing the transfer of SABRE-hyperpolarised pyridine into the aqueous phase. The circle at the middle represents axial images of a water tube, without slice selection (whole circle in grey), or with slice selection around the tube axis.

Simultaneous identification and quantification of metabolites in *Picea abies* extract by quantitative 2D ^1H - ^{13}C HSQC NMR

Lenen Lauriane, Martinez Agathe, Anthony Abou Dib, Alexis Kotland, Jean-Hugues Renault, Jane Hubert, Pedro Lameiras

Natural extracts are complex mixtures in which reliable metabolite identification and quantification remain challenging. Although ^1H 1D NMR is inherently quantitative, spectral overlap often prevents selective integration. Quantitative ^1H - ^{13}C HSQC (qHSQC) addresses this limitation by spreading correlations over two dimensions, improving resolution while preserving quantitative information when experimental parameters are carefully adjusted.

In this context, qHSQC was used to simultaneously identify and quantify metabolites in a methanolic extract of *Picea abies*. The method relies on the proportionality between cross-peak volumes and molar concentrations when polarisation transfer, J_{CH} evolution, and relaxation delays were optimised. Particular attention was paid to T_1/T_2 relaxation, delays and coupling constants to minimise signal distortions and ensure quantitative response.

The workflow combined metabolite identification using a dedicated 2D spectral database within an NMR-based dereplication strategy and optimised qHSQC experiments. Well-resolved cross-peaks were selected for absolute quantification using reference standards recorded under identical experimental conditions. The concentrations of target analytes (*E*-astringin, *E*-piceid, and (+)-catechin) in the mixture were determined, yielding excellent agreement with LC-MS results (deviations < 1%). When structurally related proxy standards were used instead, deviations remained within 5-10%, demonstrating the robustness of the workflow.

This study reveals that qHSQC extends NMR-based chemical profiling beyond structural elucidation by providing reliable quantitative data in a single experiment, offering a robust analytical approach for quantifying metabolites in complex natural extracts.

Fast and resolved NMR for deciphering the metabolism of microorganisms to sustain spationauts

Iris Hallegouet, Anastasiia Shvetsova, Delphine Drouin, Jérémy Calhabres, Patrick Giraudeau, Olivier Gonçalves, Jonathan Farjon

The MELiSSA project promotes the development of closed ecosystems. *Limnospira indica* (*LI*) was chosen by the MELiSSA project to be cultivated in space in the long term, for its oxygen production and nutritional intake.

In this context, we investigate the potential of multi-scale NMR approaches to characterize the metabolism of *LI* starting with its lipid metabolism, including high-field (HF) NMR to decipher the lipid composition, as well as low-field (LF) NMR towards more accessible and online analysis of an *LI* culture.

To achieve this goal, it is essential to study complex mixtures embedding various lipids changing in the course of a cultivation. Thus, there is a need of sensitive, resolved and fast methods. As a proof of concept of our approach, standard and N-starved extracts from a microalgae model, *Parachlorella kessleri* known for its lipid productivity, were first investigated. Lipid profiling was performed from cells extracts with HF and LF NMR. The complexity of such samples makes it difficult to identify lipids with 1D ^1H NMR because of numerous overlapping signals. Thereby, the lipid identification was performed with faster 2D experiments using Non-Uniform Sampling (NUS), such as ^1H - ^1H TOCSY, ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC as well as more resolute methods such as Pure shift HSQC. Then, to further speed up 2D experiments for monitoring needs, faster NMR methods using selective pulses were implemented. Fast 2D NMR was used to finely annotate signals. This allowed to identify isolated signals suited for quantification through 1D ^1H quantitative experiments. The quantification of major lipids was performed with deconvolution using COLMAR1d at HF and LF. The same approach was applied to *LI* extracts, and the potential of LF flow NMR for monitoring is currently evaluated.

Complicated NMR Spectra

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NMR spectra contain a wealth of information, but in complex spectra the limited chemical shift dispersion and large number of resonances frequently lead to severe spectral crowding and peak overlap. This obscures individual signals and complicates both spectral and mixture analysis, making the separation of signals a continuing challenge to modern NMR spectroscopy.

Here we introduce **MIND (Multiple Independent NMR Dimensions)**, a class of multidimensional experiments that exploits independent NMR dimensions such as diffusion, chemical shift, and TOCSY t_1 -evolution to provide spin-system-specific signal modulation. Because all signals belonging to a given spin system share the same modulation behaviour in these dimensions, overlapping resonances can acquire distinguishable signatures.

After conventional processing, **PARAFAC decomposition** separates a MIND dataset into the individual spectra of the components, recovering conventional 1D spectra that cannot be directly resolved. The experiments are implemented using a modular “Lego-like” pulse sequence design via the **MIND Module Sorter** and analysed using **MINDGATE (Multiple Independent NMR Dimensions: General Analysis Tool for Evaluation)** within the **GNAT** environment for high-dimensionality NMR data.

Applications will be demonstrated for structural analysis, diffusion measurements, NOE experiments, and other multidimensional methods where signal overlap limits interpretation, illustrating how **MIND** disentangles complex spectra. Figure 1 depicts an example for the drug amikacin, where the 3.2–3.4 ppm region was selected to separate overlapping TOCSY network for each ring. A comparison with **GEMSTONE** will also be presented to highlight the conceptual differences of the two approaches for resolving spectral overlap in complex NMR data.

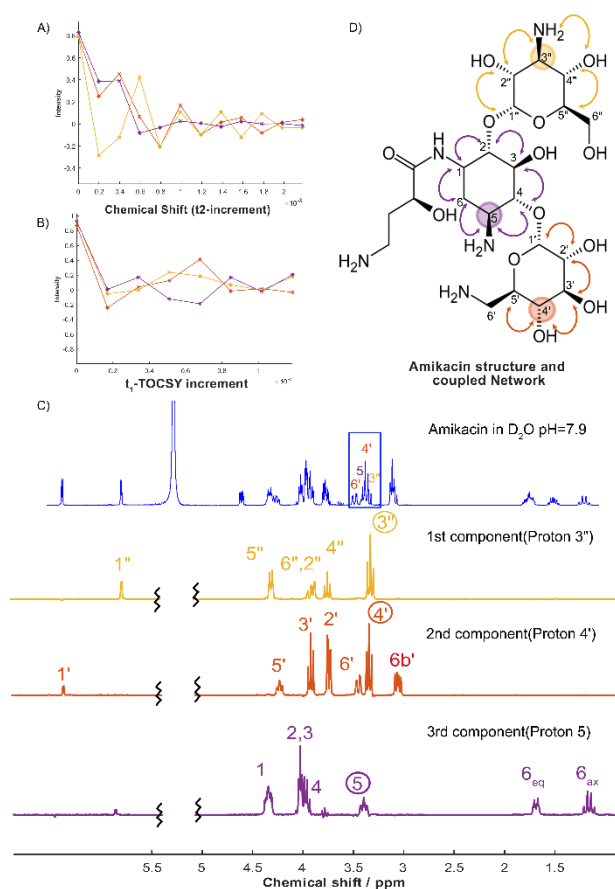
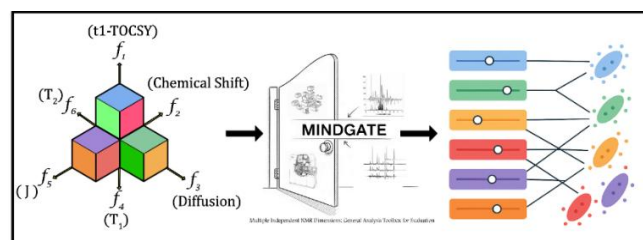


Figure 1: Demonstration of MIND framework using t_1 -TOCSY and chemical shift module to separate three distinct TOCSY network for all rings. Evolution of A) chemical shift B) t_1 -TOCSY dimensions distinguishes these components easily.

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

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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* ^1H - ^{13}C HSQC spectra. Here, the ^{13}C transverse relaxation rates, R_2 , of the four methyl lines, corresponding to protons in $\alpha\alpha\alpha$, $\alpha\alpha\beta$, $\alpha\beta\beta$ and $\beta\beta\beta$ 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.

The structure determination of organic molecules largely is based on unambiguously identified carbon connectivities. The HMBC experiment is the work horse for ^1H , ^{13}C -correlations, but in many cases the distinction of two- vs. three-bond correlations is essential for a reliable assignment, for which additional spectra are needed. H2BC and H2OBC pulse sequences are commonly used for defined two-bond correlations, but also HSQC-COSY-type experiments can be used for the task. Here we introduce fast pulsing and relayed HSQC-CLIPCOSY, resulting in spectra of particularly high quality and sensitivity, providing the desired information within minutes.

Molecules with few protons, however, require as a last resort INADEQUATE-type ^{13}C , ^{13}C -correlations. The experiment suffers from extremely low sensitivity and improvements of any kind are highly desirable. We present the 2D INADEQUATE-LBR as a pulse sequence with higher robustness and/or sensitivity compared to previously published ones.

Correlation Spectroscopy Across 300 kHz Bandwidths Using Chirps

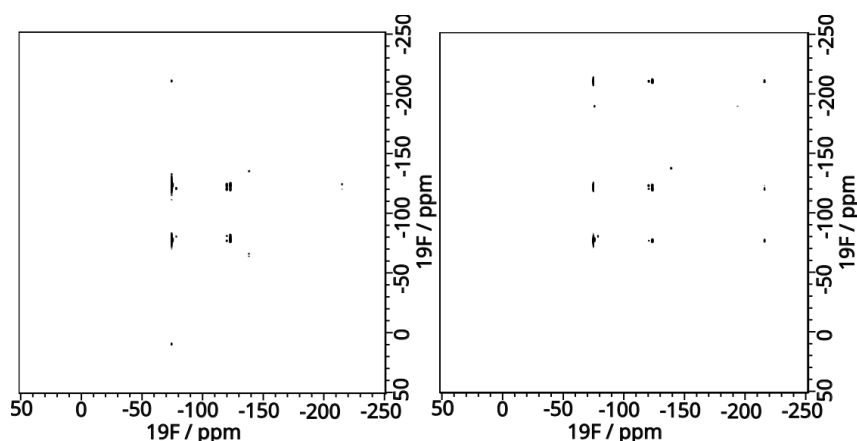
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Fluorine NMR is an area of growing importance in structural biology, drug discovery and environmental monitoring. ^{19}F is a sensitive nucleus which exhibits multiple through-space and through-bond heteronuclear and homonuclear couplings across a very large spectral width. Measuring ^{19}F — ^{19}F and ^{19}F — ^1H correlation spectra with conventional hard rectangular pulses requires higher radiofrequency power than is generally practical.

Instead, linearly frequency-swept pulses are able to achieve uniform transverse excitation across essentially arbitrary bandwidths, but at the cost of a complex phase profile in the final spectrum.[1] Wideband spectroscopy necessitates very short dwell times in the indirect dimension, and therefore additional delays are needed to enable coherence transfer. Such delays also complicate the final phase profile. Magnitude-mode processing presents an attractive and straightforward solution to the phase problem. In the context of ^{19}F COSY, the modest resolution penalty, as compared to phase sensitive processing, is not generally a problem due to the natural sparsity of fluorine spectra, and signal-to-noise is good due to the high γ of the ^{19}F nucleus.[2] In this work, homo and heteronuclear absolute-value COSY spectra across 300 kHz spectral widths are presented.



References

- [1] J.-M. Bohlen, M. Rey and G. Bodenhausen, *J. Magn. Reson.*, 84, 191–197 (1989).
- [2] W. R. Dolbier, *Guide to Fluorine NMR for Organic Chemists*, 2nd edn., 2016.

***in situ* NMR Monitoring of Heterogeneous Reactions**

Evans, Robert, Adams, Ralph

NMR spectroscopy can provide valuable insight into chemical reactions, including detailed information on reaction kinetics and mechanisms. While homogeneous reactions are readily studied by *in situ* NMR spectroscopy, studying heterogeneous reactions is significantly more challenging since the reaction now takes place at the interface of two, or more, phases. These reactions require well-controlled, efficient mixing to confirm that the observed kinetics are controlled by the reaction rather than by the mass transport between the phases.

Designing and building suitable stirring devices for use within standard NMR spectrometers is a notable challenge. A key restriction is the need to use only NMR-compatible materials, neither magnetic nor electrically conducting, due to the high magnetic fields involved. The tall, narrow shape of standard NMR tubes and the limited available space inside NMR instruments add additional complications, particularly in limiting the effectiveness of mixing. Mixing devices can be either plunger- or stirrer-based.

Our research team has recently developed a stirring device, compatible with most commercial NMR spectrometers. Powered either electrically or pneumatically, a glass shaft is rotated within a standard 5 mm NMR tube, which rapidly stirs the sample. Mixing speeds in excess of 20 kRPM have been achieved. This allows for biphasic reactions, both liquid-liquid and solid-liquid reactions to be effectively studied, with accurate kinetic data acquired.

T_{1ρ}-Enhanced Ultrafast Laplace NMR: A New Contrast Mechanism for Multidimensional Relaxation Analysis

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NMR relaxation and diffusion measurements provide versatile information about molecular dynamics in systems such as porous materials and biological tissues. Because the data consists of exponentially decaying components, processing requires an inverse Laplace transformation (ILT) to extract the distributions. Thus, these methods are referred to as Laplace NMR (LNMR).

Multidimensional approach further increases the information content by correlating different dynamic parameters. However, such experiments are often time-consuming because the data must be collected by repeated measurements with incremented evolution delays or gradient strengths. This drawback has been addressed using spatial encoding, originally introduced in ultrafast NMR spectroscopy and later in LNMR. These methods enable multidimensional acquisition in a single scan, reducing experiment times to seconds.

Rotating frame relaxation (T_{1ρ}) probes molecular motions in the micro- to millisecond range and is widely used to study slow dynamics, exchange, and biomolecular processes. Recently, we introduced the SPIn-lock CYcle (SPICY) to measure T_{1ρ} relaxation in a single scan by looping spin locking and detection similarly to a CPMG.

Here we focus on recent developments combining SPICY with other ultrafast LNMR methodologies. The approach enables rapid multidimensional experiments correlating T_{1ρ} with other dynamic parameters. Applications to porous model systems and biomaterials show how T_{1ρ} can resolve different environments and dynamical regimes in heterogeneous materials. Beyond biomaterials, T_{1ρ} relaxometry has gained interest in cementitious and porous materials research, complementing traditional T₂ and solid echo analyses while used in medical imaging as a sensitive probe of slow molecular motions.

Prethermalization in a tube of water: Dynamic phase transitions in steady-state NMR induced by radiation damping

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Prethermalization is a phenomenon whereby a periodically-disturbed system reaches a quasi-steady-state equilibration, distinct from its thermal equilibrium. We discuss a simple system supporting such behaviour: steady-state free precession (SSFP) NMR on a water sample. SSFP is commonly used on water –e.g. in cardiac MRI–where it has always been treated in the linear regime. As shown by Carr, the magnetization converges to a unique steady state determined by the pulse angle, the offset and the T_1/T_2 ratio. The interaction between water's transverse magnetization and the detection circuit—leading to radiation damping (RD) effects—introduces nonlinear feedback that can fundamentally modify such SSFP dynamics. It is shown theoretically and experimentally that in such cases two qualitatively distinct SSFP regimes may arise: a prethermal one dominated by RD, and the established one predicted by Carr. The transition between these two regimes corresponds to a dynamical bifurcation, with a sharp dependence on offset, flip angle and a strong dependence on repetition time that is absent in conventional SSFP treatments. The associated transition time increases asymptotically with respect to control (e.g., flip-angle) parameters, a hallmark of nonlinear systems. Consequences of such effects are briefly discussed.

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Unraveling Chemical Exchanges Through Steady State Free Precession NMR

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NMR line shapes are uniquely suited to probing molecular dynamics. Methods such as Chemical Exchange Saturation Transfer (CEST) and multipulse relaxation dispersion provide additional insight: CEST reveals slowly exchanging partners, including their number, chemical shifts, and populations, while relaxation dispersion yields exchange rates when these quantities are known. This study shows that Steady-State Free Precession (SSFP) experiments—based on pulse trains separated by repetition times (TR)—combine key advantages of both approaches. In the presence of chemical exchange, SSFP can report on the number, chemical shifts, and populations of exchanging sites, even for multiple intermediates with very different abundances. Through its TR dependence, SSFP can also provide kinetic information across slow, intermediate, and fast exchange regimes. This sensitivity arises from the intrinsic properties of SSFP, whose response depends strongly on chemical shifts and on the ratio between TR and competing decoherence processes (T_2 relaxation and exchange). Theory shows that chemical exchange markedly alters the SSFP response, enabling the extraction of exchange rates, chemical shifts, and site populations. Experimental validation and least-squares fitting procedures for parameter estimation are presented, and pseudo-3D implementations for probing “invisible” states in proteins and nucleic acids are discussed.

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Ultrafast Laplace NMR for probing molecular dynamics, aggregation, and microstructure across disciplines

Telkki, Ville-Veikko

NMR relaxation and diffusion measurements – collectively known as Laplace NMR – offer powerful, label free, and non-invasive access to molecular translational and rotational dynamics. When extended into multiple dimensions, these experiments reveal correlations between distinct motional modes and enable quantitative characterization of exchange, aggregation, and microstructural features in complex systems. Their versatility makes them valuable across chemistry, materials science, geoscience, biology, and industrial research.

This contribution presents recent advances that dramatically accelerate single- and multidimensional Laplace NMR using spatial encoding and related ultrafast methodologies. These single scan approaches not only reduce experiment times by orders of magnitude but also integrate seamlessly with modern hyperpolarization techniques, enabling sensitivity enhancements that were previously inaccessible. Because the methods rely on spin echo-based detection, they are inherently compatible with low field and portable NMR platforms.

We will highlight new applications spanning arctic mining tailings, aerosol–climate interactions, battery materials, biosensing, cellular metabolism, protein–ligand binding, lignocellulosic biomaterials, sustainable cement formulations, surfactant aggregation, and the characterization of rubber and tire materials – illustrating how ultrafast Laplace NMR opens new windows into molecular dynamics and microstructure in real world, multidisciplinary contexts.

Beyond the limits of solution NMR: A new ^1H edition scheme to overcome current size limitations

Cracco, Lorenzo; dos Santos Moreira, Jorge; Boisbouvier, Jérôme

Investigating very large protein assemblies using solution-state NMR spectroscopy is severely limited by poor spectral resolution and sensitivity, due to rapid transverse relaxation and spectral crowding. However, by exploiting the favourable relaxation properties of methyl groups in deuterated proteins, methyl-TROSY experiments, in combination with selective methyl labelling strategies, enabled significant progress in the NMR spectroscopy study of proteins and protein complexes ranging from several hundred kDa to 1 MDa. Nevertheless, despite these improvements, application has often been limited to symmetric complexes from (hyper-)thermophilic organisms. Application to large asymmetric complexes of human proteins is often hampered by overcrowded spectra and low sensitivity, necessitating the development of a new generation of NMR methodologies to overcome these challenges.

Based on a new concept in NMR spectroscopy, we have developed novel acquisition schemes and a new processing strategy that significantly reduce the ^1H signal linewidth for high-molecular-weight systems. This new approach enables the optimization of resolution and sensitivity, without compromising neither one nor the other. So far, this new methodology has enabled up to a 5-fold reduction in methyl ^1H linewidths in 2D and 3D NMR experiments for a 150 ns tumbling protein. Furthermore, the resolution gain is expected to increase with the molecular weight of the proteins under investigation, which highlights the importance of this new strategy in pushing the limits of NMR spectroscopy towards characterising protein complexes over 1 MDa.

InNMR: Direct *in situ* studies of transport phenomena enabled by an innovative NMR-tube insert

A novel discoid insert has been developed that can be inserted into a standard NMR tube using an associated plunger tool. By attaching any type of semi-permeable barrier to the bottom of the insert, the detection volume of the tube can be divided into two or more chambers, separated by a barrier of choice. Slice selective experiments can then be utilized to study a plethora of transport phenomena in real-time by NMR spectroscopy.

The technological solution is presented together with proof-of-concept for three different applications: permeability studies, diffusion-controlled pH titrations and diffusion-controlled protein-ligand titrations.

The insert technology unlocks new tools for scientists working in the fields of for example: drug discovery, organic chemistry, biochemistry, chemical biology, microbiology and electrochemistry, that will enable new types of experiments to be performed by NMR spectroscopy. We look forward with excitement to what the creativity of scientists in different research disciplines will be able to achieve with this new tool at their disposal.



NMR Spectroscopy for Systems Chemistry and Sustainable Biotechnology

Meier, Sebastian; Warthegau, Stefan; Maragani, Ramesh; Andersen, Emil Rysholt Hendrup; Jensen, Pernille Rose

We will discuss the use of a versatile NMR toolbox to contribute to urgent societal issues and rationally optimize sustainability. Throughout, NMR supports our systems chemistry philosophy by allowing us to approach chemical and biochemical reactions as versatile networks, which can be visualized, understood, and optimized by tailored NMR assays. Quantitative NMR, ultrahigh-resolution approaches, hyperpolarized dissolution dynamic nuclear polarization (dDNP), PREs and stable isotope tracking are used to decode the complex, interconnected reaction networks that underpin sustainable chemistry and biotechnology. A central theme in our work is the systems-level understanding of carbohydrate valorization and aqueous chemistry: using positional isotope labeling, we reveal hypercomplex pathways, through which useful products can be formed under mild conditions. Quantitative isotope tracking reveals product provenance and catalyst optimization strategies in abiotic reactions that strongly resemble metabolism and shed light on the beginning of life. The use of NMR to discover new, sustainable chemistries and to optimize the formation of new materials shows the value of NMR in approaching sustainability goals. dDNP-NMR, delivering signal enhancements exceeding 30,000-fold, is shown to uncover transient intermediates and mechanistic routes that remain invisible to conventional detection. In biological backgrounds, we develop dDNP for in-cell NMR to capture the dynamics of extended metabolic pathways in living systems. These studies show the versatility of whole cell catalysis and the enhancement of little known pathways by almost 1,000-fold through suitably designed substrate mixtures, while providing clues about the evolution of metabolism.

New methods to measure the homo- and heteronuclear couplings within methylene groups

Norzagaray, Elisa, Montgomery, James R. D., **Sinnaeve, Davy**

Scalar couplings (J) and residual dipolar couplings (RDCs) are indispensable for configurational and conformational analysis of organic compounds. The couplings within prochiral methylene moieties are attractive targets in this respect, as the two one-bond ^1H - ^{13}C and the two-bond ^1H - ^1H RDCs provide complementary orientational information. For optimal precision, couplings are best extracted individually as simple doublets, *i.e.*, in the absence of other splittings. This can be done using selective J-resolved experiments, such as SERF and PSYCHEDELIC for ^1H - ^1H couplings, or F_1 -coupled HSQC experiments and heteronuclear 2DJ experiments for ^1H - ^{13}C couplings. For methylene groups, however, such experiments often fail for multiple reasons. First, the geminal protons can be strongly coupled with overlapping multiplets, prohibiting the use of selective methods to let evolve just one of them. Second, broadband measurement of one-bond ^1H - ^{13}C couplings in the molecule in absence of homonuclear splittings requires the use of the BIRD element, but this breaks down for geminal ^1H - ^1H couplings.

We will present two new experiments to overcome these limitations. The first is 'Inverse PSYCHEDELIC', a modification of PSYCHEDELIC that can selectively deliver the geminal ^1H - ^1H coupling in case of strong coupling. The second is CYBORG, a modification of the BIRD element that does control geminal homonuclear coupling evolution in methylene groups, allowing for the design of heteronuclear J-resolved sequences for individual one-bond ^1H - ^{13}C couplings. Both experiments will be valuable tools for organic structure analysis.

Pure Shift 2D NMR with Water Suppression for Structural and Dynamic Analysis of Flexible Molecules.

El Hayek Mosbah¹, Xiaojing Wu³, Denis-Quanquin Sandrine², Roux Amandine⁴, Bertho Gildas¹, Dumont Elise³, Maury Olivier^{2*} and Giraud Nicolas^{1*}.

Achieving high-resolution proton NMR spectra in aqueous media remains a major challenge for structurally complex and conformationally flexible molecules, where homonuclear scalar couplings and limited chemical-shift dispersion lead to severe spectral congestion. Here we introduce two multidimensional pure-shift pulse sequences, TOCSY-presat-PSYCHE-Excitation Sculpting and NOESY-presat-PSYCHE-Excitation Sculpting, which combine broadband homonuclear decoupling with efficient dual solvent suppression. By integrating presaturation, a PSYCHE-based decoupling element, and excitation sculpting within a unified pulse-sequence architecture, these experiments enable ultrahigh-resolution two-dimensional correlation spectroscopy under biologically relevant aqueous conditions while preserving spectral purity and sensitivity. Application to the diamagnetic Y–Gadopiclenol complex allows the unambiguous discrimination and assignment of coexisting diastereoisomers that remain unresolved in conventional spectra, in agreement with DFT chemical-shift calculations. Extension to the flexible crystallophore LuXo4 further demonstrates that pure-shift EXSY experiments provide quantitative access to slow conformational exchange processes consistent with a ligand-arm rotational mechanism identified by DFT. Beyond resolution enhancement, this work establishes water-compatible two-dimensional pure-shift NMR as a general platform for simultaneously probing stereochemistry, structure, and dynamics in complex supramolecular and bioinorganic systems.

Spins stuck in traffic: the stop-and-go of pulsed FlowNMR

Yuan Gao, Catherine Lyall, John Lowe, and Ulrich Hintermair*

NMR spectroscopy is a powerful technique for reaction monitoring due to its high chemical specificity, quantitative nature, and non-invasive character. FlowNMR extends these capabilities to *operando* measurements under process-relevant conditions. However, NMR is inherently insensitive, and under continuous flow conditions this limitation can be further exacerbated by flow effects, particularly for slowly relaxing nuclei. In addition, advanced NMR experiments based on spatial encoding are compromised by spin movement during acquisition. Manual pausing of flow can mitigate these issues but is laborious and time-inefficient. We have developed a pulsed FlowNMR approach in which sample flow is repeatedly stopped and restarted in an automated fashion using precisely controlled displacement volumes and timing, synchronised with NMR acquisition through an electronic trigger (**Figure 1**).

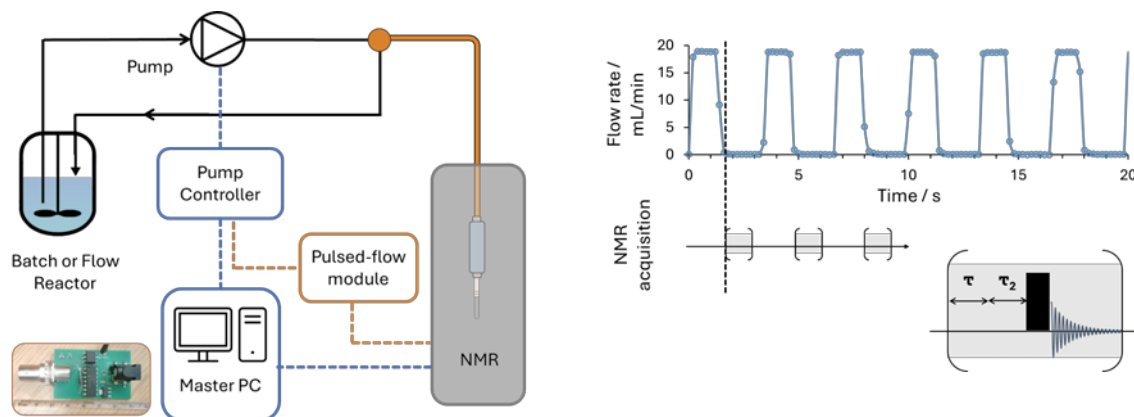


Figure 1: Left: schematic of a recirculating FlowNMR setup with a pulsed flow module. Right: example of a pulsed FlowNMR showing flow modulation and synchronised NMR acquisition.

This high degree of control allows tuning of in-flow and out-flow effects to maximise effective sensitivity (signal-to-noise per unit time, $S/N/t$) relative to conventional continuous flow operation and even static acquisitions that are limited by long relaxation delays. Preliminary experiments demonstrate excellent reproducibility and robust synchronisation between flow control and NMR acquisition. Pulsed FlowNMR also enables experiments that benefit from a static sample environment, including DOSY and ultrafast 2D NMR. It therefore provides a practical route to more sensitive, reproducible, and versatile reaction monitoring under pulsed flow conditions.

In situ monitoring of plasmon driven chemical reactions by NMR spectroscopy

Bier, Niklas, Srinivasalu, Hari H., von Chamier-Glisczinski, Sarah, Manglani-Dreßler, Jannes, Nediellkov, Ruslan, Pacholski, Claudia, Schmidt, Bernd, Lu, Yan and Möller, Heiko M.

Plasmonic excitation of noble metal nanostructures paves the way to new or more selective chemical pathways by harnessing excited electrons, electron holes, localized heat or electromagnetic fields for driving chemical reactions. Typically, plasmon driven reactions have been studied by surface-enhanced Raman spectroscopy (SERS) and UV-Vis spectroscopy taking advantage of their high sensitivity and easy application. Our goal is to widen our view and to unravel the dependence of photocatalytic properties such as reactivity and selectivity on nanostructure size, surface coating and morphology in bulk solution. To gain detailed insight, we are employing in-situ and flow NMR to monitor reactions on colloiddally dispersed and immobilized nanoparticles.

To characterize the catalytic properties of the applied nanostructures, we study model reactions such as the plasmonic degradation of methylene blue or the reduction of 4-nitrophenol via in situ real-time ^1H NMR spectroscopy. The irradiation of the samples is carried out in the NMR tube through fibre-coupled LEDs. Recently, we started looking into cross-coupling reactions such as Suzuki-Miyaura coupling and Heck coupling on Au@Pd core-shell nanoparticles. We could follow the Suzuki coupling of 4-iodotoluene and phenylboronic acid to 4-methylbiphenyl and the Heck coupling of iodobenzene and methyl acrylate to methyl cinnamate in situ. Under certain conditions we see an increase of the reaction rates by illuminating the sample with white light.

Future investigations will include the application gold nanoparticle-functionalized NMR tubes, the implementation of an NMR-coupled flow cell and the investigation of interactions of the reactants and products with the applied structures through saturation-based NMR techniques i.e. STD NMR.

Potential and Limitations of NOAH Super Sequences in Dynamic NMR Studies

Jasmin Hack and Robert Geitner.

NOAH (NMR by Ordered Acquisition using ^1H -detection) super sequences enable the combination of multiple 2D NMR experiments within a single pulse sequence. By efficiently using proton magnetisation, either acquisition time can be reduced or the signal-to-noise ratio (SNR) improved. This makes NOAH particularly suitable for in situ monitoring of dynamic reactions, allowing time-resolved 2D spectra.

The present study explores the application of NOAH super sequences for accelerated in situ analysis of dynamic reactions, focusing on data quality at varying acquisition times compared to standard 2D experiments. In particular, it investigates the influence of the ratio between the NOAH acquisition time and the reaction kinetics on possible signal distortions.

Reproducible imine formation from benzaldehyde and aniline serves as the model system. Reaction kinetics were tracked using NOAH sequences and ^1H NMR spectra, and the results were compared with data sets from classical 2D experiments. NOAH FID data are processed using a customised protocol. An R script automates the analysis of the spectra, evaluating the 2D spectra in the direct and indirect dimensions and determining parameters such as intensity maxima, FWHM and 95% signal limits.

Results show that reliable processing of NOAH data is possible even during an ongoing reaction. The aldehyde peak is narrower in the standard 2D measurement (0.02 vs. 0.05 ppm), while the imine peak has a width comparable to that in the NOAH measurement (0.17 ppm). The standard measurement has a higher number of scans (31,000 vs. 3,100), improving the signal-noise ratio by a factor of 10. The study is intended to provide a methodological basis for the use of NOAH sequences in complex, dynamic, and catalytic systems.

Contact-free magnetic resonance imaging and spectroscopy with acoustic levitation

Argyri, Smaragda-Maria, Leo Svenningsson, Feryal Guerroudj, Diana Bernin, Lars Evenäs & Romain Bordes

Conventional magnetic resonance studies often rely on the use of sample containers. This limits the implementation of time-resolved studies at the molecular level of liquid samples (*e.g.*, evaporation, interfacial phase transition, *etc.*), where having a large gas-liquid interface is central. Very few attempts have been performed on surface resting droplets, which however disrupt the homogeneity of the magnetic field, due to droplet asymmetry on the *xy*-plane. This shortcoming can be avoided, in an ideal case where the droplet is suspended in air. Acoustic levitation is a technique that employs ultrasonic transducers to generate an acoustic pressure field able to levitate small droplets (volume $\sim 2\text{--}10\ \mu\text{L}$). In this study, we developed a demagnetized acoustic levitator to perform magnetic resonance studies on liquid samples, in a contact-free manner. The performance of the levitator inside a 7.05 T magnetic field was examined and magnetic resonance images of the levitator and the levitated samples were acquired. Then, we collected magnetic resonance spectra of the levitated droplets by applying localized and non-localized pulse sequences and we examined the effect of the droplet shape on the chemical shift. Additionally, we conducted time-resolved experiments on pure solvents and mixtures, and captured physical and chemical molecular interactions, in real-time. This approach enables contact-free studies at the molecular level of dynamic phenomena on a microliter droplet using magnetic resonance techniques.

Investigation of Water-Soluble Polymers in Complex Water-Surfactant Matrices by ^1H NMR Spectroscopy and Diffusion Measurements

Eckhardt, Paul; Nunn, Christopher; Claussen, Jan

NMR spectroscopy can be used to understand post-consumer usage water-soluble polymer behaviour in complex matrices where other techniques such as light scattering or imaging might face limitations. This study investigates the structure of polyvinyl alcohol (PVA) and its interactions with surfactants in complex matrices expected during and after cleaning cycles in washing machines. A comparative analysis of hydrogels, original PVA films, and raw material resins was performed. Results show that the degree of hydrolysis remains unchanged, confirming the polymer's chemical structure is preserved during the washing process. Furthermore, diffusion measurements reveal no evidence of permanent aggregation or cross-linking. Reversible interactions between PVA and surfactants can be detected at high concentrations. In conclusion, PVA's fundamental macromolecular structure remains robust within these complex matrices, with observed polymer-surfactant interactions being reversible and concentration-dependent.

Monte Carlo-like peaks assignment using a time-resolved in situ NMR approach for complex reaction monitoring: a case study of the photodegradation of retinyl acetate

Farwa Khalid, Chunchesh Malangi Gajendramurthy, Franz F.

Westermair, Lena Miler, Ruth M. Gschwind, Tomasz Ratajczyk, **Mateusz Urbańczyk**

Photoactive molecules are of central interest due to their roles in light–matter interaction and in biological systems, and their applications in technology and medicine. Many important photoactive molecules undergo photodegradation, which is a complex, multi-path process involving many molecular species. One compelling case of photodecomposition involves retinyl acetate, which is necessary for vision processes. Here, we demonstrate a comprehensive approach in which the informativity of NMR can be harnessed for the in-depth investigation of the way in which photodegradation proceeds via a combination of two approaches: the interleaved time-resolved non-uniform sampling and time-resolved diffusion NMR. Specifically, the mass evolution, which was estimated from the first approach, was compared with the mass calculated from the diffusion coefficient estimated from the second approach, and the correlation between them was identified, enabling NMR signal assignment. This comparison is used for the Monte Carlo-like assignment, in which a vast library of potential reactants is scanned to determine the proper assignment. The presented approach is general-purpose and can be easily implemented for the investigation of many important photodegradation processes.

[1] F. Khalid, C.M. Gajendramurthy, F.F. Westermair, L. Miler, R.M. Gschwind, T. Ratajczyk, M. Urbańczyk, Monte Carlo-like peaks assignment using a time-resolved in situ NMR approach for complex reaction monitoring: a case study of the photodegradation of retinyl acetate, *Analyst* (2026). <https://doi.org/10.1039/D6AN00072J>.

Probing Self-Association of (+)-Catechin Coupled with Hydrogen-Deuterium Exchange by Solution NMR Spectroscopy

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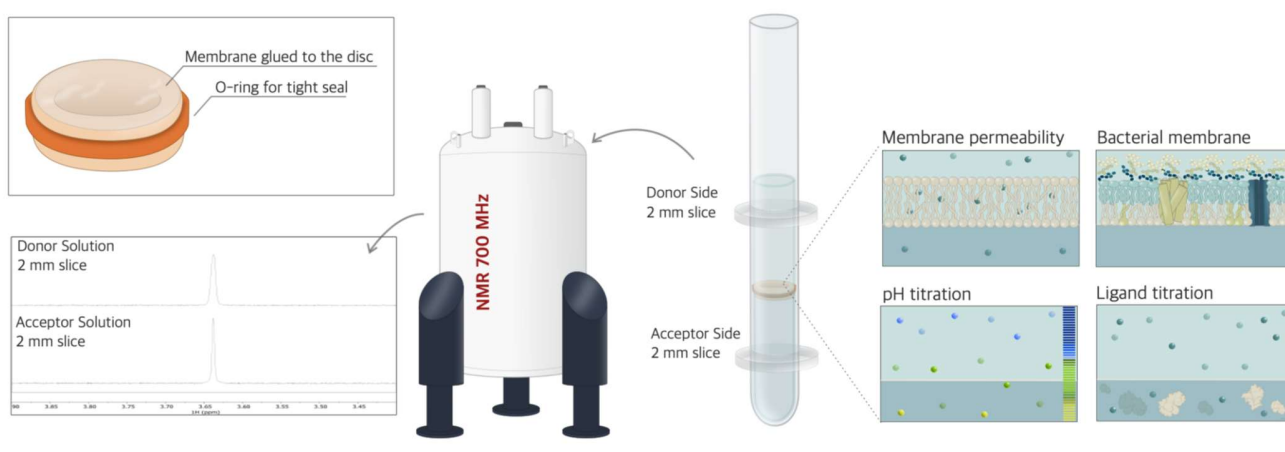
Hydrogen–deuterium exchange at aromatic carbon centers provides a sensitive probe of local electronic structure and transient chemical states, yet its dependence on intermolecular dynamics remains poorly understood. Here, we investigate the H/D exchange of (+)-catechin at the C6 and C8 positions using quantitative solution NMR, combining time-resolved ¹H signal attenuation, exchange-induced chemical shift perturbations (δ_{ex}), and Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion measurements. At low concentration, exchange kinetics follow a two-step pseudo-first-order mechanism consistent with independent site-specific deuteration. However, increasing catechin concentration results in a pronounced acceleration of exchange rates that cannot be explained by pD variations, indicating the involvement of intermolecular exchange processes. Analysis of concentration-dependent δ_{ex} and CPMG dispersion profiles reveals a weak monomer–dimer equilibrium in the millisecond timescale, operating in the intermediate-to-fast exchange regime on the NMR timescale. Global kinetic modeling demonstrates that the observed exchange kinetics arise from population-weighted contributions of monomeric and dimeric species, with the dimer exhibiting an enhancement of deuteration rates by up to two orders of magnitude relative to the monomer. This acceleration is consistent with stabilization of the reactive keto tautomer in the associated state. These results establish a direct connection between supramolecular exchange processes and chemical reactivity, and demonstrate that carbon-centered H/D exchange, when analyzed within an NMR exchange framework, provides a quantitative probe of transient self-association and its impact on reaction kinetics.

InNMR: Direct *in situ* studies of transport phenomena enabled by an innovative NMR-tube insert

A novel discoid insert has been developed that can be inserted into a standard NMR tube using an associated plunger tool. By attaching any type of semi-permeable barrier to the bottom of the insert, the detection volume of the tube can be divided into two or more chambers, separated by a barrier of choice. Slice selective experiments can then be utilized to study a plethora of transport phenomena in real-time by NMR spectroscopy.

The technological solution is presented together with proof-of-concept for three different applications: permeability studies, diffusion-controlled pH titrations and diffusion-controlled protein-ligand titrations.

The insert technology unlocks new tools for scientists working in the fields of for example: drug discovery, organic chemistry, biochemistry, chemical biology, microbiology and electrochemistry, that will enable new types of experiments to be performed by NMR spectroscopy. We look forward with excitement to what the creativity of scientists in different research disciplines will be able to achieve with this new tool at their disposal.



HSQC-*J*: Pure shift HSQC with retained coupling information

Davies Ellie, Morris Gareth A., Roy Soumya S., Adams Ralph W.

Analysis of ^1H NMR spectra is commonly hindered by extensive signal overlap due to the narrow chemical shift range and broad multiplet structures. Despite the inherently higher resolution afforded by multidimensional techniques, such as HSQC, issues of signal overlap can persist. Previous work has successfully mitigated this issue by employing pure shift methods to simplify the appearance of the direct dimension. However, in doing so, valuable coupling information is lost, making elucidation of molecular structure and conformation more difficult.

Here, we introduce a new 3D NMR experiment, HSQC-*J*, and apply it to a series of small molecules. By adding an incremented spin echo and BIRD (Bilinear Rotational Decoupling) element to a typical gradient-enhanced HSQC, we are able to encode spin-spin coupling information in an orthogonal spectral dimension. A subsequent 45° shear simultaneously aligns multiplet components in the *J*-dimension and collapses multiplet structure in the HSQC spectrum. This produces a pure shift resolution HSQC whilst retaining absorption mode ^1H multiplet structure in an additional dimension.

The technique presented has particular relevance in the study of mixtures, complex molecules and biomolecules, where signal overlap is most prevalent.

Ultrasensitive NMR Experiments in Flow

Harbor-Collins Harry, Davies Ellie, Kiraly Peter, Adams Ralph W.,
Morris Gareth A., Nilsson Mathias

The GEMSTONE family of ultrasensitive single-scan NMR experiments enables rapid extraction of individual multiplets from overlapped spectra, providing access to key structural information. The GEMSTONEs variant was developed to improve robustness to the deleterious effects of translational diffusion, but suffers from lineshape distortions and relaxation losses. The JND-GEMSTONE sequence (in review) overcomes these problems while preserving sensitivity.

In this work, the performance of the GEMSTONE pulse sequence family under flow conditions is investigated both theoretically and experimentally. Using a convecting sample, we demonstrate that the JND-GEMSTONE sequence offers the best overall performance in terms both of lineshape quality and of sensitivity. These results highlight the potential of ultrasensitive NMR experiments for applications in flow NMR.

Development of Novel 2D Longitudinal ^1H Relaxation NMR Methods for Probing Protein–Ligand Interactions

Farkas László Bence, Tardi Zsuzsanna, Gáti Tamás, Timári István

Nuclear Magnetic Resonance (NMR) Spectroscopy is an essential tool in modern drug discovery and plays a significant role in studying protein–ligand interactions at atomic level providing structure–activity relationships. Ligand-detected NMR methods enable the identification of ligand epitopes involved in binding, thereby allowing the determination of molecular regions that act a key role in the establishment of interaction, as well as those that can be structurally modified to improve binding affinity or other pharmacological properties. The T_1/R_1 -based 1D ^1H DIRECTION method developed by Mizukoshi et al. exploits differences in the relaxation rates of ligand proton signals, measured with and without selective irradiation of the protein, to map the ligand moieties involved in binding. One of the major limitations of this ^1H NMR based technique is the relatively low spectral resolution arising from the small chemical shift dispersion. Proton resonance signals often overlap, especially in larger or structurally similar molecules, which makes it difficult to accurately identify and quantitatively evaluate the ligand sites involved in the interaction. We have extended the basic 1D DIRECTION sequence with a 2D ^1H – ^1H CLIP-COSY relay experiment, as well as a homonuclear broadband proton-decoupled (“pure shift”) ^1H – ^{13}C HSQC module, enabling the determination of longitudinal ^1H relaxation times through correlation peaks with improved spectral resolution. The advantages and limitations of our methods developed will be demonstrated on two well-established and widely studied biological systems through epitope mapping of the interactions between *human* serum albumin (HSA) and (*S*)-ibuprofen, as well as *human* galectin-1 (*hGal*-1) and methyl- β -lactoside.

Double BIRD Filter for the Detection of ^{13}C -labeled Metabolites against a Background of Metabolites at Natural Abundance

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To gain insight into active cellular pathways, cells can be fed with ^{13}C -labeled glucose as part of metabolomic flux analyses. As the glucose is metabolized, metabolites further down the specific pathways become enriched in ^{13}C , which can then be detected and quantified by NMR spectroscopy. Of particular interest here could be the quantification of alanine, which plays a crucial role in anabolic and catabolic processes.

The quantification of ^{13}C -labeled metabolites by NMR can be challenging when there are large differences in signal intensity and signal overlap. For example, the methyl group signal of ^{13}C -labeled alanine is adjacent to an often intense lactate signal. This complicated the quantification of ^{13}C -labeled alanine at low concentrations in a conventional $^1\text{H}^{13}\text{C}$ -HSQC spectrum, regardless of whether it was recorded in 2D or 1D mode.

In need of a robust and highly sensitive pulse sequence for quantification of ^{13}C -labeled metabolites, we developed a new BIRD-based NMR experiment. Bilinear rotation elements can be used to detect protons directly coupled to ^{13}C and suppress those coupled to ^{12}C . A double BIRD^{d,X}-element with excitation sculpting proved most effective. Chirp pulses for ^{13}C provided a broader bandwidth and enhanced signal intensities. Selective inversion was achieved by matching the delays of the BIRD-element with the magnitude of the heteronuclear coupling constant $^1J_{\text{CH}}$. Since accurate selection of the $^1J_{\text{CH}}$ -value is crucial for optimal inversion of spins, a mean value had to be chosen out of the variety of coupling constants of the various metabolites. Decoupling was achieved with bilevel adiabatic decoupling.

The resulting experiment convinces with strong signal intensities, flat baseline and minimal peak overlap compared with a conventional $^1\text{H}^{13}\text{C}$ -HSQC pulse sequence.

Quantitative ^{13}C NMR analysis for technical lignin: sample preparation, acquisition parameters, spectra processing, and data validation

Liu, Liyang; Gu, Xunhong;

Technical lignin can be isolated from biomass through acidic, alkaline, or organic solvent-based processes. However, its heterogeneous chemical structure poses significant challenges to have reliable characterization methods. Quantitative ^{13}C NMR spectroscopy is a powerful tool for analyzing lignin macromolecules, enabling the assessment of more than 80 structural motifs, including hydroxyl groups, interunit linkages, and lignin subunits. Here, we investigate and optimize quantitative ^{13}C NMR analysis of technical lignin with respect to sample preparation, lignin type, acquisition conditions, and spectral processing. 800 MHz NMR is used to analyze organosolv hardwood lignin with emphasis on spectral sensitivity, signal-to-noise ratio (S/N), and accurate quantification of functional groups and lignin subunits. Increasing lignin concentration improved spectral quality and reduced experimental time for samples with good solubility, with S/N increasing across the tested concentration range. Good agreement was observed between the hydroxyl contents determined by ^{31}P NMR and ^{13}C NMR. A strong correlation was also found between total syringyl and guaiacyl unit content and methoxy group content. However, the internal standard signal decreased at higher lignin concentrations, indicating that direct signal-intensity-based quantification requires caution. Overall, reliable quantitative ^{13}C NMR analysis requires careful control of lignin concentration, molar mass, functional group composition, acquisition parameters, and spectral processing, with further optimization needed for chemically derivatized lignins.

Two-field ^1H - ^{19}F heteronuclear Overhauser spectroscopy

Licciardi, Giulia (1), Laníková, Alice (1,2), Siemons, Lucas (1), Vu, Duc Duy (1), Marcos, Emmanuel (1), Garibay, Jorge (3), Kast, Peter (4), Arthanari, Haribabu (5), Kay, Lewis E (6), Bouvignies, Guillaume (1), Pelupessy, Philippe (1), Ferrage, Fabien (1)

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Heteronuclear Overhauser Effect Spectroscopy (HOESY) is a powerful NMR method for probing intra- and intermolecular contacts, but the efficiency of ^1H - ^{19}F cross relaxation is reduced in large macromolecules. Here, we present two-field HOESY (2F-HOESY) in which the mixing time occurs at fields lower than the high-field (14.1 T, 600 MHz) where chemical shift evolution takes place. A fast sample shuttle transfers the sample between the magnetic center of the spectrometer and chosen positions in the stray field, where the spectral density at the difference of resonance frequencies approaches that at zero frequency: $J(\omega_H - \omega_F) \approx J(0)$, enhancing heteronuclear cross-relaxation. Combining low-field mixing with high-field detection improves efficiency, with no impact to sensitivity and resolution.

We implemented 2F ^1H - ^{19}F HOESY for the monomeric variant of the α subunit of the proteasome from *Thermoplasma acidophilum* labelled with ^{19}F -phenylalanine, in aqueous buffer and in 30% glycerol to mimic larger proteins with increased correlation times. 2F-HOESY build-up curves recorded from 0.8 to 14.1 T showed two- to threefold higher intensities when mixing occurred at low field. We acquired 2D 2F-HOESY correlations with optimal delays and magnetic fields, enabling the observation of cross-relaxation pathways.

2F-HOESY offers new opportunities for studying conformational equilibria and interactions in slowly tumbling systems, and a potential new strategy for assignment. Combining shuttle-based field-cycling NMR and selective ^{19}F labeling provides a versatile platform for probing biomolecular structure and dynamics over multiple timescales while preserving the high resolution and sensitivity required for biomolecular NMR spectroscopy.

Time saving qTROSY concatenation of 3D HNCX & HNCY experiment pairs

Diercks, Tammo; Orekhov, Vladislav

Queued TROSY (qTROSY) is a scheme to *specifically* recover and exploit the 50% anti-TROSY polarisation, discarded in conventional TROSY experiments, in an *immediately* appended second scan, thus saving every other long interscan delay ($d1$) required for *unspecific* polarisation recovery. Central to qTROSY is *orthogonal* spin state separation (oS^3) of the ^{15}N anti-TROSY *polarisation* ($\text{N}_z\text{H}^\alpha$) from exploited TROSY *coherence* (N_yH^β) by means of an inserted S^3E module prior to any frequency modulation. The final ST2-PT heteronuclear $\text{N} \rightarrow \text{H}$ transfer module used in TROSY experiments is *isotropic* and achieves spin state selective (S^3) transfer of both evolved $\text{N}_{xy}\text{H}^\beta$ TROSY coherence and retained $\text{N}_z\text{H}^\alpha$ anti-TROSY polarisation to their amide ^1H counterparts. Thus restored $\text{H}_z\text{N}^\alpha$ anti-TROSY polarisation largely survives the FID acquisition period and is then exploited in an *immediately* appended second TROSY scan where the initial $\text{H} \rightarrow \text{N}$ INEPT is replaced by a modified ST2-PT module for S^3 conversion into N_yH^β TROSY coherence.

While qTROSY has so far been used to concatenate identical 2D TROSY scans for sensitivity enhancement by simple addition, we here explore its potential for concatenating distinct 3D HNCX and HNCY experiments, as in $2 \times 3\text{D}$ HNCA/HN[CO]CA qTROSY. This affords a $[2\tau_{\text{scan}} - d1]/[2\tau_{\text{scan}}]$ time saving relative to recording two conventional TROSY scans of total duration τ_{scan} each, and allows to record such pairs of complementary assignment experiments simultaneously, thus, under identical conditions (sample state, temperature etc.) for best spectral comparability. By recording the inherently more sensitive experiment in the appended scan, incomplete anti-TROSY polarisation recovery in qTROSY may be well compensated for, especially when smartly distributing the reciprocal enhancement by natural and γ_{H} -hyperpolarised ^{15}N polarisations. Finally, losses from pulse imperfections, which add up especially for the appended qTROSY scan, may be largely avoided by use of optimised pulse shapes.

Multilinear analysis of DOSY spectra across time and concentration dissects transient oligomer formation.

Anders Malmendal & Kristine Steen Jensen

Macromolecular self-assembly processes often involve the transient formation and disappearance of species of different size, posing a general challenge for mechanistic characterization. The formation of transient oligomers during fibril formation of human superoxide dismutase 1 (SOD1) in amyotrophic lateral sclerosis is one such case.

Using translational diffusion NMR combined with multilinear analysis, we previously resolved loss of the native monomer, the emergence of a partially unfolded intermediate with diffusion coefficients consistent with a trimer or tetramer, and the formation of a distinct, similarly sized species that persisted to the end of the experiment together with the build-up of amyloid fibrils.

Here, we extend this work across 0.5–2 mM SOD1, incorporate chemical-shift assignments of the folded and unfolded protein, and refine the extraction and interpretation of time-dependent amplitudes and peak contributions.

Across concentrations, the behavior is broadly consistent, except a substantially larger intermediate that appears at 2 mM and persists throughout. Kinetics are concentration-dependent, with faster formation and decay of the intermediate at lower protein levels. Diffusion constants decrease slightly with increasing concentration, consistent with higher viscosity or collision rates.

Signals from unfolded regions are enhanced relative to the folded core in the intermediate, and further in the final species. Except for the intermediate at 2 mM, relative intensities are similar across concentrations.

More broadly, this approach enables *resolution and overview* of time-evolving macromolecular ensembles spanning multiple size regimes, offering mechanistic insight into complex processes that are difficult to access by other methods.

Low field/Benchtop

Beyond Imaging: Benchtop NMR Spectroscopy of Intracranial Hemorrhages.

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Cerebrovascular diseases are the second leading cause of death worldwide. Diagnosis relies mainly on neuroimaging techniques. While these methods detect structural alterations, they provide limited biochemical information and may restrict early diagnostic and prognostic insights. NMR-based metabolomics has emerged as a promising strategy to identify metabolic signatures associated with cerebrovascular events. However, its clinical adoption is limited by the cost and infrastructure requirements of High-resolution (HR) NMR systems. Benchtop NMR (bNMR) could enable the translation of metabolomics into real clinical environments. This study aimed to identify metabolic biomarkers capable of discriminating different types of intracranial hemorrhage and to evaluate the feasibility of translating this approach to clinical settings using benchtop NMR.

A total of 90 patients admitted to the ICU of Getafe University Hospital and 44 healthy subjects were enrolled. Serum samples were analyzed using both HR Bruker AVIII 500 MHz and benchtop Magritek Spinsolve 80 MHz.

Partial Least Squares Discriminant Analysis (PLS-DA) models were developed for three scenarios: (i) healthy controls vs intracranial hemorrhage, (ii) intraparenchymal vs subarachnoid hemorrhage (SAH), and (iii) aneurysmal vs non-aneurysmal SAH. Both spectrometers achieved perfect discrimination between controls and hemorrhage patients based

on 15 biomarkers. Hemorrhage localization reached accuracies of 85% with HR-NMR and 68% with bNMR, while aneurysmal vs non-aneurysmal SAH discrimination reached 72% and 70%, respectively.

These findings highlight the potential of NMR-based metabolomics for the diagnosis of cerebrovascular hemorrhages and the feasibility of translating this strategy to clinical environments using bNMR.

Water binding in dairy matrices

Pérez Mendigorri, Maria, Afrough, Armin, Vosegaard, Thomas

Low field NMR (LF-NMR) relaxation techniques have increased in popularity across food science over the past decades. The resulting data is typically converted into relaxation time distributions using multi-exponential analysis methods based on the inverse Laplace transformation. The assignment of these distributions can be often complex, particularly in heterogeneous systems where each environment can contribute to more than one relaxation distribution. As a result, there is a common conviction in the field that the interpretation of the relaxation time distributions is difficult and not always correct.

LF-NMR relaxation time distributions are particularly powerful tools for dairy gels, where relaxation can give direct insights about the sample microstructure and can help address quality related challenges as textural instability. A clear example is syneresis which is defined as the spontaneous expulsion of whey during time. This phenomenon is considered a technological defect that results in undesirable sensory characteristics, making the product less appealing to consumers. At an industrial scale, the most common solution is using additives that increase the viscosity of the aqueous phase. However, there is currently no standardized methods that assess syneresis, complicating comparison across studies. Moreover, most approaches measure direct bulk water, not its dynamics.

We show that LF-NMR relaxation is a promising analytical approach for understanding and quantifying syneresis and present our efforts in developing an NMR-based toolbox that enables the translation of NMR relaxation parameters into meaningful food science descriptors, bridging the gap between magnetic resonance data and functional properties relevant to food systems.

Probing Molecular Motions with Field-Cycling Relaxometry: From Viscous Liquids to Advanced MRI Contrast Agents

Parigi, Giacomo

Field-cycling relaxometry represents a powerful methodology for investigating molecular dynamics in complex systems, providing direct access to spectral density functions across a frequency range spanning over four orders of magnitude. This methodology is uniquely suited to probe the multiscale mobility of diverse systems, ranging from complex viscous liquids, such as olive oil, to high-efficiency MRI contrast agents.

Fast field-cycling relaxometry (0.01–40 MHz) and high-resolution measurements acquired using an advanced sample shuttle system installed on a 600 MHz spectrometer were performed to determine the field dependence of the longitudinal relaxation rates of individual hydrogen atoms in olive oil. The analysis of the relaxation profiles revealed mobility on timescales from microseconds to tens of picoseconds, providing insights into the site-specific dynamics.

Relaxometry is extensively employed to characterize paramagnetic systems based on gadolinium(III) complexes. A primary strategy to reduce clinical dosages and associated risks focuses on increasing relaxivity by restricting reorientation dynamics. Significant enhancements were achieved by conjugating Gd(III) complexes to nanogels or large protein assemblies, such as asparaginase or transthyretin. These systems exhibit relaxivities up to six times higher than standard clinical agents, primarily due to the optimized correlation times, thus emerging as promising theranostic platforms. Enzymatic activation (bio-responsiveness) has also proven effective in modulating relaxivity by triggering changes in molecular dynamics. This capability makes these platforms ideal for the non-invasive detection of cancer-specific biomarkers and the real-time monitoring of tumour progression or response to therapy.

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

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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.

NMR-Based Metabolomics and Lipidomics for Eosinophilic Esophagitis Diagnosis: Clinical Translation Using Benchtop NMR.

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Eosinophilic esophagitis (EoE) is a chronic inflammatory disease that currently requires invasive endoscopy and biopsy for diagnosis. NMR-based metabolomics represents a promising non-invasive alternative capable of identifying disease-associated metabolic signatures. However, the clinical implementation of metabolomics has been limited by the cost and infrastructure requirements of high-field NMR systems. The emergence of benchtop NMR (bNMR) spectrometers offers a potential route to translate metabolomics into routine clinical practice.

The aim of this study was to identify metabolomic and lipidomic biomarkers associated with EoE and to evaluate the feasibility of benchtop NMR for clinical diagnosis.

40 EoE patients and 28 healthy controls were recruited at Tomelloso General Hospital. Serum samples were analyzed using high-resolution NMR (Bruker AVIII 500 MHz) and two benchtop spectrometers (Magritek Spinsolve Ultra 80 MHz and Bruker Fourier80).

Partial Least Squares Discriminant Analysis (PLS-DA) enabled classification between EoE patients and controls with an accuracy of 86.3% using HR-NMR and above 70% with both benchtop spectrometers. AUC-ROC values were 0.83 for HR-NMR and 0.74 and 0.64 for Spinsolve and Fourier bNMR systems.

Lipidomic analysis using Fourier80 identified nine significant lipoprotein parameters with an AUC-ROC of 0.712. Additionally, metabolomic profiling discriminated EoE patients according to the presence or absence of esophageal eosinophilic infiltration, reaching up to 95% accuracy with the Fourier80.

These results highlight the potential of NMR-based metabolomics as a diagnostic approach for EoE and demonstrate the feasibility of translating this strategy to clinical environments using benchtop NMR.

Compact Magnets for Portable Magnetic Resonance Applications

Alnajjar, Belal, Bernhard Blümich, Jens Anders

The miniaturization of magnetic resonance (MR) systems is essential to expand the use of NMR and ESR in portable, cost-effective sensing applications. A primary challenge in scaling down these systems is maintaining high magnetic field homogeneity while reducing the weight and footprint of the magnet. This work presents a comprehensive framework for the design, optimization, and realization of compact magnets tailored to MR applications, including ESR, NMR spectroscopy, relaxometry, and second-generation quantum sensing. The framework integrates advanced manufacturing techniques, numerical optimization, and machine learning.

We introduce 3D printing as a versatile approach for fabricating passive shimming structures and key magnet components using steel-particle composite filaments. This enables rapid prototyping of complex geometries and a weight reduction of up to 60% compared to conventional iron-based designs, while maintaining competitive performance. Using numerical size and free-form shape optimization based on non-uniform rational B-splines, customized shim structures are realized, achieving NMR spectral linewidths down to 54 ppm. In addition, a compact single-sided stray-field NMR sensor is developed and optimized. The system integrates a tailored RF coil design, genetic-algorithm-based magnet shimming, and precise 3D-printed alignment structures, enabling high-resolution relaxometry with a spatial resolution of 50 μm and dead times below 20 μs . Measurements on heterogeneous materials demonstrate the robustness and versatility of the approach.

Furthermore, miniaturized Halbach-like permanent magnets and 3D-printed electromagnets are designed for NV-based magnetometry, addressing mechanical and material tolerances to ensure stable and homogeneous fields at the millimeter scale. Finally, deep learning models combined with finite element simulations are employed to accelerate magnet design, enabling the realization of compact magnets ($\sim 9\text{ mm} \times 9\text{ mm} \times 9\text{ mm}$) with a field homogeneity of 200 ppm.

The presented approaches establish a scalable pathway toward lightweight, cost-effective, and high-performance magnet systems, opening new opportunities for portable MR and quantum sensing technologies.

Coupling Benchtop NMR and EPR for Studying Redox Flow Batteries in Operando

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Abstract Text:

Redox flow batteries (RFBs) are a promising solution for grid-scale energy storage, due to their scalability and flexibility. While high-field *operando* nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) analyses have provided valuable insights into RFB processes, the high cost has limited their availability to the broader electrochemistry community. Here, we present a novel benchtop NMR and EPR method to study multiple phenomena observed in RFBs.

First, we evaluated the performance of a new class of ion-selective fluorine-free membranes, using ⁷Li-NMR to investigate their role in facilitating Li⁺ transport as a supporting electrolyte. We show that Li⁺ can be transported through the membranes. By integrating the intensity of the observed resonance, we could quantify the concentration of Li⁺ in solution directly. Furthermore, we applied the membranes in a symmetric battery to study the electromigration of Li⁺. Changes in chemical shift and peak intensity in the NMR spectra provided insights about the state of charge of the battery via the Evans method. The Li⁺ concentration was monitored and quantified as a function of the charge and discharge cycles.

Next, we coupled benchtop NMR-EPR instrumentation, which serves as complementary techniques for studying both diamagnetic and paramagnetic species generated inline in *operating* electrochemical systems. We tested our dual methodology by applying it to the well-known methyl viologen (MV²⁺)/TEMPOL redox-active pair. Our novel setup enabled simultaneous monitoring of MV²⁺ by studying the ¹H-NMR during charge and discharge cycles, while parallelly detecting the generation and consumption of MV^{•+} radical cations via EPR. The electronic structure and concentration of MV^{•+} radicals were quantified.

Our results highlight the molecular-level insights that the coupled benchtop NMR-EPR technique can offer. This method offers a general and accessible tool for exploring diverse redox chemistries for flow battery applications and many other electrochemical systems that involve diamagnetic and paramagnetic redox-active species.

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Broadband Benchtop NMR Spectroscopy for Research and Industrial Applications

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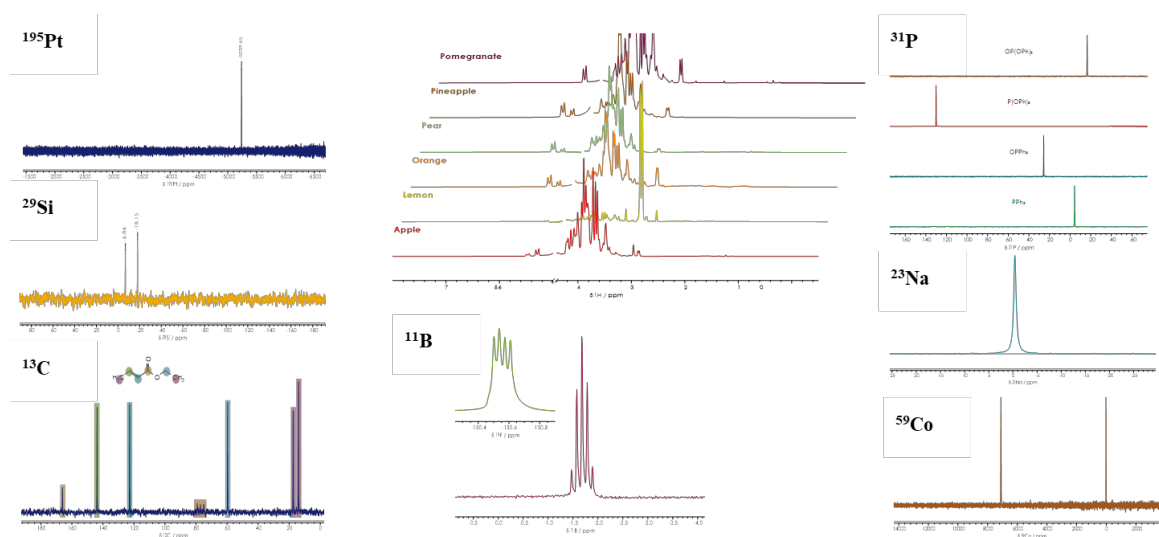
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The X-Pulse 90 is a benchtop NMR spectrometer built around a 90 MHz permanent magnet. It combines advanced features typically associated with high-field systems with the simplicity and user-friendly design of a compact instrument. With full broadband capability, adjustable temperature control, and automated operation, the X-Pulse provides both flexibility and precision across a wide range of NMR applications.

In this work, we demonstrate the performance of the X-Pulse system in several analytical contexts, including the suppression of undesired signals using a range of solvent suppression sequences. This capability is illustrated using different batches of fruit juice samples prepared in buffer solution, in which the dominant water signal is effectively suppressed. As a result, signal overlap is reduced, enabling improved quantification of key components such as organic acids and sugars. The suppression sequences can also be extended to target multiple residual signals, offering enhanced flexibility for diverse analytical challenges.

In addition, the X-Pulse is well-suited for real-time reaction monitoring due to its fully tuneable broadband capability, making it applicable across a wide variety of reaction types. When combined with flowNMR and an external lock, the system enables the acquisition of quantitative kinetic and mechanistic data, providing insight into reaction pathways and catalytic efficiency. Furthermore, the instrument operates over a broad temperature range (0–60°C), allowing investigation of dynamic processes and the determination of thermodynamic and kinetic parameters.



Proving the Power of Benchtop NMR with Advanced Experiments on the Fourier 80

Sakas, Justinas; Beaton, Alec; Ferrer, Maria-Jose; Goerling, Benjamin; Haber, Agnes

Benchtop NMR is making NMR spectroscopy more accessible to an increasing number of users by removing the need for dedicated rooms, cryogen-cooled magnets, and even deuterated solvents. However, the lower field strength of benchtop systems introduces its own challenges, most notably reduced spectral dispersion and sensitivity compared to floor-standing spectrometers.

In this poster, we demonstrate that the Fourier 80 can easily run advanced experiments originally developed for high-field systems available in the TopSpin library. We use these methods to directly address the aforementioned spectroscopic challenges at 80 MHz.

Selective experiments such as 1D selective TOCSY can be used to investigate a particular resonance and its corresponding scalar-coupled network. The use of sophisticated shaped pulses in GEMSTONE, and DREAMTIME enables the isolation of individual compounds from a mixture of overlapping metabolites. These experiments can be set up intuitively with a few clicks on the Fourier 80.

The SHARPER experiment is employed to achieve sensitivity gains by removing homo- and heteronuclear coupling from a select signal, with decoupling possible even for nuclei without a dedicated hardware channel. A more recent variant of SHARPER is used to obtain substantial sensitivity gains by collapsing entire spectra into a single sharp singlet. Using this approach, DOSY spectra at sub-millimolar concentrations can be acquired in just 8 minutes at 80 MHz.

Finally, NOAH supersequences with NUS are easily implemented, demonstrating that impressive time-saving strategies commonly applied in high-field NMR perform equally well at 80 MHz. All experiments presented are fully compatible with automation and can be acquired hands-off by less experienced users.

Investigation of Molecular Relaxation Times in Ripening Cheeses by Fast Field-Cycling NMR Technique

Zalewski, Tomasz, Markiewicz, Roksana, Ferrante, Gianni

Cheese ripening is a complex biochemical process involving continuous structural and molecular transformations that strongly influence its texture, flavour, and water mobility. Understanding this molecular dynamics is essential for controlling product quality and optimizing ripening conditions. In this study, molecular relaxation behaviour in ripening cheeses was investigated using NMR Relaxometry with a fast field-cycling (FFC) approach.

Relaxation measurements were performed at different locations within the cheese matrix to evaluate changes in proton mobility and molecular interactions as a function of distance from the surface. The longitudinal relaxation times (T_1) were analyzed as a function of magnetic field strength, providing information about water distribution, fat-protein interactions, and molecular dynamics occurring during ripening. Variations in relaxation dispersion profiles were associated with progressive changes of the cheese structure, including protein network reorganization and moisture redistribution.

The results demonstrate that FFC-NMR relaxometry is a sensitive and non-destructive method for monitoring molecular-level changes during cheese ripening. The technique enables characterization of dynamic processes that are difficult to detect using conventional analytical methods and may provide valuable indicators for assessing ripening stage and product quality. This work highlights the potential of fast field-cycling NMR as a valuable analytical tool in dairy science and food quality research.

Acknowledgements

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magnETHical: Democratizing NMR with an open-source low-field spectrometer

Maximilian Stabel, , Takuya Segawa, Sebastian Kozerke, Roland Riek

High-field NMR spectroscopy remains inaccessible to much of the global scientific community due to prohibitive costs and cryogenic liquid requirements. To address this disparity, we developed "magnETHical," an open-source, cryogen-free 25 MHz ^1H NMR spectrometer with a construction cost of approximately \$10,000. Unlike closed-source commercial benchtop systems, our platform is designed for modularity and repairability, utilizing off-the-shelf components, 3D printed parts, and Software-Defined Radio (SDR) architecture.

The hardware centerpieces are a 0.6 T permanent dipole magnet and a RedPitaya SDRlab 122-16 console, controlled via a Python-based interface. A critical challenge in low-field instrumentation is achieving sufficient field homogeneity for chemical shift resolution. By implementing a custom shim driver and active shimming protocols, we achieved a spectral resolution of less than 1 Hz (<0.04 ppm) linewidth for water in standard 5 mm tubes. This performance allows for the clear observation of scalar J-couplings in Ethanol in a single scan.

The flexible SDR front-end allows for multi-purpose use and future innovation, including photochemically induced dynamic nuclear polarization (photo-CIDNP) and Nuclear Quadrupole Resonance (NQR) experiments at frequencies that are currently commercially unavailable. This will allow us to compensate for the inherent sensitivity limits for specific radical reactions. By releasing the full hardware and software specifications, we aim to foster a global community of developers. This platform enables affordable chemical analysis and education, bridging the gap between high-end research and local infrastructure needs in the Global South.

TD-NMR Analysis of Phase Composition, Mobility, and Domain Size in Semicrystalline Polymers

Baranauskaite Valeria, Ivanova Maria, Erkki Lähderanta, Grunin Leonid

This work presents a TD-NMR approach to characterization of the phase composition, segmental mobility, and nanoscale morphology in commodity polymers, such as polyethylene, polypropylene, and polystyrene. The semicrystalline polymers have heterogeneous structures with rigid crystalline domains embedded in amorphous phases of varying mobility. These structural differences are reflected in distinct dipolar interactions and relaxation behaviors, what makes them the perfect object for TD-NMR methods.

Free induction decay and solid echo experiments were used to measure phase fractions. The apparent transverse relaxation time (T_2^*) and the second moment analysis were correlated with crystallinity with the model-free approach. Double quantum–Multiple quantum (DQ–MQ) NMR experiments allowed to estimate residual dipolar couplings distributions (D_{res}), to quantify structural heterogeneity. The MQ signal was used to estimate highly mobile defect fractions. The novel methodology to recover the MQ overall relaxation curve from the DQ build up data was proposed and tested on the wide set of various polymer samples.

To investigate nanoscale morphology, Goldman–Shen spin diffusion experiments were combined with DQ data. The spin diffusion coefficients and characteristic domain sizes were determined without relying on spectral deconvolution. The results demonstrate that softer amorphous domains and interfacial regions can be effectively detected through magnetization transfer dynamics.

Overall, the combined TD-NMR methodology provides a model-free approach for simultaneous determination of crystallinity, molecular mobility, and domain structure. The findings show the capability of low-field TD-NMR to extract detailed structural and dynamic information in semicrystalline polymers.

Structural Evolution of β -Cyclodextrin based Nanosponges during drug release: An insight from multifield NMR analysis

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β -Cyclodextrin-based nanosponges (β -CDNS) have attracted considerable attention owing to their wide-ranging applications in food technology, wastewater treatment, and, most notably, drug delivery systems[1]. The present study utilizes an array of multifield NMR techniques to comprehensively characterize the key pore parameters and evaluate the effect of drug loading–release of 5-fluorouracil for a set of three synthesized β -CDNS. ^1H NMR Restricted diffusion and ^1H transverse relaxation measurements at high field (11.7T) have been employed to obtain average pore size across macroporous scale, porosity, tortuosity (τ) and adsorption capacity of the nanosponges[2]. On the other hand, NMR cryoporometry performed at low field ($\sim 0.5\text{T}$) using ^1H transverse relaxation measurement of water as a probe liquid has unveiled the porosity, pore size distribution in microporous regime ($1 \leq \mu\text{m}$)[3] and evolution of pore structure of all three different β -CDNS during drug release. A Loading of 5-FU in to these nanosponges is confirmed using ^{19}F solid-state NMR spectroscopy complemented with a selected set of analytical techniques. Subsequently, drug encapsulation efficiency and release profiles are quantified using high-field ^{19}F NMR spectroscopy.

Diphenyl carbonate (DPC) and epichlorohydrin (EPI) are used as cross-linking agents at varying molar ratios (DPC at 1:2 and 1:4, EPI at 1:15) for synthesis of β -CDNS with variable pore networks. NMR data analysis demonstrates that β -CDNS possesses a tunable porous architecture and undergoes significant structural modifications during synthesis. Key pore parameters have shown variation with the choice of crosslinking agent. DPC-based nanosponge exhibits larger pores and a more tortuous network, whereas the EPI-based nanosponge shows enhanced microporosity compared to the DPC system. Further, the effect of drug loading on the porous architecture of nanosponges is assessed using low field cryoporometry that clearly indicates significant changes in pore size of the nanosponges after release of 5-FU. Throughout the drug loading-release analysis, the dynamic evolution of the pore structure is monitored to capture any structural changes occurring during these processes,

thereby providing a comprehensive understanding of the relationship between pore architecture and functional performance in β -CDNS materials.

Keywords: Multifield NMR, Restricted diffusion, Porous materials, beta-Cyclodextrin

Exploring T_1 -based contrast upon protein-ligand interaction in Earth's magnetic field

Felicia Cilenov, Vineeth Francis Thalakkotloor Jose Chacko, Nicola Salvi, Kirill F. Sheberstov

Drug screening plays a crucial role in early drug discovery, particularly for identifying protein-ligand interactions. Among other analytical techniques, nuclear magnetic resonance (NMR) stands out for its non-invasive nature and ability to operate under near-physiological conditions. Conventional ligand-observed NMR screening methods rely primarily on transverse relaxation (T_2) measurements due to their simplicity and sensitivity to binding events. On the other hand, longitudinal relaxation (T_1) measurements at high fields typically offer poor contrast and are rarely used in binding studies. In this study, we explore the T_1 contrast achieved at Earth's field and compare it to the contrast obtained by standard T_2 -based methods. We use an experimental setup combining a 60 MHz benchtop NMR spectrometer with a robotic arm to perform mechanical field-cycling experiments. The sample is initially polarized at 1.4 T, then physically transported to Earth's magnetic field, where longitudinal relaxation occurs, before returning to the spectrometer for detection. Using the model system trimethylsilylpropanesulfonate (DSS) and bovine serum albumin (BSA), we performed iterative titration experiments and compared relaxation-based contrast across different concentrations of BSA. At the highest protein concentration (10 μ M BSA), low-field T_1 measurements achieved ~90% contrast, significantly outperforming T_2 measurements (~60% contrast) and T_1 measurements at 1.4 T. Therefore, our automated, low-cost approach offers a practical alternative to expensive high-field NMR, making sensitive protein-ligand screening accessible to academic laboratories and early-stage drug discovery research projects.

Materials NMR

Probing the dynamics of polymers at the surface of cosmetic pigments using solid-state NMR spectroscopy

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Since 2017, regulatory actions at the EU level have been implemented to limit the use of microplastics due to growing concerns about their environmental impact. All cosmetic pigments containing solid polymers covering their entire surface will be considered as microplastics according to the definition added to REACH, and will be banned from manufacture and sale from 2035. A derogation is however possible for non-solid polymers.

In this context, we investigated how the mobility of polymers at the surface of pigments can be probed using solid-state NMR spectroscopy and linked to the liquid or solid state of the polymers. A combination of ¹H, ¹³C and ²⁹Si solid-state NMR experiments were used to investigate atomic-scale dynamics of polymers. In particular, millisecond-scale motions were probed through the measurement of ¹H transverse relaxation times and the comparison between NMR spectra of ¹³C and ²⁹Si isotopes acquired using direct acquisition, CPMAS and refocused *J*-INEPT. A signal is observed for rigid segments in CPMAS spectra based on ¹H→¹³C and ¹H→²⁹Si polarization transfers *via* dipolar interactions, whereas *J*-based refocused INEPT experiments only yield signals for mobile species.

The validity of the approach was first tested on pigments treated with linear alkanes with different chain lengths and melting points (T_m), including hexadecane (C₁₆H₃₄ with T_m = 18 °C), eicosane (C₂₀H₄₂ with T_m = 37 °C) and triacontane (C₃₀H₆₂ with T_m = 66 °C). It was then applied to gain unique insights into the atomic-level dynamics of silane compounds, including triethoxy(octyl)silane, polydimethylsiloxane and polymethylhydrosiloxane, near the surface of cosmetic pigments. These NMR results will be useful to identify cosmetic pigments which should be classified as microplastics.

In-Situ and *Ex-Situ* NMR Reveal Temperature-Dependent Degradation mechanisms in Organic Redox Flow Battery Electrolytes

Singh K. and Grey C. P

Aqueous organic redox flow batteries (AORFBs) are promising for grid-scale energy storage, but performance is limited by temperature-dependent electrolyte degradation of redox-active species. Temperature-dependent chemical transformations are difficult to resolve without real-time characterization under operating conditions.

Here, we demonstrate that *in-situ* and *ex-situ* 1D and 2D NMR spectroscopy, with systematic sampling, provides molecular-level insight into the electrochemical degradation of DHAQ and DHPS analytes cycled against a $\text{Fe}(\text{CN})_6$ catholyte between 25 and 65 °C.

Real-time 1D ^1H NMR revealed temperature-dependent electrochemical degradation pathways responsible for battery capacity loss (Fig. 1). Quantitative *in-situ* NMR under flow enabled kinetic modeling to predict capacity fade as a function of time. Multidimensional NMR (MQ/SQ, EXSY, NOESY) resolved proton exchange, keto–enol tautomerization, dimerization, and π – π interactions, and determined activation energies.

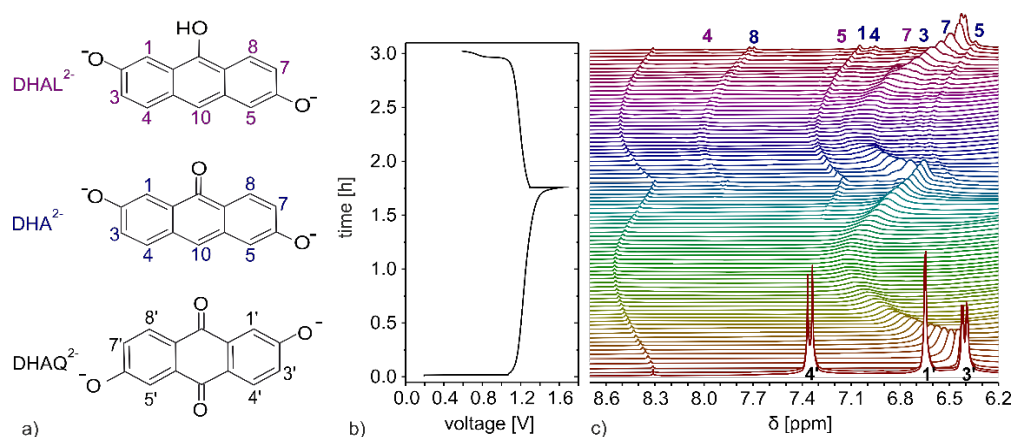


Figure 1. In situ ^1H NMR during electrochemical cycling at 65 °C. (a) Molecular structures of DHAQ²⁻ and its degradation products. (b) Cell voltage during the first cycle. (c) *In-situ* ^1H NMR spectra of the anolyte.

This framework links molecular transformations, temperature-dependent kinetics, and electrochemical behavior, offering predictive capability and design guidelines to enhance stability, performance, and cycle life of DHAQ- and DHPS-based AORFBs.

NMR Analysis of Carbon-Capturing Mg-based Cements

Bochert, Ida; Nguyen, Hoang; Kinnunen, Päivö; Telkki, Ville-Veikko

The cement industry is responsible for around 8% of global anthropogenic CO₂ emissions, making the development of low-carbon alternatives an urgent environmental priority. Hydrotalcite- and magnesium-silicate-hydrate-based cements offer promising pathways toward sustainable construction materials with reduced CO₂ footprints and the ability to bind CO₂. However, their microstructure and the formation of the pore network, which governs durability, moisture transport, and CO₂ uptake, remain insufficiently understood. To address this, we employ advanced NMR techniques to probe the porous structure of individual components and final cementitious materials using the water signal of the samples in their native, hydrated state. ¹H NMR relaxometry provides insights into solid-liquid water ratios and is used to qualitatively understand the pore size distribution. Relaxation-exchange experiments allow us to assess pore connectivity, while diffusion measurements characterize moisture dynamics in the porous system. The study is currently being extended to include cryoporometry for quantitative pore-size distributions, and ultrafast Laplace NMR is being implemented to significantly reduce experiment times. Preliminary results resolve interlayer, mesopore, and macropore populations and indicate that the water distribution in the pores of the individual cement components change measurably on timescales of days to weeks. By combining state-of-the-art NMR methodology with targeted materials research, the project contributes to the development of durable, carbon-capturing cements and expands the analytical toolbox for studying complex porous materials relevant to global environmental challenges.

Electrochemistry and Anisotropy of Sn(II) MOFs

Helena Loan, Ryan Bragg, Caitlyn Walton, Xiangting Xiao, Richard Walton, Michael Hope

Tin-based anode materials in Li-ion batteries have high theoretical capacities. Unfortunately, most Sn-based anodes have poor cyclability, due to volume changes during lithiation and delithiation. Here, we explore flexible Sn(II) metal–organic frameworks (MOFs) as potential Li-ion anode materials using solid-state NMR.

Sn(II) has a stereoactive lone pair which results in unusual MOF structures and causes the nucleus to exhibit high chemical shift anisotropy (CSA). The CSA is very sensitive to the local coordination environment; however, large CSAs are challenging to measure. We measured the ^{119}Sn CSA of 6 Sn(II) carboxylate MOFs, with between 1 and 3 crystallographically distinct Sn sites per structure, using a combination of variable magic-angle spinning and static wideline BRAIN-CP-WCPMG experiments. To relate the measured NMR parameters to the local structure, we performed GIPAW DFT calculations. We find good agreement for the isotropic shift, but DFT systematically underestimates the CSA. By accounting for the anisotropic effect of the Sn(II) lone pair on the shielding tensor, the experimental CSAs can be accurately calculated. We then tracked the changes a Sn(II) MOF upon cycling in a lithium-ion battery using ^1H , ^7Li , ^{13}C , and ^{119}Sn NMR, revealing an unusual mechanism of charge storage. This work demonstrates the strength of multinuclear (wideline) NMR and DFT to investigate battery materials, especially those exhibiting large anisotropies.

Solid-state NMR spectroscopy reveals confinement effects in MOF-based quasi-solid-state electrolytes

Lan, An; Yu, Han; Yuhang Yang; Laurence Middlemiss; Robert Dryfe; Sihai Yang; Martin Schröder; Daniel Lee

Understanding how electrolyte solvation is modified under nanoconfinement is essential for the rational design of high-performance quasi-solid-state electrolytes for energy storage devices. Metal-organic frameworks (MOFs) provide high surface areas and well-defined pore environments, but these can fundamentally alter ion-solvent interactions; resolving these effects at the molecular level remains challenging with most conventional techniques.

Here, multinuclear solid-state NMR spectroscopy is used to directly probe how MOF pore chemistry affects $\text{NaBF}_4/\text{glyme}$ electrolyte structure under confinement. Studying two structurally distinct frameworks, MFM-300(Sc) and MIL-125(Ti), reveals that pore geometry and metal-site chemistry impose markedly different fates on the confined electrolyte: heterogeneous, restricted solvent environments and anion decomposition in MFM-300(Sc) contrast sharply with liquid-like solvent dynamics and intact anion speciation in MIL-125(Ti).

Building on this framework, low temperature magic angle spinning (LTMAS) 2D correlation experiments are used to probe Na^+ coordination and solvent local environments within the pores. Applied to a series of MIL-125(Ti) variants (pristine, defected, and Zn-modified), these measurements reveal preferential Na^+ association with framework metal-oxo sites, providing direct evidence for confinement-driven solvation modifications.

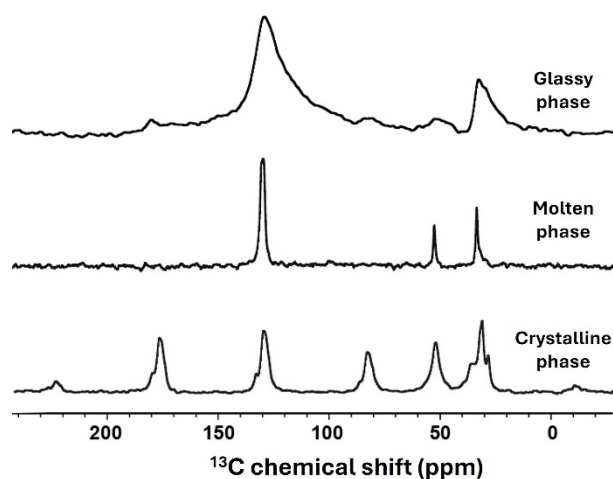
Together, these results establish a molecular-level understanding of how framework chemistry affects electrolyte organisation under confinement, from solvent dynamics and anion speciation to cation coordination, and demonstrate the power of multinuclear solid-state NMR spectroscopy as a tool for understanding and designing MOF-based quasi-solid-state electrolytes.

Structural dynamics of melting and glass formation in 2D metal halide perovskites from ^1H and ^{13}C VT solid-state MAS NMR

Shrestha Banerjee, Benjamin Gallant, Dominik J. Kubicki (*University of Birmingham, UK*)

Hybrid organic–inorganic metal-halide perovskites (HOIPs) have garnered significant interest, owing to their of tuneable optoelectronic properties. Initially explored primarily as crystalline semiconductors for photovoltaics, these materials are emerging for their ability to access melt and glassy states. The transition from the crystalline to melt and further to a glassy (amorphous) state by vitrification opens new dimensions in these materials by broadening their chemistries, processing options and improving mechanical robustness. Although, there are not many reports of HOIP glasses, (S-(-)-1-(1-naphthyl)ethylammonium) $_2\text{PbBr}_4$ is the most widely known 2D HOIP that melts at a moderate temperature ($<200\text{ }^\circ\text{C}$).

Here, we design a new class of 2D HOIP glasses with record low melting temperatures based on a simple ammonium cation and lead and tin halides. The tin-rich materials have melting points ranging from 45 to $65\text{ }^\circ\text{C}$, and glass transition temperatures close to $0\text{ }^\circ\text{C}$, which are the lowest recorded for HOIP glasses. To understand the local structural changes enabling such low melting behavior and vitrification we carried out *in situ* by ^1H and ^{13}C variable-temperature solid-state MAS NMR, as well as ^{119}Sn and ^{207}Pb RT solid-state MAS NMR studies to elucidate the atomic-level transformations associated with melting of the crystalline phase and vitrification. In combination with DSC-TGA data, we found that MAS NMR provides essential insights into phase purity, dynamics, and order-disorder transitions upon vitrification. We believe that this new class of low-melting glasses will open a new chapter in the halide perovskite research.



Variable-pressure ^{129}Xe NMR setup with a J-Young valved NMR tube

Nencini, Ricky; Telkki, Ville-Veikko

^{129}Xe has been widely used in NMR research since Ito and Fraissard proposed it as an ideal probe due to its high chemical shift sensitivity. It has been extensively applied to the study of porous materials, where variations in pore size and connectivity give rise to distinct chemical shifts and line shapes. Many important material properties can be deduced by performing experiments at different temperatures and pressures, including relaxation and exchange measurements.

Historically, higher xenon pressures were achieved by condensing xenon into an NMR tube using liquid nitrogen, after which the tubes were flame-sealed. Later, with the commercialization of J-Young valved NMR tubes, this preparation became a convenient alternative for experiments at lower pressures. Both methods, however, provide samples with only a single xenon density, making it impossible to conveniently record isobars or isotherms.

Subsequently, special systems containing sapphire NMR tubes were developed for high-pressure xenon NMR experiments, allowing pressure regulation directly inside the spectrometer.

Here, we present a very simple system for variable-pressure experiments at pressures up to 10 atm, using a J-Young valved NMR tube connected directly to a high-pressure Xe cylinder and a vacuum pump. We demonstrate the feasibility of this setup by measuring isothermal pressure series, and T_1 relaxations, for various carbon materials, including gas-storage polymers, carbon nanotubes, and carbons with different conductive properties.

In Situ Light-induced Degradation of Hybrid Perovskites by Solid-state NMR Spectroscopy

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Hybrid organic-inorganic perovskite materials are promising candidates for next-generation photovoltaic materials due to their outstanding optoelectronic properties. However, their instability is the major bottleneck preventing their industrialization. Therefore, gaining a detailed atomic-level understanding of exact degradation mechanisms of perovskite absorber layers is vital for improving long-term stability. Among the various environmental factors, photoinduced degradation represents the most important mechanism limiting the operational stability of the perovskite solar cells.

Here, we coupled a laser system to a solid-state NMR spectrometer to investigate degradation products and kinetics of methylammonium lead iodide (MAPbI₃) and formamidinium methylammonium lead iodide (FA_{0.95}MA_{0.05}PbI₃) scraped thin-film samples. Real time ²⁰⁷Pb experiments under controlled illumination conditions were performed to directly monitor *in situ* degradation of the perovskite samples. Our results indicate that after 130 hours of intense illumination, the MAPbI₃ perovskite completely degrades into PbI₂ at a rate of 64 nmol/h, with no evidence of metallic lead formation. In contrast, under similar conditions, the FA_{0.95}MA_{0.05}PbI₃ perovskite degraded by only approximately 50%, proceeding at a slower rate of 30 nmol/h. The degradation follows zeroth-order kinetics, is primarily light-induced and is accelerated by temperature. These results clearly indicate that incorporation of formamidinium into the perovskite lattice improves photostability of the material compared to pure MAPbI₃ composition.

Overall, the *in situ* NMR method presented here constitutes a new way to determine degradation products and kinetics, and compare photostability of lead-halide perovskites with different compositions.

Probing structural evolution of paramagnetic lithium transition-metal phosphates during battery cycling process using solid-state MAS NMR spectroscopy and DFT calculations

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Lithium-ion batteries underpin modern technology, powering applications from portable electronics to large-scale energy storage. Among next-generation cathode materials, $\text{LiMn}_x\text{Fe}_{1-x}\text{PO}_4$ (LMFP), ($x = 0.6, 0.7, 0.8$) an olivine-structured compound, has attracted considerable interest due to its strong electrochemical performance and inherent thermal stability. A major obstacle to improving battery longevity, however, is capacity degradation during repeated cycling. One dominant pathway involves transition metal dissolution from the cathode, which can induce structural rearrangements, promote electrolyte decomposition, lead to metal deposition on the anode solid electrolyte interphase (SEI), reduce the active lithium inventory, and ultimately result in capacity loss. Gaining a detailed understanding of the structural and chemical changes driving these processes is therefore critical for the design of more durable materials.

Despite its promise, the structural evolution of LMFP during cycling is not yet fully resolved. In this work, we examine LMFP at different states of charge and cycle numbers using solid-state nuclear magnetic resonance (NMR) spectroscopy and in-situ NMR experiments, employing ^{31}P and ^7Li as probes. To support spectral interpretation, we use density functional theory (DFT) calculations to compute NMR chemical shifts, enabling correlation of experimental resonances with local structural environments. Through detailed analysis of the observed species during the cycling process, we uncover important structural features and transformations. Notably, we find that the material does not follow a single reaction pathway during cycling, but instead switches between a uniform (solid-solution) process and a two-phase (biphasic) transformation, offering clearer insight into how the reaction progresses.

Together, these findings shed light on the dynamic structural evolution of LMFP and its impact on long-term electrochemical performance, offering guidance for the development of more stable lithium-ion battery cathodes.

Solid-State ^1H NMR Using an FGMAS Probe

Ando Shota¹, Ito Kohzo

¹The University of Tokyo

In recent years, marine microplastic pollution has emerged as a pressing global environmental issue, with increasing reports of plastic bags and PET bottles being retrieved from the gastrointestinal tracts of marine organisms. Among the primary microplastics that are considered difficult to recover, tire wear particles from automobiles account for approximately 28%. The main constituent of tires, vulcanized rubber, is virtually non-biodegradable in marine environments, largely due to the three-dimensional network structure formed through crosslinking. Conventional vulcanized rubber design typically emphasizes dense crosslinking to achieve high mechanical strength; consequently, mechanical performance and biodegradability are generally considered mutually exclusive properties.

In contrast, polyrotaxane (PR) elastomers, classified as topological supramolecular compounds, possess crosslinking points that can slide like pulleys, allowing the network to deform while dispersing applied stress. This unique architecture enables the enhancement of mechanical properties through an approach distinct from conventional rubber materials. In this study, we conducted marine biodegradation testing on polyrotaxane-based materials—potential candidates to overcome the traditional trade-off between mechanical robustness and biodegradability—and sought to elucidate their degradation mechanism. Specifically, high-resolution solid-state ^1H NMR measurements using a fast magic-angle spinning (FGMAS) probe were employed to identify the molecular components associated with biodegradation.

Identification of the Surface Hydrogen Functionalities on Colloidal Zinc Oxide Nanocrystals with Multinuclear Solid-State NMR Spectroscopy

Guolong Sun, Xueqian Kong,* and Yizheng Jin*

The surface hydrogen species of zinc oxide (ZnO) nanocrystals, including hydroxyl groups and water, play critical roles in determining their chemical reactivity, colloidal stability, and optoelectronic properties. However, direct structural identification of these surface species remains challenging due to their complex nature and surface disorder of the nanocrystals. Here, we present a comprehensive analysis of surface functionalities on sol-gel synthesized ZnO nanocrystals using advanced multinuclear and multidimensional solid-state nuclear magnetic resonance (NMR) spectroscopy, combined with density functional theory (DFT) theoretical calculations. By employing ^1H , ^7Li , ^{13}C , ^{17}O , and ^{31}P NMR, alongside the use of deuterated precursors, ^{17}O isotope labeling, probe molecules, defect analysis, and electron paramagnetic resonance (EPR) spectroscopy, we resolve and assign eight distinct hydrogen species, including acetate and lithium acetate ligands, chemisorbed and physisorbed water, terminal hydroxyls (OH_T), tribridged hydroxyls ($\mu_3\text{-OH}$), and defect-associated hydroxyls. Furthermore, ^1H - ^1H homonuclear correlation SSNMR experiments elucidate the crystal facet selectivity and spatial interactions of these surface hydrogen species. Our study establishes an in-depth understanding of the rich surface hydrogen landscape on ZnO nanocrystals and offers a framework for the rational design and functional tuning of ZnO-based nanomaterials.

NMR relaxometry up to 300°C on polymers and adhesives

Mörchel, Philipp; Distler, Michael; Nestle, Nikolaus

Using a high-temperature low magnetic field strengths (0.5T) NMR tabletop system (HT-NMR system), NMR relaxometry measurements were performed at temperatures up to 300°C and compared with data from DSC, DEA, and rheometer measurements on the same samples. The samples do not have to be prepared, and the statistical reliability is increased since the quantity (few grams) is significantly higher.

With the developed dedicated measurement and evaluation software, temperature cycles from 40°C to 300°C can be realized while simultaneously performing solid-echo relaxometry measurements. A new parameter based on the integral signal ratios (RMI) was defined and calculated. When plotting the RMI versus the temperature, the melting processes and melting temperature, as well as the glass transition temperature can be directly read from the curve. The NMR data correspond very well with the values from DSC reference measurements ($\pm 2.9\%$).

The RMI is well suited for measuring the curing curves of adhesives over several days. Moisture-, heat-, and UV-curing adhesives were examined. The plots of the RMI values versus time immediately show the same characteristics as the DEA and rheometer measurements.

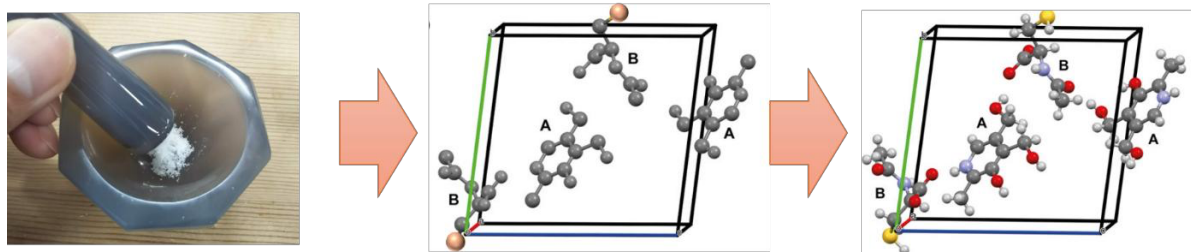
The system presented can also be used to determine the degree of cross-linking in polymers very accurately ($\pm 2\text{--}5\%$) after a calibration measurement.

To our knowledge the presented NMR system is the world's first tabletop NMR system that allows measurements of up to 300°C (450°C is planned). It enables the collection of measurement data that would otherwise require three measurement systems (DSC, DEA, rheometer). The HT-NMR system thus offers various advantages and can be used profitably by material manufacturers, processors, recyclers, and testing service providers.

De novo Structure Solution of Nano-Crystalline Powders

Yusuke Nishiyama

Structural solution of nanocrystalline powders remains a formidable challenge in crystallography. While three-dimensional electron diffraction (3D ED) has emerged as a powerful tool for structure determination, it inherently suffers from two critical limitations: (1) the ambiguous assignment of elements with similar atomic numbers (e.g., C, N, O, and F / Si, P, S, and Cl) and (2) the poor sensitivity of hydrogen atoms in electrostatic potential maps. To overcome these bottlenecks, we propose an integrated de novo analytical framework that synergizes 3D ED with high-resolution mass spectrometry (HRMS), nuclear magnetic resonance (NMR), X-ray fluorescence (XRF), and database mining. In this approach, 3D ED provides the initial structural skeleton, albeit with poor or no elemental assignments. XRF definitively identifies elements heavier than fluorine, while HRMS with soft ionization yields the precise molecular weight. By combining these data, the chemical formula can be unambiguously derived. This formula, when cross-referenced with the structural skeleton via database mining, narrows the structural candidates to one or only a few structures. Furthermore, precise hydrogen positions are optimized by DFT-D computation and quantitatively measured through internuclear distance measurements using solid-state NMR. Finally, the integrity of the proposed structure is rigorously validated by comparing experimental and calculated NMR spectra. This integrated methodology enables the reliable structure solution of unknown nanocrystalline materials without any prior chemical information.



When do f-orbitals participate in bonding? f-element covalency from NMR spectroscopy and quantum chemical calculations

Baker, Cameron F.; Bonham, Katherine L.; Hurd, Joseph; Du, Jingzhen; Davies, Matthew; Seed, John A.; Wooles, Ashley J.; Adams, Ralph W.; Liddle, Stephen T.; **Lee, Daniel**

The extent to which f-orbitals participate in chemical bonding remains a central question in f-element chemistry, with implications for our understanding of covalency across the lanthanide and actinide series. Resolving this issue is critical for predicting and controlling reactivity, electronic structure, and selectivity of f-element compounds in applications from catalysis to advanced materials. In particular, understanding the degree of actinide–ligand covalency is key to the design of selective ligands for nuclear fuel reprocessing and waste separations, where subtle differences in bonding between actinides and lanthanides can be exploited.

Solid-state NMR spectroscopy provides a powerful experimental probe of local electronic structure through ligand chemical shifts and anisotropies sensitive to metal–ligand orbital interactions. When combined with relativistic density functional theory calculations, these observables can be directly related to spin density, orbital mixing, and bonding contributions, enabling a detailed picture of f-element covalency.

Within this framework, the principal components of the chemical shielding tensor (σ_{11} , σ_{22} , σ_{33}) are interpreted using the Ramsey formulation relating the paramagnetic shielding contribution to virtual excitations between occupied and unoccupied orbitals, which is thus strongly influenced by the HOMO–LUMO energy gap and the spatial extent of the orbitals. Magnetic coupling between rotationally related orbitals provides a model for how these tensor components respond to local bonding environments.

Here, a chemical shift tensor analysis of cerium(IV), yttrium(III), and uranium(VI) diphosphonioalkylidene complexes will be described, where the Y=C and U=C linkages display extremely different bond character, with ^{13}C isotropic chemical shifts of 55 and 402 ppm, respectively. These differences will be rationalised and a broader correlation of NMR parameters with bond order for transition metal, rare earth, and actinide complexes containing formal M=CR₂ bonds will be presented.

Evaluation of transverse relaxometry data sets of polymers. Comparing classical fitting approaches and integral descriptors

Nestle, Nikolaus, Koppenhagen, Isabell, Funke, Lena-Marie, Pöselt, Elmar, Reichel, Tobias, Drießle, Toni, Mörchel, Philipp

Transverse relaxation decay curves of polymers can be recorded over 7 orders of magnitude in time in a quasi single-shot measurement by combining a solid echo or MSE with a CPMG sequence. Except for very low temperatures, polymers usually exhibit a wide distribution of different transverse relaxation times, representing proton environments of different local mobility. It is well-known that these local mobilities are essential for the mechanical and other application properties of polymeric materials and also for their processing behavior. However, extracting relevant information from classical evaluation procedures for NMR relaxation data is difficult as ILT-like approaches turn a curve into a curve and data obtained by multicomponent fits with different numbers of components are also hard to compared directly. These issues are avoided for model free descriptors based on the integrals over different parts of the signal decay or on the ratio of such integrals.

In our contribution we demonstrate the application of such descriptors to a data set for different TPU materials studied over a wide range of temperatures and present the correlation of these results with component amplitudes and relaxation times obtained from classical multicomponent evaluations and with information on application and processing data.

The results indicate that such integral descriptors can be used for fast identification or TPU grades, quality control or ageing studies of the materials.

Revealing the Potential of Quadrupolar Nuclei in Catalysis Through Ultra-High Field Solid-State NMR Spectroscopy

Delevoye Laurent, Gauvin Régis M., Taoufik Mostafa, Scott Susannah, Autschbach Jochen, Trébosc Julien, Szeto Kai, Motta Alessandro, Fayon Franck, Gajan David, Zurek Eva,

This presentation will show the impact of ultra-high field NMR (up to 28.2 T) for the characterization of quadrupolar nuclei in catalytically relevant materials. We will demonstrate how the combination of extreme magnetic fields and methodologies enhances spectral resolution and sensitivity, enabling the identification and quantification of distinct sites, in order to link directly their structure to reactivity. Examples will be drawn from our recent work on supported catalysts, including gallium-, zinc- and molybdenum based materials, highlighting the relevance of this approach to major industrial processes relying on such types of catalysts.

Furthermore, a special focus will be placed on NMR investigations on methylaluminoxane (MAO), a cornerstone co-catalyst in olefin production. Despite its industrial importance, MAO's molecular structure remains elusive due to its amorphous nature and to some specific structural and spectroscopic features. Using ultra-high field ^{27}Al MAS NMR and cutting-edge techniques, such as coslp-MQMAS pulse sequence, we have identified and quantified five distinct aluminum environments, including a site with an exceptionally large quadrupolar coupling constant ($C_Q = 27$ MHz). Reactivity studies with Lewis bases and metallocene pre-catalysts further validate these assignments, providing new paths for the rational design of cocatalysts. These findings, supported by DFT calculations, provide a comprehensive picture of MAO's structure and reactivity, including in catalyst activation and polymerization process.

These results demonstrate that ultra-high field NMR combined with DFT and reactivity studies can open new avenues for understanding and improving this class of industrial reagents, going from a black box to a molecular-level understanding.

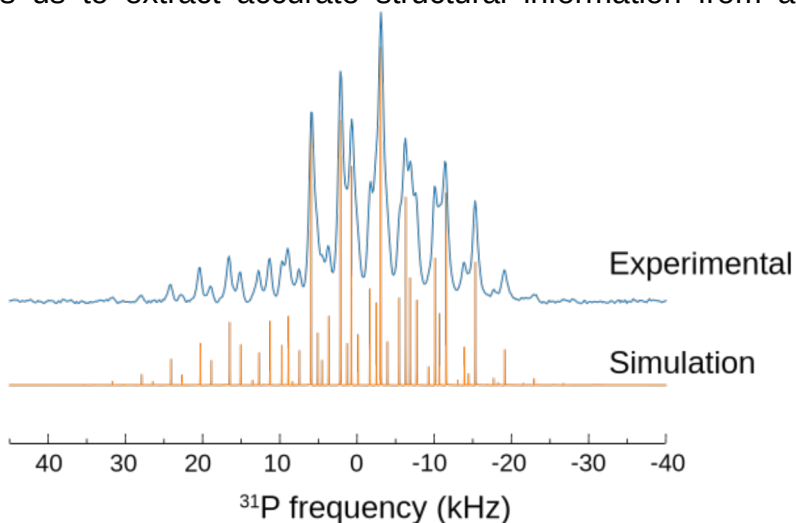
Interplay of Chemical Shift, Dipolar, and Scalar couplings tensors: Study of Aluminophosphite.

Claire Roiland, Lisa Nicole, Clément Lebastard, Véronique Alonzo, Thierry Bataille, Régis Gautier, Eric Le Fur

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Abstract

This study examines the interplay between chemical shift anisotropy (CSA), dipolar coupling (D), and scalar coupling (J) tensors in the solid-state NMR spectra of aluminum phosphite ($\text{KAl}(\text{HPO}_3)_2$). By combining experimental NMR techniques, Simpson simulations, and first-principles calculations based on density functional theory (DFT), we disentangle the individual contributions of these tensors to the observed ^{31}P NMR lineshapes. Our analysis allows us to extract accurate structural information from a system where multiple anisotropic interactions coexist and overlap. The findings underscore the critical role of accurately characterizing CSA, dipolar, and scalar coupling tensor contributions for reliable spectral interpretation and structural elucidation in solid-state NMR studies.



Keywords: Solid-state NMR, Chemical Shift Anisotropy, Dipolar Coupling, Scalar Coupling, Aluminum Phosphite, GIPAW-DFT Calculations, Simspon Simulations

Multi-Exponential Analysis of Magnetic Resonance Decay and Recovery Signals: Principles, Pitfalls, and Practice

Afrough, Armin, Vosegaard, Thomas

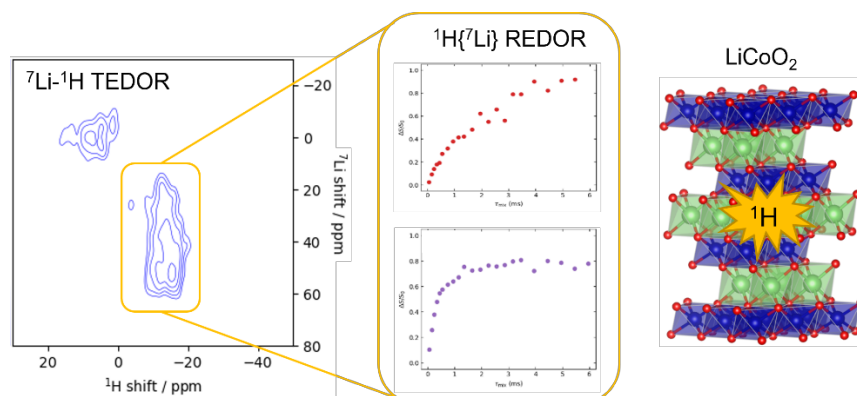
Multi-exponential decay and recovery signals are prevalent in magnetic resonance spectroscopy and imaging. Inversion of such signals to continuous lifetime distribution can provide insight into the dynamics of chemical species, health and disease in biological tissue, and pore size distribution in technological materials. Despite opportunities such inversions offer, the magnetic resonance community has remained hesitant to adopt continuous lifetime distributions widely. This hesitancy comes from concerns that (1) the inversion is sensitive to noise, (2) recovered distributions depend on regularization and are not unique, (3) typical magnetic resonance data are not acquired with sufficient quality for reliable inversion, and (4) the assignment of distribution components is difficult. Here, we argue that concerns (1)-(3) are often overstated. We show that careful experimentation, quality control, and signal inversion enables recovering reliable lifetime distributions from noisy magnetic resonance signals. First, we demonstrate successful inversion of multi-exponential signals containing up to five components spanning four orders of magnitude in lifetime at SNRs between 10 to 1000. Second, we show that current open-access tools can correctly determine optimal smoothing for relaxation time distributions. Third, we provide practical guidelines for acquisition and quality control of time-domain magnetic resonance data intended for such inversions. We acknowledge that component assignment, concern (4), remains an open problem for the community. Nevertheless, a selection of benchmark problems show satisfactory inversion quality and illustrative applications are presented from solid-state NMR of food, T_1 - T_2 relaxation correlation of rocks, and analysis of DNP build-up curves.

Solid-state NMR of aqueous Li-ion batteries: Understanding the behaviour of LiCoO_2 electrodes in water-based electrolytes

Lana Abboud, Ankan Nath, Gaël De Paëpe, **Katharina Märker**

While the importance of Li-ion batteries (LIBs) is undisputed, the flammability of their organic electrolyte remains a major safety concern. The use of water-based electrolytes has therefore been proposed as a safer alternative, but such aqueous LIBs suffer from limited energy density and cycling stability. One reason is that most LIB electrode materials have been developed for use with organic electrolytes, meaning that their interactions with water are not well understood. A common assumption is that H^+ can replace Li^+ in the electrode materials. However, reliable experimental methods to directly confirm this process and to evaluate its consequences for battery performance are still lacking.

In this work, we show that solid-state NMR spectroscopy provides a powerful tool to probe this phenomenon. We demonstrate this through ex situ solid-state NMR studies of the positive electrode material lithium cobalt oxide (LCO) in aqueous LIBs. ^7Li NMR spectra encode information on state-of-charge within the electrode, while ^1H NMR spectra reveal a number of different ^1H environments after prolonged battery cycling. With the help of ^1H - ^7Li dipolar recoupling experiments, specific ^1H signals can indeed be assigned to hydrogen located within the LCO layers, providing direct evidence of Li^+/H^+ exchange. Having shown that this process can be detected, we further investigate its dependence on electrolyte pH, cycle number and state-of-charge of the battery, before finally evaluating its impact on the performance of aqueous LIBs employing LCO.



Real-Time NMR Window into Biopolyester PHBV Extraction from Biomass

Martinho, Ricardo P., Barrulas, Raquel V., Vermeer, Chris, Brandt, Boudewijn P., Sousa, João A. B., Wurm, Frederik R., Cornelissen, Jeroen J. L. M., Schuur, Boelo

Bioplastics such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are promising alternatives to petrochemical plastics due to their biobased origin and biodegradability; however, their large-scale adoption is hindered by inefficient and poorly understood extraction processes from complex biomass matrices [1]. Conventional analytical techniques, including chromatography, and standard NMR, lack the capability to resolve molecular-scale events in heterogeneous systems under realistic conditions [2-3].

We introduce the use of slice-selective NMR spectroscopy for real-time, spatially resolved monitoring of PHBV extraction from organic-rich residual streams. This approach combines chemical specificity with spatial resolution, enabling in situ observation of solvent diffusion, polymer dissolution, and side-product formation without sample homogenization [4-5]. Extractions were performed using model solvents between 25 and 120 °C and monitored for up to 24 hours on a 600 MHz spectrometer. The method provided kinetic insights into extraction efficiency and polymer stability, as well as information on impurities, offering a mechanistic basis for solvent and process optimization.

These results demonstrate that slice-selective NMR is a powerful tool for characterizing biopolymer recovery in complex systems and holds broad potential for advancing sustainable polymer processing through improved analytical access to dynamic extraction mechanisms.

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Solid-state NMR of chemical transformations in functional materials

Benjamin M. Gallant, **Dominik J. Kubicki** (*School of Chemistry, University of Birmingham, UK*)

Many transformations in advanced materials proceed through disordered, short-lived, or non-crystalline states that cannot be captured by diffraction techniques. Solid-state NMR offers direct, element-specific access to local structure in both crystalline and amorphous phases. Here, I will show how we use multinuclear solid-state NMR to study such transformations.

In the first case, we looked at the coordination chemistry of the ambidentate phosphaehtynolate anion (PCO^-) bound to manganese within a metal-organic framework. While molecular Mn(I)-PCO complexes rapidly degrade, encapsulation within a porous framework stabilizes the elusive Mn(I)-PCO motif. We used ^{55}Mn and ^{31}P MAS NMR to establish the presence of this binding mode and to understand its geometry (Fig. 1A,B).

Next, we address the chemical persistence of polytetrafluoroethylene (PTFE). This fluoropolymer is produced on the scale of $\sim 200,000$ tonnes per year and largely destined for landfill at end of life owing to its exceptional chemical inertness. We set out to recycle it under mild conditions and, as a result, developed a straightforward protocol that only uses sodium metal as a reagent (Lowe et al. *JACS* **2025**, *147*, 40895–40899). ^{19}F and ^{13}C MAS NMR enabled us to determine the structure of the products. The development of PTFE recycling strategies is increasingly important in light of the environmental persistence of PFAS and fluoropolymer-derived microplastics.

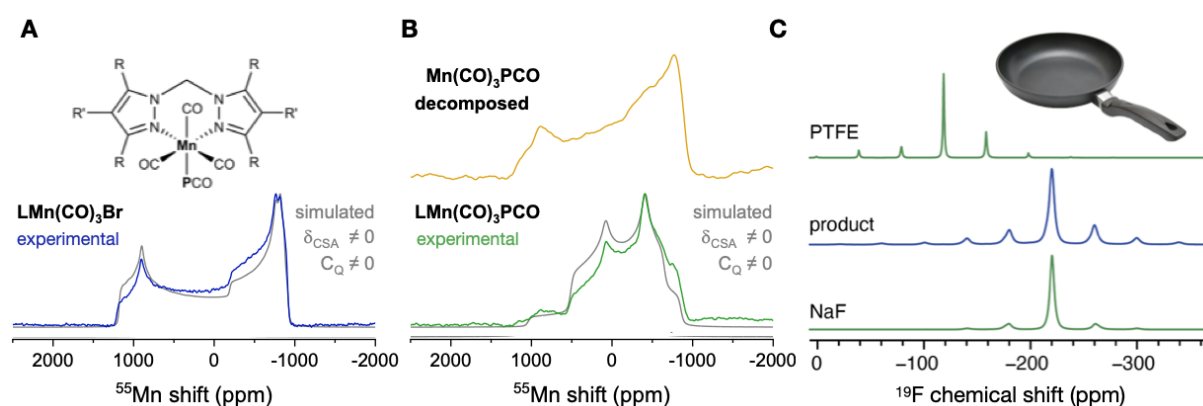


Figure 1. Solid-state characterization of the materials. (a) The precursor Mn(I) complex inside a MOF, (b) the Mn(I) precursor after PCO coordination, and after decomposition (^{55}Mn , static, 11.7 T, RT); (c) the product of the mechanochemical degradation of PTFE in a reaction with sodium metal (^{19}F , 15 kHz MAS, 9.4 T, RT).

Halogen-free ionogels as electrolytes and lubricating greases: solid-state and diffusion-NMR studies.

Antzutkin, Oleg N., Filippov, Andrei and Shah, Faiz Ullah

Isotropic and anisotropic ionogels (IGs) based on non-halogenated ionic liquids (ILs) and various gelating agents were studied by solid-state and diffusion (PFG) NMR techniques. IGs in the research focus do have negligible vapour pressure and are non-flammable. Therefore, IGs are attractive materials for soft-solid electrolytes in electrochemical energy storage devices and as emerging next generation space lubricants [EP4484526C0]. As gelators we employed tetraalkylsilicates [PCCP (2025) 27, 8221] and a variety of organic polymers including next-generation polyhydroxyurethane polymers cross-linked by dioxazaborocanes forming vitrimers [Chem Mater (2025) 37, 7049]. Our choice of ionic liquids in IGs was: for electrolytes - surface active ionic liquids (SAILs) synthesised in our group and validated in MWCNT-based supercapacitors, while for lubrication - orthoborate-ILs with outstanding friction and wear reducing properties [US9518243B2].

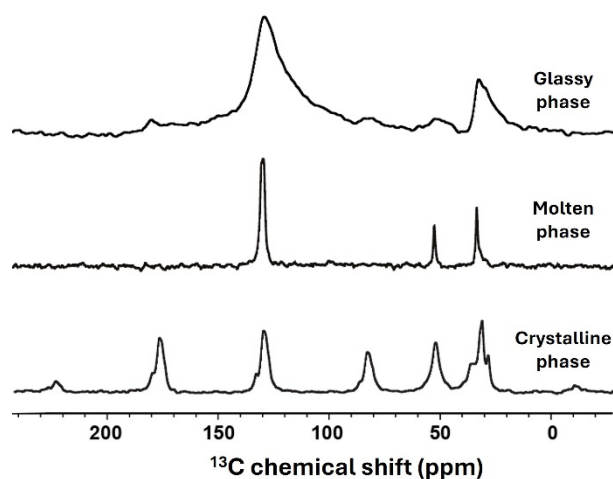
As a specific example, the translational dynamics of ions in IGs prepared from an IL lithium salt and PVA are discussed in detail. ^1H and ^7Li PFG NMR are performed on thin films (0.2-0.7 mm) of IGs at different orientations with respect to the external magnetic field and magnetic gradients. Two diffusional decay components are observed: a slow mode linked to PVA network oscillations and a fast mode from mobile ions. Diffusivities of ions have an orientational dependence of the films and are higher in thick films at the normal orientation with respect to the external magnetic field. Interestingly, an elongation of IG films by stretching reduces Li^+ diffusivities and leads to broadening of ^7Li NMR resonances, suggesting that transport properties of ions are strongly governed by structural constraints and internal stresses in the IG network.

Structural dynamics of melting and glass formation in 2D metal halide perovskites from ^1H and ^{13}C VT solid-state MAS NMR

Shrestha Banerjee, Benjamin Gallant, Dominik J. Kubicki (*University of Birmingham, UK*)

Hybrid organic–inorganic metal-halide perovskites (HOIPs) have garnered significant interest, owing to their of tuneable optoelectronic properties. Initially explored primarily as crystalline semiconductors for photovoltaics, these materials are emerging for their ability to access melt and glassy states. The transition from the crystalline to melt and further to a glassy (amorphous) state by vitrification opens new dimensions in these materials by broadening their chemistries, processing options and improving mechanical robustness. Although, there are not many reports of HOIP glasses, (S-(-)-1-(1-naphthyl)ethylammonium) $_2\text{PbBr}_4$ is the most widely known 2D HOIP that melts at a moderate temperature ($<200\text{ }^\circ\text{C}$).

Here, we design a new class of 2D HOIP glasses with record low melting temperatures based on a simple ammonium cation and lead and tin halides. The tin-rich materials have melting points ranging from 45 to $65\text{ }^\circ\text{C}$, and glass transition temperatures close to $0\text{ }^\circ\text{C}$, which are the lowest recorded for HOIP glasses. To understand the local structural changes enabling such low melting behavior and vitrification we carried out *in situ* by ^1H and ^{13}C variable-temperature solid-state MAS NMR, as well as ^{119}Sn and ^{207}Pb RT solid-state MAS NMR studies to elucidate the atomic-level transformations associated with melting of the crystalline phase and vitrification. In combination with DSC-TGA data, we found that MAS NMR provides essential insights into phase purity, dynamics, and order-disorder transitions upon vitrification. We believe that this new class of low-melting glasses will open a new chapter in the halide perovskite research.



Advances in solid-state NMR of electrolyte and electrode materials: method development and application to battery systems

Raiker Witter, Ulrich Sternberg, Soutam Panja, Guruprakash Karkera, Rémi Blinder, Sibylle Riedel, Mai-Liis Org, Fedor Jelezko, Ago Samoson, Maximilian Fichtner

Solid-state NMR is uniquely suited to probe local structure, ion dynamics and interfacial processes in electrochemical materials, but many battery-relevant systems remain challenging because of disorder, quadrupolar broadening and limited sensitivity. Here, we present recent advances in experimental and theoretical concepts for solid-state NMR applied to electrolyte and electrode materials for post-lithium batteries. In chloride-ion conductors based on substituted CsSnCl_3 , ^{35}Cl MAS NMR relaxation analysis combined with DFT and molecular dynamics reveals that aliovalent substitution stabilizes a dynamically disordered cubic framework and promotes chloride mobility through lattice flexibility, anharmonic vibrations and reduced migration barriers. These findings directly connect local structural fluctuations with fast ion transport and electrochemical performance.

For the organic anode material PTCDA, we explore chemical-shift-driven structure analysis by comparing experimental ^{13}C shifts with calculated values derived from candidate crystal structures, providing a first step toward NMR-assisted crystal-structure determination in multivalent battery systems.

In parallel, pulse-sequence development is extended toward surface-sensitive hyperfine mapping relevant for battery materials and hyperpolarization concepts, building on RAI-based recoupling strategies to probe hyperfine interactions and polarization transfer pathways at interfaces. This opens perspectives for sensitivity enhancement in studies of critical low-signal electrode and electrolyte materials. Together, these results demonstrate how combined NMR methodology, modelling, and materials application can uncover ion transport, local structure, and interfacial mechanisms in next-generation battery materials.

ABSTRACT MISSING

Solvent- and Concentration-Independent Molecular Weight Determination of Polysaccharides by Diffusion NMR

Francisco M. Arrabal-Campos, Marta González-Lázaro, Ignacio Fernández

Polysaccharides are biopolymers of increasing importance in biomedicine, food science and materials engineering due to their biocompatibility and versatile functional properties. Accurate molecular weight determination is essential for controlling these properties; however, size-exclusion chromatography (SEC) presents well-known limitations when applied to polysaccharides, including calibration requirements and solubility constraints. Diffusion NMR based on PFGSE experiments represent an attractive alternative, yet conventional approaches require highly diluted samples to avoid concentration-dependent distortions of the diffusion coefficient. Recently, we introduced an iterative methodology that overcomes this limitation by incorporating the concentration and viscosity dependence into a unified Arrabal-Fernandez scaling law: $D\eta|_c = e^{-\kappa c^v} D\eta|_{1/\infty}$. This strategy was validated for polystyrene and polypropylene glycol across four deuterated solvents (1.5–150 mg/mL), yielding molecular weight estimates with errors below 4%, independently of solvent and concentration. Here, we extend this methodology to polysaccharides for the first time. Ten monodisperse dextran standards (180 Da – 322 kDa) were measured in D₂O at six concentrations per sample (1.5–150 mg/mL). Diffusion coefficients were processed with DiffAtOnce and TRAIn to construct the first concentration-independent Kappa and infinite-dilution calibration curves for a polysaccharide system. These curves were applied to oligochitosan and chitosan, biomaterials widely used in drug delivery, wound healing and tissue engineering, enabling molecular weight determination directly at working concentrations. These results demonstrate that diffusion NMR combined with the proposed methodology provides a robust tool for biopolymeric molecular weight determination.

Deciphering the Topology of Less Ordered Materials Using an NMR Assisted Approach

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Profound knowledge of the molecular structure and supramolecular organization of organic molecules is essential for understanding their structure–property relationships. In this talk, I will highlight the importance of unraveling the molecular structure and supramolecular organization of organic molecules to gain insights into their structure–property relationships. I will demonstrate how the structure of partially disordered systems can be elucidated through an interdisciplinary approach that integrates solid-state NMR, 3D electron diffraction (3D ED), and DFT calculations (Fig 1).^{1–3} The synergy between experimental and theoretical methods holds significant promise for investigating novel materials with unique properties that are challenging to study using conventional techniques due to their inherent disorder. By effectively addressing these challenges, our methodology paves the way for new avenues in material characterization, driving exciting advancements in the field.

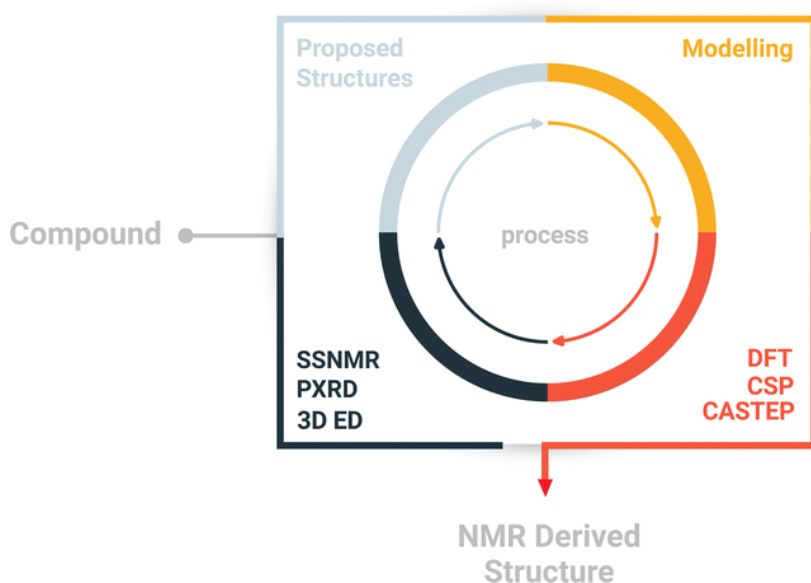


Fig 1: Schematic diagram of the structure determination approach. The approach combines complementary techniques like solid-state NMR, powder X-ray diffraction and electron diffraction on a quantum mechanical platform to integrate the results.

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Multinuclear Solid-State NMR Study of Novel MOFs for Combined Physical and Chemical H₂ Storage and CO₂ Incorporation

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Metal-Organic Frameworks (MOFs) have emerged as premier candidates for efficient hydrogen storage and carbon incorporation. This work presents a comprehensive solid-state NMR (ssNMR) investigation of three new MOF systems: a lightweight beryllium-based framework, [Be₄O(BDC-NH₂)_{2.5}(OAc)] (**Be_BDC**; BDC = 2-aminoterephthalic), two zirconium-based MOF, [Zr₆O₄(OH)₄(TzTz)₆] (**Zr_TzTz**) and [Zr₆O₄(OH)₄(PyPy)₆] (**Zr_PyPy**), and a new Aluminium-Aspartate MOF (**Al-Asp**) similar to Aluminium Fumarate (Al-FUM). To increase the hydrogen content we employed a strategy based in the nanoconfinement of light-weight hydrides like ammonia borane (AB) and hydrazine bis(borane) (HBB). We employed multinuclear ssNMR (¹H, ⁹Be, ¹¹B, ¹³C, ¹⁵N) to elucidate the host-guest interactions and chemical state of confined AB and HBB. For the **Be_BDC**, ⁹Be NMR provided rare insight into the environment of the beryllium nodes, while ¹¹B and ¹⁵N NMR revealed that the amino-functionalized linkers trigger partial chemical transformations of AB. This material demonstrated a remarkable gravimetric H₂ density of 8.0 wt% at 77 K. In the zirconium MOFs, NMR evidence proved that nanoconfinement induces a reaction between the hydrides and the acidic MOF hydroxyl groups, resulting in direct B-O bond formation and a significantly reduced the hydrogen release temperature. A similar mechanism was observed also for the incorporation of AB in **Al-Asp**. Notably this MOF shows interesting carbon capture capacity that reach 3.2 mmol/g of CO₂ at 273 K and 1 bar. Our findings highlight solid-state NMR as an indispensable tool for deciphering the complex interfacial chemistry in MOF-based hydrogen storage materials, guiding the rational design of more efficient energy carriers.

Solid-state MAS NMR and Dynamic Nuclear Polarization of nitroxide-templated metal halide perovskites

Luis Simbari, Dominik J. Kubicki (*School of Chemistry, University of Birmingham, UK*); Olivier Ouari (*Institut de Chimie Radicale UMR 7273, Aix-Marseille Université / CNRS*)

Metal halide perovskites are a rapidly developing class of semiconductors, widely known for their applications in solar cells. Solid-state NMR has already delivered significant atomic-level insights into these materials, notably on their local structure, ion dynamics, and speciation of dopants. In device architectures, halide perovskites are used as thin films and studying them with MAS NMR in this form is a challenge due to their limited mass. Dynamic Nuclear Polarization (MAS DNP) would be the ideal solution, but its application to this class of solids has been largely limited to fully inorganic perovskites (Mishra et al. *J. Phys. Chem. C* **2023**, *127*, 11094–11102) owing to the challenges associated with hyperpolarization transfer efficiency through ^1H - ^1H spin diffusion in the organic-inorganic materials when exogenous polarizing agents are used (Hanrahan et al. *Chem. Mater.* **2018**, *30*, 7005–7015).

Here, we proposed another approach: incorporating polarizing agents directly into the structure of metal halide perovskites to provide an endogenous source of polarization. We present the synthesis and full characterization (XRD, EPR, MAS NMR, MAS DNP) of hybrid halide perovskite materials incorporating ammonium-nitroxide radicals. We use the radical concentration dependent Paramagnetic Relaxation Enhancements of ^1H T_1 relaxation as atomic-level evidence for the incorporation of the nitroxides into the halide perovskite structures. We anticipate that this approach will enable MAS DNP on mass limited samples, such as μg -level single thin films.

Solid-state NMR studies on fluoride-ion conducting mechanism in $\text{Cs}_2\text{RbBiF}_6$

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1 Kyoto University, 2 Mie University

A fluoride ion conductor attracts attention as an electrolyte for a so-called all-solid fluoride-shuttle battery, which is expected to give high energy density. Structures of most of the fluoride ion conductors may be classified as fluorite-type, tysonite-type, and PbSnF_4 related materials. In this work, we examine ion conducting mechanism in $\text{Cs}_2\text{RbBiF}_6$ whose structure is double perovskite. ^{19}F MAS NMR spectra of $\text{Cs}_2\text{RbBiF}_6$ show the characteristic lineshape reflecting the J interaction with ^{209}Bi . The fact that the J coupling is observed indicates that the BiF_6 octahedron undergoes rapid isotropic reorientation in solids. From the lineshape analysis of 1D and 2D exchange NMR spectra, we estimate the exchange rate of the fluoride ion. We obtain the diffusion coefficient for the fluoride ion from the observed conductivity using the Nernst-Einstein relation, and the jumping distance of the fluoride ions is evaluated by using the exchange rate obtained by ^{19}F NMR. The distance is much longer than the nearest F-F distance among the different BiF_6 units. So we postulate that the ion conducting mechanism of $\text{Cs}_2\text{RbBiF}_6$ is the Grötthuss type; the exchanged fluoride ions are brought further by isotropic reorientation of octahedral BiF_6 .

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NMR Crystallography of Curcumin-Based Supramolecular Systems

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Curcumin is an active ingredient in the Indian spice turmeric and is mainly utilized for its anti-oxidant and anti-inflammatory properties. However, curcumin has a low solubility in body fluids and is unstable at physiological pH, which may be mitigated by exploiting cocrystals, which are supramolecular aggregates of two molecular species.

The crystal structures of curcumin, its cocrystal with resorcinol (1:1 stoichiometry), and the binary eutectic with hydroquinone (1:1) were refined by plane-wave DFT calculations. We will contrast the DFT-derived ^1H and ^{13}C chemical shifts with experimental data from MAS NMR. A ^1H – ^1H internuclear distance-analysis based on double-quantum (2Q)–single-quantum (1Q) ^1H NMR experiments performed at 60 kHz MAS will also be presented.

Understanding competitive adsorption in CALF-20 via ^1H , ^2H and ^{17}O NMR spectroscopy for carbon capture applications

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The removal of carbon dioxide (CO_2) from our atmosphere is pivotal in the battle against climate change. CALF-20 is a metal-organic framework that is a highly promising material as a solid sorbent of CO_2 .¹ It is durable, scalable and has a relatively high capacity and selectivity for CO_2 , even in wet gases. The competitive adsorption of CO_2 and H_2O is an important factor in its use as a carbon capture material, however, the interaction between the water and the CALF-20 framework is poorly understood.² In this work, we use static and Magic Angle Spinning (MAS) NMR to experimentally determine, for the first time, the atomic-level structure of confined water in this material, characterise its absorption with isotope specificity and quantify its dynamics, as a function of relative humidity and time.

We prepared CALF-20 samples, exposed to a range of relative humidities (%RH = 0 - 100) and employed ^1H , ^2H and ^{17}O NMR to monitor local structure. Figure 1a shows a set of representative static and MAS ^1H spectra at selected %RH. We find that ^1H NMR line shapes are highly sensitive to %RH, suggesting changes in the underlying local structure at different humidity levels. Finally, a combination of static variable-temperature ^2H and ^{17}O MAS measurements quantified water dynamics, which indicated water is substantially hindered compared to bulk liquid water.

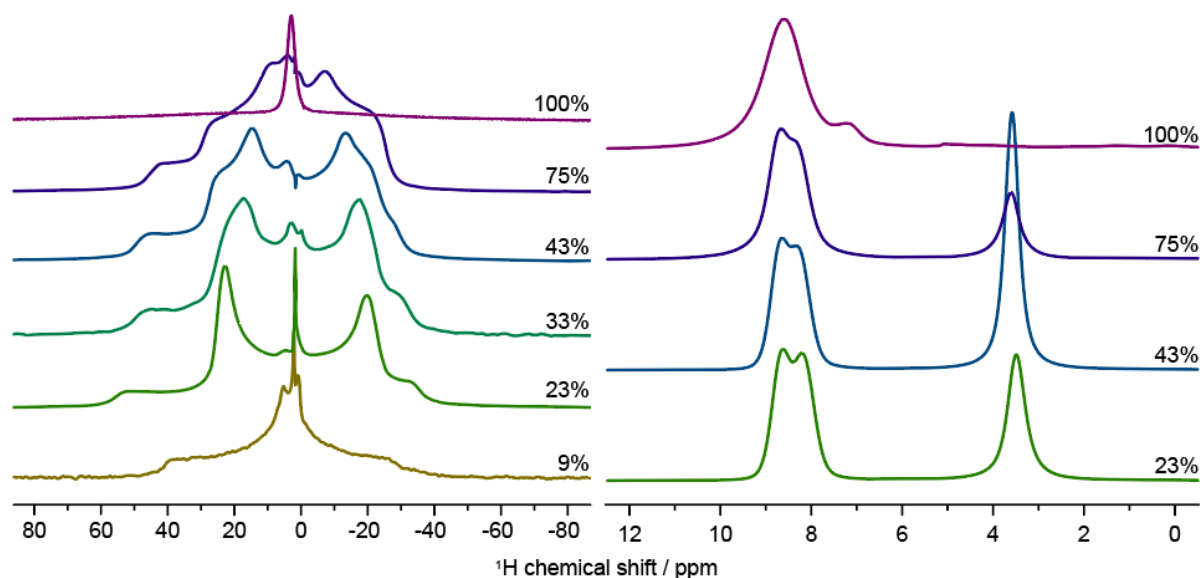


Figure 1. (a) Static and (b) MAS ^1H NMR spectra of CALF-20 after exposure to a range of relative humidities.

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Following Polymer Circularity: Insights from ssNMR Methodologies

Haber, Shira

Polymer circularity has become a major challenge in sustainable materials science due to the growing accumulation of plastic waste and the limited efficiency of current recycling technologies. While conventional mechanical recycling often leads to deterioration in polymer performance, emerging circular strategies aim to preserve, restore, or enhance material functionality through controlled design, upcycling, and deconstruction. Achieving these goals requires molecular-level understanding of polymer microstructure, chain dynamics, and chemical evolution throughout degradation and reuse.

In this work, solid-state NMR (ssNMR) spectroscopy is presented as a versatile platform for characterizing and tracking molecular-level changes associated with polymer upcycling and deconstruction. The first part focuses on polymer upcycling through linker incorporation and microstructural modification strategies designed to improve thermomechanical performance. Utilizing multinuclear ssNMR spectroscopy and relaxometry, enables correlation of changes in local structure, segmental dynamics, and phase behavior with macroscopic material properties, providing insight into how chain architecture and intermolecular interactions govern polymer performance during circular processing.

The second part addresses polymer deconstruction and demonstrates how ssNMR can monitor bond cleavage, chain mobility, and structural evolution during degradation. Two representative deconstruction systems are presented to illustrate the sensitivity of ssNMR spectroscopy and relaxation measurements to chemical and dynamic transformations during polymer breakdown. Together, these examples highlight the capability of ssNMR to bridge molecular structure, chain dynamics, and material functionality across multiple stages of the polymer circularity cycle, establishing the foundation for the rational design of next-generation upcycled materials with tailored mechanical and chemical properties.

Unlocking Nanocellulose Surface Chemistry with DNP-enhanced Solid-State NMR

Hediger, Sabine, Kumar, Akshay, Yagita, Tomohito, Demeunynck, Martine, Paul, Subhradip, Fujisawa, Shuji, De Paëpe, Gaël.

Owing to the variety of chemical pretreatments available, nanocellulose has a wide range of shapes and surface chemistries allowing fine tuning of mechanical, chemical, and rheological properties. However, optimizing its use requires a detailed understanding of atomic-scale morphology and surface chemistry. Solid-state ^{13}C cross-polarization MAS NMR can uniquely probe local atomic environments, but its low sensitivity—due to the $\sim 1.1\%$ natural abundance of ^{13}C —limits its effectiveness at natural isotopic abundance.

Recent advances in high-field dynamic nuclear polarization (DNP), along with improved polarizing agents, have significantly enhanced NMR sensitivity, notably at high magnetic fields, under fast MAS, and in protonated systems.¹ These developments have revolutionized our ability to obtain atomic-level insights on CNF surface modifications.

Thus, by combining DNP-enhanced NMR with molecular dynamics (MD) simulations and density functional theory (DFT) calculations, we were able to probe at atomic resolution the chemical structure of the nanocellulose surface, providing a precise understanding of the chemical surface topology of surface-phosphorylated-cellulose nanofibers (P-CNFs), including substitution positions, spatial distribution, and conformation.²

Furthermore, in the context of drug delivery applications, DNP allowed us to locate low-level prodrug functionalization on CNFs and unambiguously differentiate adsorption from covalent grafting. Such information, uniquely obtained thanks to DNP, provides a direct and powerful way to compare various coupling agents and optimize the grafting process.^{3,4}

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The calcium chlorapatite $\text{Ca}_5(\text{PO}_4)_3\text{Cl}$ samples with controlled surface morphology ranging from low to high surface area were studied using $^{35,37}\text{Cl}$ MAS and static NMR. Complex $^{35,37}\text{Cl}$ NMR lineshapes obtained for all studied $\text{Ca}_5(\text{PO}_4)_3\text{Cl}$ samples, consisting of two components, were analyzed using quadrupolar and *Czjzek* models that characterize highly crystalline and glass-like moieties, respectively. TEM micrographs confirmed the formation of the glass-like material as a shell, while crystalline moieties were formed in the core of the particles. The ratio of crystalline to glass-like material is related to the morphology of the samples: smaller particles contain more glass-like species, while larger particles are composed mostly of crystalline material. The results obtained highlight the importance of quadrupolar NMR as an experimental method for correlating particle morphology with phase composition at the molecular level. Moreover, quadrupolar NMR demonstrated its robustness as a complementary technique to XRD for the characterization of complex materials, especially those consisting of several coexisting phases, some of which lack long-range order, e.g., glasses and glass-like materials.

Multinuclear NMR Study of the $\text{Na}_{2.5}\text{V}_{1.5}\text{Ti}_{0.5}(\text{PO}_4)_3$ Aqueous Electrochemical Degradation

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Transition towards sustainable and renewable energy requires development of energy storage technologies such as Na-ion based batteries based on non-critical materials. Promising sodium ion batteries (SIB) are aqueous NASICON (Na Super Ionic CONductor) based batteries. NASICON materials feature a stable three-dimensional crystal structure, high ionic conductivity, thermal stability and wide range of redox potentials. NASICON can be used as anodes, cathodes, solid electrolytes, or electrode coating materials in battery development. To efficiently and successfully use these technologies, it is necessary to understand the chemical processes that limit the effective usage time of the batteries. Solid-state Nuclear Magnetic Resonance spectroscopy can provide invaluable insights on the processes taking part in the solid-liquid interphases on the electrode. In addition, high-resolution (liquids) NMR is a powerful method to detect dissolved species in the electrolyte, thus give insights on the solubility of the active electrode materials.

In this study, we use multinuclear ^{31}P , ^{23}Na , ^{51}V , and ^{13}C *ex situ* solid state NMR of NVTP-based electrodes as well as ^{31}P and ^{51}V *ex situ* high resolution NMR of the electrolytes to analyze the degradation of the electrode material and the formation of the interphasial layers in aqueous electrolytes during electrochemical charge-discharge cycling. The obtained results provide some insights regarding the degradation products and processes in NVTP during its aqueous electrochemical operation.

Funding by the Research Council of Lithuania under grant S-MIP-23-47 is gratefully acknowledged.

Cryogenic He MAS NMR Reveals Hidden Dynamical Disorder in the Ordered Low-Temperature Phase of MAPbBr₃ Quantum Emitters

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Nanocrystalline lead halide perovskites (APbX₃) are promising quantum emitters capable of generating single, indistinguishable, and coherent photons. While hybrid perovskites containing small organic A-site cations such as methylammonium (MA) or formamidinium (FA) already exhibit pure single-photon emission at room temperature, achieving coherence and indistinguishability requires cryogenic cooling. However, the low-temperature (LT) structure of APbX₃ remain elusive, despite their direct influence on the optical and excitonic properties. In particular, MAPbBr₃ is commonly described as adopting an ordered orthorhombic structure at LT, with conflicting reports on residual dynamics potentially leading to disordered domains and altered fine-structure states.

Here, we investigate the structure and dynamics of MAPbBr₃ using multinuclear (¹H, ¹³C, ¹⁵N, ²⁰⁷Pb) variable-temperature (VT) MAS NMR down to 20 K using a home-built setup enabling cryogenic Helium MAS. The temperature dependence of the ²⁰⁷Pb chemical shift reveals a change in slope near 50 K, consistent with subtle modifications of the inorganic framework, corroborated by neutron diffraction and ⁷⁹Br NQR spectroscopy. Decrease in lattice dynamics upon cooling can be probed directly via ²⁰⁷Pb T₁ and refocused transverse decay times (T₂') measurements. ¹H lineshapes reveal persistent motional averaging, while spin-dynamics simulations demonstrate an order-of-magnitude difference between the rotational hopping rates of the CH₃ and NH₃ groups. ¹H–⁷⁹Br RESPDOR experiments reveal shorter N–H⋯Br than C–H⋯Br distances, consistent with hydrogen bonding modulating rotational barriers. Additional ¹³C, ¹⁵N T₁/T₂ measurements support these hopping rates while evidencing a complex multi-timescale dynamic. Strikingly, despite the apparently ordered LT phase, ¹³C spectra exhibit inhomogeneous broadening, revealing distributions of local chemical environments consistent with residual orientational disorder, while ¹³C T₂' suggest the emergence of additional slow μs–ms dynamics between 20 and 60 K.

Solid State NMR Study of Polybetaine Polymer Effect on Phase Composition of Biomimetically Synthesized Calcium Phosphates

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Development of new materials for biomimetic remineralization of tooth enamel is a new approach in dental materials research for prevention and treatment of enamel defects. Recent studies show that betaine-functional polymers can influence calcium phosphate mineralization and control phase formation.

Novel Polymer/Calcium Phosphate (P/CaP) hybrid materials were synthesized and characterized by solid state NMR. Hybrid P/CaP systems were prepared by *in situ* precipitation of CaP in physiological medium containing polycarboxybetaine (PCB) or polysulfobetaine (PSB) under different reaction conditions. Pure CaP synthesized under identical conditions were also studied for comparison. ^1H , single pulse ^{31}P and $^1\text{H} \rightarrow ^{31}\text{P}$ CPMAS NMR spectra provided insight on the structural characteristics and chemical composition of CaP phases as a function of preparation procedure, synthesis conditions and polymer type. The NMR data showed that pure CaP phase formed under controlled conditions (pH, temperature, maturation time) consisted of crystalline and poorly crystalline hydroxyapatite. In contrast CaP phase formed in absence of polymer under conditions used for hybrid materials, showed a complex mixture of orthophosphates and hydrogen phosphate species. The NMR spectra of polymer/CaP hybrids showed that PCB promoted formation of amorphous calcium phosphate, while in presence of PSB a mixture of dicalcium phosphate dihydrate and poorly crystalline octacalcium phosphate was formed. These results demonstrate the significant effect of polymer functionality on the composition and structural properties of P/CaP phases.

Acknowledgment: The study used research equipment from INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science.

Quadrupolar Coupling in 2D Lead Bromide

Perovskites: NQR, Ultra-high Field NMR, and DFT

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Lead halide perovskites are an up-and-coming solar cell material but have significant issues with stability. To remedy these, 2D Ruddlesden–Popper layered perovskites can be used as protective barriers on the surface of their 3D analogues in photovoltaic devices. Understanding the structure of these materials, as well as defect formation, is of key importance for optimising their protective capabilities.

^{79/81}Br nuclei have large quadrupole moments, resulting in strong coupling to the electric field gradient at the nucleus. The ^{79/81}Br quadrupolar coupling is therefore an excellent probe of the long-range order of crystalline materials and thus of defect formation. Nuclear quadrupolar resonance (NQR) spectroscopy enables extremely precise measurement of the quadrupolar coupling. This has been demonstrated for 3D perovskites, but neither ^{79/81}Br NMR nor NQR has been reported for the more complex layered perovskites, which have distinct axial and equatorial halide sites. The problem with NQR is finding the signals, which could be anywhere between 0 and 100 MHz for ^{79/81}Br. DFT would be an excellent way of predicting the NQR frequency, but requires experimental optimisation.

Here, we use ultra-high field (1.2 GHz) NMR to measure the ⁸¹Br NMR spectra of layered perovskites with different spacer cations and determine the quadrupolar parameters. This enables us to find their ^{79/81}Br NQR signals for the first time. These results are used to benchmark DFT calculations of the quadrupolar parameters, and we find that the RSCAN functional gives good predictions of the quadrupolar coupling constant (RMSE $\approx$ 5 MHz). This work paves the way for ^{79/81}Br NQR studies of emerging layered perovskites, guided by accurate DFT calculations, and demonstrates the strength of ultra-high field NMR.

Insights into Curcumin Encapsulation within ZIF-8 via Solid-State NMR

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The need for controlled drug release to reduce toxicity and enhance therapeutic efficacy has driven the development of Metal-Organic Frameworks (MOFs) as drug carriers. ZIF-8 is particularly promising due to its biocompatibility and pH-responsive nature. A major pharmacological challenge is the delivery of Curcumin (*cur*), a natural bioactive polyphenol renowned for its antioxidant, anti-inflammatory and anticancer properties, with low aqueous solubility and poor stability. Encapsulating *cur* within ZIF-8 offers a strategic solution to improve the stability of the molecule and its bioavailability. Understanding how drugs interact at a molecular level with porous nanocarriers is fundamental to guide the rational design of advanced drug delivery systems. This work exploits solid-state NMR (ssNMR) as a unique atomic-level probe of structure and dynamics in *cur*@ZIF-8 systems. This technique provides definitive evidence of whether the drug is truly encapsulated, adsorbed on the surface, or present as an amorphous/crystalline bulk phase. By analyzing samples with different curcumin initial concentrations (0.5, 1, and 2 mg/mL – referring to the amount of curcumin dissolved in MeOH for the synthesis), we provide detailed insights into host-drug interactions and structural modifications induced by loading, enabling the identification of stability thresholds and distinct confinement regimes within the framework. A multinuclear (^1H , ^{13}C , ^{15}N) high-resolution ssNMR strategy was used to investigate the structure of both the framework and the drug and, in particular, the interactions between curcumin and ZIF-8. Furthermore, spin-lattice (T_1) and spin-spin (T_2) relaxation measurements were exploited to reveal linker and guest dynamics under confinement across multiple timescales. To gain a deeper understanding of the host-guest interactions, 2D ssNMR experiments were also performed, complemented by DFT calculations to provide both structural validation and characterization of the non-covalent forces at play.

Monitoring microplastic weathering with conventional and DNP-enhanced MAS NMR spectroscopy – Influence of additives

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While plastics are indispensable in our everyday life due to their advantageous material properties, mismanaged plastic waste accumulates in the environment and poses severe ecological risks. For improved microplastic risk assessment, key questions such as polymer degradation rates and the degradation and leaching behavior of additives must be addressed. This requires highly sensitive quantitative analytical methods that are able to detect minority species with contents down to a few hundred ppm.

To analyze abiotic polymer degradation as a function of additive content, we conducted accelerated weathering studies on pristine polystyrene (PS) and PS containing the antioxidant Irgafos 168. For the additivated samples an initial induction period was observed. However, at later stages Irgafos accelerated the degradation of PS, indicating adverse long-term effects on polymer stability. Polar polymer defects, such as peroxides, were monitored by $^1\text{H}/^{13}\text{C}$ CP NMR. Defect quantification was achieved by calibrating whole sample series with selectively acquired multiCP experiments after careful parameter optimization due to a broad spread of TIS. The degradation of Irgafos was simultaneously investigated using $^1\text{H}/^31\text{P}$ MATPASS CP NMR while its leaching behavior was assessed via solid- and liquid-state NMR. Even after 3200 h of weathering, about half of the additive degradation products remained in the polymer, indicating preferential binding to polymer defects.

From Rocks to Bones: Internal Gradient NMR in Complex Porous Media

Seland, John Georg

Correlations between internal magnetic field gradients (G_0) and the free induction decay rate (FID rate) may reveal important properties of liquid saturated pore structures. The FID rate encodes both magnetic susceptibility differences ($\Delta\chi$) and spectral peak frequency dispersion ($\Delta\nu$), depending on the data analysis, Inverse Laplace or Fourier Transform, respectively. It has previously been demonstrated, by our research group and others, that G_0 – $\Delta\chi$ correlations enable estimation of pore size distributions, while G_0 – $\Delta\nu$ correlations provide information on grain size heterogeneity.

We present recent results from spatially resolved G_0 – $\Delta\chi$ and G_0 – $\Delta\nu$ correlations in porous materials ranging from rocks to bone, highlighting potential applications in geological reservoir characterization and medical imaging. The experimental set-up and methods have previously been presented in papers from our research group.

Results obtained from rock materials, represented by fluid-saturated close packings of quartz sand grains, demonstrate estimations of pore size distributions, wettability and grain size heterogeneity across varying mineral surface and fluid saturation conditions, all important parameters in reservoir characterizations. Results from bone, obtained through *ex vivo* measurements of extracted samples from lamb, enable the characterization of variations in internal porous bone structure, and highlight potential applications in osteoporosis diagnosis.

Multinuclear NMR and MRI Investigation of the Wetting and Formation Processes in Lithium-Ion Batteries

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Formation, ageing and testing (FAT) are the final steps in the battery manufacturing process and are crucial for the formation of the interphase layers, which ultimately dictate the long-term performance of a battery. The current standard for electrolyte wetting, in both academic and industrial settings, is between 12-48 hours, followed by a charge-discharge cycles which can take up to 20 hrs of time on the cycler. As such, FAT processes currently constitute a major bottleneck in lithium-ion battery (LIB) manufacturing, and are expensive, as well as energy and time intensive. However, the minimum requirements for these processes are not fully established, because wetting and the mechanistic details of formation processes remain poorly understood. Thus, there is significant demand from industry for these steps to be optimised, to reduce the cost of manufacturing LIBs.

This project is developing a better understanding of wetting, formation and ageing processes using in situ and in operando ^1H ^7Li and ^{19}F , NMR and MRI, studying LIBs comprising graphite anodes and NMC955 cathodes. By observing the increase and distribution of signal using ^1H NMR spectra and 1D profiles, we have observed the rate at which the LP57 electrolyte is able to wet both individual electrode and separator components, as well as collectively in a model pouch cell. We observe the LiPF_6 salt and electrolyte solvent independently and have investigated the relationship between type of separator, and its orientation, with respect to the rate of wetting, observing that the rate is dependent on the nature of the separator. Building on these studies, we are extending towards an exploration of the full formation and ageing process to evaluate formation protocols we develop against existing industrial standards.

Studying the interactions of the sizing agent in glass fiber – polymer composites

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Glass fiber reinforced polymers – polymers containing milled glass fiber particles – are essential to modern engineering due to their increased strength compared to the native polymer. This makes them suitable for use in fields such as the automotive industry and construction applications. A crucial component within these composites is the sizing agent: a thin chemical coating on top of the milled glass fiber that increases the durability of the fibers, improves the processing, and facilitates the adhesion between the milled glass fiber and the polymer. Despite its importance, the exact interaction mechanism of the sizing agent with the milled glass fiber and the polymer is not well understood yet. Investigating this interaction with solid-state NMR presents a significant challenge due to the very low concentration of the sizing agent (the full molecule generally comprises much less than 0.1 wt% of the composite). On this poster we present our recent work in studying this interfacial interaction. By employing swelling agents to enhance the interfacial mobility and utilizing model compounds, we demonstrate how solid-state NMR can be used to elucidate the interactions between the sizing agent and the polymer.

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Role of oxygen triclusters in enhancing the mechanical properties of Na–Mg aluminosilicate glasses: ^{27}Al MAS & 3QMAS NMR

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Aluminosilicate glasses serve as the technological foundation for high-tech applications from smartphone screens like Gorilla Glass to high-power laser fibers. However, tailoring their fracture behavior remains difficult due to non-linear structural anomalies. Optimizing these networks requires solving the long-standing dichotomy between hardness and crack resistance, a trade-off heavily governed by hidden atomic-scale mechanisms.

To resolve this challenge, we deploy advanced high-resolution ^{27}Al MAS and 3QMAS NMR spectroscopy across systematic $\text{Na}_2\text{O} \rightarrow \text{MgO}$ substitution trajectories to establish a unified, coordination-driven chemical mechanism. Structural simulation approaches based on the Cjzek model were utilized to extract the continuous Aluminum coordination speciation. Rigorous cross-correlations among the best-fitted NMR tensor parameters, including isotropic chemical shift (δ_{iso}), quadrupolar coupling product ($C_{Q,\eta}$), and isotropic chemical shift distribution (W_{iso}), reveal a systematic linear proliferation of coordination-expanded Al species.

Crucially, correlating these NMR parameters with macro-mechanical profiles allows us to propose a comprehensive reaction mechanism that tackles unresolved questions in glass science. Specifically, this work unifies the structural response to resolve the well-known viscosity anomaly, settling the contentious dilemma regarding the competitive roles of oxygen triclusters (TBOs) and high-coordinated Al species acting as charge-balancers, while elucidating the formation of non-stoichiometric non-bridging oxygens (NBOs). Concurrently, this model perfectly rationalizes mechanical evolution, establishing a direct correlation between crack resistance and an effective energy-dissipation index accounting for TBO proliferation and the progressive expulsion of residual sodium. Ultimately, advanced solid-state NMR serves as a uniquely powerful tool to engineer high-performance glasses that simultaneously optimize hardness and fracture resistance.

Fire, Charcoal & Charring: Analysis of Charcoal Production Sites in Northern Sweden via NIR, NMR & XRD

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The Swedish Iron Industry saw a mostly stable supply of charcoal from the mid-18th century to the 1830s, with a particularly prolific period of charcoal consumption between 1898 and 1900. The chemical and structural analysis of charcoal from archaeological sites is important to better understand how humans advanced industrialised charcoal production in Fennoscandia.

However, the dark nature of charcoal makes it difficult to analyse through traditional spectroscopy methods, such as Raman and FT-IR. Our proposed solution is NMR which does not suffer from fluorescence or light absorbing problems.

Our case-study is a presumed mid- to late-19th century charcoal production site located in Gunnarsbyn (Norrbotten county), in Northern Sweden. Soil samples and charcoal pieces were collected via coring and separated based on soil horizon. Both soil samples and individual charcoal pieces were analysed via ¹³C MAS NMR in conjunction with NIR-HSI (near-infrared hyperspectral imaging) for functional group confirmation and XRD to confirm phase identification and the extent of amorphous/crystalline phases.

Our preliminary results indicate that full carbonisation occurred, which correlates with XRD results that indicate the presence of amorphous carbon, as well as the presence of crystalline graphitic carbon within the same charcoal piece. These results are important for creating a multi-analysis toolbox that can be used to understand the carbon cycle in soils and sediments and charcoal production. This analysis has application in areas where carbon is present, including modern forensics, batteries, and biomass production from wood, crops, and waste, which is used for energy production.

Structural characterisation of transition metal phosphites $AM(HPO_3)_2$ with $A = K, NH_4$ or Rb and $M = Al$ using XRD, ss-NMR and RAMAN spectroscopy.

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Transition metal phosphites (Al ; Fe ; V, ...) are promising cathode candidates for batteries, due to their multiple oxidation state, low dimensionality, and the presence of hydrogen atoms. Single-crystal X-ray diffraction (XRD) is commonly used to characterize the crystalline structure through Fourier transform map of electronic densities, allowing precise atom positioning. However, this method is generally inefficient for locating hydrogen atom. In phosphites-based material, this lack of information on hydrogen positioning is a significant issue, as hydrogen atoms can strongly influence material's properties. Therefore, developing a reliable method to properly accurately determined hydrogen atom positions and their local environment is crucial to a better understanding of the relationship between structure and properties in phosphites-based materials.

This study focuses on developing a methodology to locate hydrogen atom in diamagnetic compounds using solid-state NMR (ss-NMR), by measuring the distance between phosphorus and hydrogen atoms (r_{P-H}). Hodgskinson et al. previously measured the dipolar coupling interaction between ^{31}P and 1H by CP-MAS under Hartmann-Hahn conditions in a simple tin phosphite. The measurement was obtained after Fourier transform of the CP build-up curve, resulting in a "Pake-like" pattern, where separation between the horns is related to the value of the dipolar coupling constant value (D_{P-H}). The study of the $AM(HPO_3)_2$ -type phosphites materials appears to be more complex than that of tin phosphite due to the presence of two inequivalent phosphorus atoms in the structure. Spectra showed two well-defined ^{31}P signals, to which the method could be applied to determine both distances (r_{P-H}). These distances were then compared with values from neutron diffraction for validation. Finally, in order to distinguish between the two ^{31}P signals, NMR and RAMAN computational simulations were

performed. Combining all these information allowed to complete the crystallographic structure with accurately positioned hydrogen atoms.

The transition to sustainable energy systems relies on efficient energy storage and has motivated research into next generation, high-capacity battery technologies such as lithium–sulfur batteries. A powerful technique to study batteries is in-situ NMR spectroscopy. This technique enables direct correlation between electrochemical behaviour and the structural and chemical information offered by NMR, providing insights into dynamic processes.

One of the most effective techniques to investigate lithium batteries is ^7Li NMR. However, a major limitation of static operando ^7Li NMR is poor spectral resolution near the diamagnetic lithium region. The narrow chemical shift range of ^7Li causes signals from solid lithium-containing species to overlap with those from the liquid electrolyte. Consequently, single-pulse ^7Li NMR experiments are insufficient for resolving and monitoring solid-state species during battery operation.

In this work, ^7Li dipolar and quadrupolar based multiple-quantum NMR experiments were explored to selectively observe solid lithium species. First, density functional theory calculations were run to estimate the range of quadrupolar and dipolar coupling constants for relevant lithium species, and SIMPSON simulations were run to predict the excitation efficiencies of different multiple-quantum transitions.

We then implemented an operando double quantum filter experiment and demonstrated its application to lithium–sulfur batteries. Here, the reversible formation of solid Li_2S on the cathode could be distinguished from irreversible degradation and we identified at which points during cycling degradation occurred in cells with and without the LiNO_3 electrolyte additive. This technique is broadly applicable to investigations of SEI formation, degradation, and conversion chemistries in lithium battery systems.

Magnetic resonance spectroscopy and imaging of ionic liquid-based electrolytes in zinc-ion batteries.

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Beyond lithium- and sodium-ion batteries, zinc-ion and zinc-air batteries emerge as exceptionally promising next-generation energy storage systems offering the advantages of low cost, high safety, environmental sustainability, and high energy density. However, aqueous caustic electrolytes are currently dominating these systems despite significant drawbacks: toxic harsh reagents, low electrochemical windows and reduced current efficiencies (lower cell voltages). Consequently, green alternatives, like - ionic liquids (IL), are essential for advancing battery design and performance. In this study, we have harnessed the power of magnetic resonance spectroscopy and imaging to directly visualize and characterize the real-time distribution, transport, and deposition of Zn^{2+} ions within an electrochemical cell containing zinc triflate salt dissolved in imidazolium-based ionic liquid. The triflate anion (TfO^-) emerges as a critical player, significantly controlling both the kinetics and thermodynamics of Zn^{2+} ion behaviour in solution. Relaxation and diffusion NMR measurements proved instrumental in revealing the molecular interactions and dynamic behaviour of both cationic and anionic species. We have demonstrated that ^1H and ^{19}F nuclear relaxation times (T_1 and T_2) are sensitive to Zn^{2+} ion concentration and even trace amounts of water - the latter having a pronounced impact on zinc deposit quality at the electrode surface. Through ^1H and ^{19}F MRI, we have visualized real-time Zn-dissolution and deposition processes via T_1 and T_2 relaxation maps as contrast mechanisms. Thus, we have unravelled the detailed dynamic interplay of all the ionic species during Zn-electrochemical processes via *in-operando* and *in-situ* relaxation imaging techniques.

Exploring Interfacial Interactions in Ammonium Ionic Liquids: From Molecular Dynamics to Water Remediation

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Ionic liquids (ILs) represent a promising media for removal of various contaminants thanks to their structural diversity and tuneable physicochemical properties, which are enabled by the wide range of cation-anion combinations. Appropriately designed ILs with tailored hydrophobicity, interfacial activity, and extraction performance have already demonstrated high efficiency in extraction and purification applications, making them attractive for nano- and microplastics removal as well. Understanding interfacial interactions in ILs is essential for linking molecular organization and dynamics with their performance in complex systems. Based on our earlier studies on alkyl chain length-dependent ILs' nanostructuring and molecular dynamics, we investigated a broader group of ammonium ILs in bulk and at solid interfaces by means of FFC NMR relaxometry, NMR diffusometry, FTIR, DLS, and SAXS. Particular attention was given to IL-microplastic and cellulose-immobilized IL-microplastic systems in order to identify changes in mobility and interfacial structuring induced when in contact with a polymer surface.

The combined results reveal dynamically distinct IL fractions and their exchange between bulk-like and interfacial environments, highlighting the role of chemical structure in governing these interactions. The results show that cation architecture strongly affects IL dynamics and interfacial response, which ultimately contributes to the molecular understanding of ionic liquid behaviour at polymer interfaces and supports the design of ionic-liquid-based systems for microplastic capture and water remediation.

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^{29}Si and ^1H paramagnetic NMR structural study of chrysocolla, an amorphous copper silicate: application to archeological copper metallurgy.

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This work was conducted as part of an archaeological study of pre-Hispanic metallurgical processes. At Ujina-Collahuasi (Tarapacá, Chile) an Inca mining camp and over 50 furnaces were discovered, constituting the largest known pre-Hispanic copper production site in northern Chile. This unique archaeological site provides valuable insights into the functioning of furnaces employed to reduce ores into metal in the pre-Hispanic metallurgical processes. Among the copper ores processed in these furnaces, chrysocolla is one of the main minerals - a natural amorphous paramagnetic "mineraloid" with a proposed formula unit of $\text{Cu}_2\text{H}_2(\text{Si}_2\text{O}_5)(\text{OH})_4 \cdot n\text{H}_2\text{O}$. The local structure of chrysocolla remains poorly understood and is still a subject of debate. This mineral has even been described as a nanoscale association of silicates and copper hydroxides.

To gain a better understanding of chrysocolla's local structure, we performed ^1H - ^{29}Si NMR correlation experiments with a TEDOR sequence using adiabatic double echoes —primarily to ensure proper inversion of the broad ^{29}Si signals (spanning ~ 1000 ppm). These experiments evidenced nuclear dipolar interactions between ^1H and ^{29}Si . We also performed ^{29}Si variable-temperature (VT) NMR experiments and combined the NMR results with bulk magnetic susceptibility measurements. These experiments revealed the presence of a hyperfine coupling between the paramagnetic copper ions and silicon atoms.

Our NMR results clearly demonstrate that protons, silicon, and copper atoms coexist in the same phase in chrysocolla at the atomic length scale. These results provide useful information for a better understanding of the thermal decomposition of chrysocolla, enabling a more accurate description of the archaeological metallurgical process.

ABSTRACT MISSING

Metabolomics

Exhaled Breath Condensate and Urine NMR Metabolomics Reveal Metabolic Signatures of Asthma in a Birth Cohort Study

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Abstract

In this study, nuclear magnetic resonance (NMR)-based metabolomic analyses of urine and exhaled breath condensate (EBC) samples were integrated to identify potential biomarkers for asthma. The investigation was conducted among asthmatic and non-asthmatic participants from a birth cohort at their “age 8” visit. Ethanol and propylene glycol in EBC, and citrate, hippurate, and glucuronate in urine exhibited differential trends in the two groups. Pathway analysis indicated the involvement of ascorbate and aldarate metabolism, pentose and glucuronate interconversions, and the citrate cycle. The strongest correlation was observed between methanol in EBC and trigonelline in urine. These findings suggest that multi-biofluid NMR metabolomics represents a useful strategy for advancing the understanding of asthma pathophysiology.

Keywords NMR, metabolomics, asthma, exhaled breath condensate, urine

Distinct Urinary Metabolic Signatures Differentiate Adult ADHD Subtypes and OCD

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Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental condition characterized by inattention, hyperactivity, and impulsivity. In adults, these symptoms appear more subtle than in children, which complicates the diagnosis. In addition, the high rate of comorbidities, including obsessive-compulsive disorder (OCD), may result in overlapping symptoms between ADHD and OCD, obscuring accurate identification and treatment of each condition. Furthermore, the heterogeneity of ADHD presentations in adults and considerable variability in treatment response further complicates diagnosis and personalized management. In this context, ^1H NMR-based metabolomics offers a promising avenue for improving diagnostic precision through detailed metabolic profiling. This study aimed to identify metabolic differences in urine among ADHD presentations, OCD and healthy controls with the goal of enhancing diagnostic accuracy and understanding of these overlapping disorders. A total of 105 urine samples were analysed. Significant metabolic differences were observed among all groups, including between ADHD presentations and when comparing each with OCD and healthy controls. Altered levels of microbiota-related metabolites (phenylacetylglycine, p-cresol sulfate, formic acid), amino acid derivatives linked to neurotransmission (threonine, *N*-acetylglutamine, taurine), and other metabolites associated with energy metabolism (creatinine, 2-propanol) suggest disruptions in gut–brain axis signalling, excitatory/inhibitory balance, and cellular energy homeostasis. These findings highlight distinct metabolic signatures across ADHD subtypes and OCD, supporting the quantification of selected metabolic biomarkers as a complementary tool to improve differential diagnosis and to advance the understanding of the neurobiological mechanisms underlying these disorders.

From 600 to 100 MHz: Developing Reliable Benchtop NMR Protocols for Urine Metabolomics

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Metabolomics is the comprehensive analysis of low molecular weight metabolites in a biological system, providing insight into health and disease by reflecting genetic and environmental interactions(1). Among analytical techniques, Nuclear Magnetic Resonance (NMR) spectroscopy is valued for robustness, reproducibility, and minimal sample preparation. High-field NMR spectrometer operating at 600 MHz remains the gold standard(2), but its high cost and infrastructure requirements limit clinical use. Portable low-field benchtop NMR systems offer a promising cost-effective alternative(3).

We developed standardized protocols for urine metabolomics combining high-field (600 MHz) and low-field (100 MHz) NMR spectroscopy. To overcome solvent suppression issues and low sensitivity of low-field NMR instruments, urine samples were lyophilized and reconstituted in D₂O, enabling water removal and sample concentration.

This work presents optimized procedures for urine sample preparation, lyophilization, and NMR acquisition. Urine samples were systematically evaluated for lyophilization time, storage conditions, and reconstitution protocols to establish a robust and reproducible workflow.

Samples were acquired at both low- and high-field NMR to validate protocol reliability. Statistical analyses identified the most suitable strategy. Lyophilization for 18-24 h was sufficient, whereas 6 h was inadequate. A 3 M KH₂PO₄/K₂HPO₄ buffer in D₂O improved pH stabilization across samples. Vortexing followed by centrifugation under controlled conditions was optimal, and acquisition and spectral processing parameters were refined.

These results provide a reliable methodological framework for benchtop NMR metabolomics and support integrating low-field NMR systems into routine workflows.

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Predicting Sepsis Prior to Clinical Onset using NMR-based Lipidomics and Metabolomics.

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Sepsis is a dysregulated host inflammatory response to infection that leads to organ dysfunction and is associated with high mortality worldwide. Its heterogeneous nature and rapid, time-dependent progression highlight the need for early detection; however, identification close to the time of admission remains limited with current diagnostic tools. NMR-based metabolomics and lipidomics offer a promising strategy for early prediction and detection.

In this study, we evaluated the potential of NMR metabolomics and lipidomics to predict sepsis in a clinical emergency setting.

A total of 173 patients admitted to the Emergency Department of Hospital Parc Taulí (Sabadell, Spain) with suspected infection were included. In total, 291 serum samples were collected at three time points (0, 4, and 24 hours) after admission. Lipidomic analysis was performed on untreated serum using benchtop Bruker Fourier80 spectrometer. Metabolomic analysis was carried out on ultrafiltered samples using both 500 MHz Bruker AVIII and benchtop Fourier80. Spectral processing was performed using Mnova software, and multivariate statistical analyses were conducted with MetaboAnalyst 6.0.

Partial Least Squares Discriminant Analysis (PLS-DA) was used to differentiate patients who developed sepsis from those who did not. High-resolution NMR data enabled discrimination with an accuracy of 72% ($R^2 = 0.87$, $Q^2 = 0.13$) and an AUC-ROC of 0.78, identifying 18 discriminant metabolites. Benchtop NMR analysis identified 19 lipoprotein parameters and 8 metabolites, achieving a classification accuracy of 73% ($R^2 = 0.29$, $Q^2 = 0.20$) with an AUC-ROC of 0.76.

These results highlight the potential of benchtop NMR spectroscopy as an accessible and rapid strategy for the early prediction of sepsis in emergency care settings.

METABOLIC FINGERPRINTING OF BOVINE TUBERCULOSIS: TRANSLATING BENCHTOP NMR FROM LAB TO BARN.

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Bovine tuberculosis diagnosis is based on the detection of the host immune response, primarily through tests such the interferon-gamma release assay (IGRA). However, this response can be influenced by multiple factors, including exposure to environmental mycobacteria, which may lead to false-positive results and reduced diagnostic specificity.

NMR-based metabolomics represents a promising approach for identifying metabolic profiles that could improve bovine tuberculosis diagnosis. Its application has traditionally been limited by high costs and infrastructure demands. However, the emergence of benchtop NMR spectrometers offers a novel and accessible alternative.

The aim this study was to identify metabolomic biomarkers associated with bovine tuberculosis infection and to evaluate the implementation of benchtop NMR.

Two groups were analysed: the first comprised calves from herds with confirmed bovine tuberculosis ($N = 20$), classified as true positives; the second included calves from tuberculosis-free herds that tested IGRA-positive ($N = 20$), classified as false positives. NMR-based metabolomic analysis were performed from plasma samples with high-field (Bruker AVANCE III 500 MHz) and low-field (Magritek Spinsolve Ultra 80 MHz) spectrometers.

Partial Least Squares Discriminant Analysis (PLS-DA) enabled discrimination between true-positive and false-positive animals, achieving an accuracy of 92.5% using high-resolution NMR and 90% with benchtop NMR spectrometers. The high-field approach fulfilled an AUC-ROC of 88.1%, with 89% sensitivity and 81% specificity, while benchtop NMR achieved an AUC-ROC of 92%, with 94% sensitivity and 86% specificity.

These findings highlight the potential of NMR-based metabolomics as a more specific diagnostic approach for bovine tuberculosis.

Exploring the urinary metabolomic fingerprint of human cytomegalovirus: a ^1H -NMR study on congenitally infected newborns

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Gallo Angelo

Human cytomegalovirus (HCMV) is the leading cause of congenital infections resulting in severe morbidity and mortality among newborns worldwide. Currently, the most significant prognostic factor of cCMV is the time of maternal infection, with a more severe clinical phenotype if the mother's first outbreak occurs during the first trimester of pregnancy. Nonetheless, the pathogenesis of cCMV infection has still to be completely characterized. Little is known about the metabolic response triggered by HCMV in congenitally infected newborns. As such, urinary metabolic profiling by ^1H -NMR might represent a promising tool to be exploited in the context of cCMV infection.

This study aims to investigate the impact of HCMV infection on the urine metabolome in a population of newborns by ^1H -NMR spectroscopy combined with multivariate statistical analysis.

Thirty-three newborns diagnosed with cCMV infection were recruited at the Neonatal Unit of the University of Turin and for each of them a urine sample was collected during admission medical examination. Seventeen healthy newborns without cCMV infection were also included as healthy controls.

The ^1H NMR spectra of patients ($n=35$) and controls ($n=15$) allowed the identification of an overall amount of 55 metabolites. Principal Component Analysis (PCA) and clustering correctly assigned 49 out of 50 newborns into the infected and control groups. Partial Least-Squares-Discriminant Analysis (PLS-DA) revealed that newborns with cCMV resulted in having increased betaine, citrate, 3-hydroxybutyrate, 4-hydroxybutyrate, acetoacetate, formate, glycolate, lactate, succinate, and threonine levels in the urine. On the other hand, healthy controls showed increased 4-aminohippurate, creatine, creatinine, fumarate, mannitol, taurine, and dimethylamine levels. These results showed a clear difference in metabolomic fingerprint between newborns with cCMV infection and healthy controls. Thus, metabolomics can be considered a new, promising diagnostic and prognostic tool in the clinical management of cCMV patients.

Serum metabolic and lipoprotein signatures differentiate young ischemic stroke from stroke mimics

Tristán, Ana Isabel, García-Mañas, Celia, González-Lázaro, Marta, Abreu, Ana Cristina, Amaya-Pascasio, Laura, García-Pinteño, José, Martínez-Sánchez, Patricia, Fernández, Ignacio.

Ischemic stroke in young adults represents a growing clinical challenge. In emergency settings, several conditions known as stroke mimics present neurological symptoms similar to ischemic stroke, complicating rapid and accurate diagnosis. Identifying metabolic alterations associated with these conditions may improve understanding of stroke pathophysiology and support differential diagnosis. Here, serum NMR-based metabolomics was applied to characterize metabolic differences among young ischemic stroke patients, stroke mimics, and healthy controls.

Serum samples from individuals under 55 years of age were analyzed using ^1H NMR spectroscopy. Metabolite and lipoprotein profiles were evaluated through multivariate and univariate statistical approaches. Partial least squares discriminant analysis (PLS-DA) showed group separation, while linear models adjusted for age and sex were used to assess metabolite associations. Corresponding fold change and receiver operating characteristic (ROC) analyses were performed to evaluate diagnostic performance.

Significant metabolic differences were observed between ischemic stroke patients and controls, including alterations in lipoprotein subclasses and metabolites related to energy metabolism, such as phenylalanine, fatty acids, 3-hydroxybutyrate, and acetoacetate. In addition, the supramolecular phospholipid composite (SPC) emerged as a discriminant feature. Comparisons between stroke mimics and controls revealed differences in amino acids, glucose, citrate, fatty acids, and lipid profiles. Distinct metabolic signatures were also identified between stroke mimics and ischemic stroke, including SPC, lipoproteins, glutamine, glutamate, and glycerol, showing moderate discriminative performance (AUC 0.60–0.80).

NMR-based serum metabolite and lipoprotein profiling for endometriosis across clinically relevant and physiological comparator settings

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Background: Reliable non-invasive biomarkers for endometriosis remain unavailable in routine practice. We evaluated ¹H NMR-based serum metabolite and lipoprotein profiling at 600 MHz for endometriosis across clinically relevant and physiological comparator settings, with parallel exploratory analyses of systemic biological variation.

Methods: Serum samples from women with surgically confirmed endometriosis, symptomatic controls, and healthy volunteers underwent a quantitative in vitro diagnostics research (IVDr) NMR platform, able to absolutely quantify up to 38 metabolites and 112 lipoprotein parameters. Performance was assessed using fully nested repeated cross-validation and an independently processed temporal cohort. Parallel exploratory analyses included covariate-adjusted group comparisons, weighted correlation network analysis and paired pre-/post-operative comparisons.

Results: While discrimination was high comparing endometriosis against healthy controls, (AUC 0.994), diagnostic performance dropped when comparing endometriosis against symptomatic controls (AUC 0.620). The clearest biological differences were characterized by decreased amino acids (aromatic and branched chain amino acids, alanine) and LDL6 lipoprotein subfractions. Part of the amino-acid pattern was also present in symptomatic controls, consistent with shared symptom-state physiology.

Conclusions: NMR-based serum metabolite and lipoprotein profiling showed strong apparent discrimination against healthy volunteers, while performance was limited in the clinically relevant symptomatic-control cohort. The decrease of LDL6 particles in endometriosis warrants deeper lipidomics investigations also on the tissue level. Such work is currently in progress.

NMR foodomics of Austrian honey.

Mares, Jiri, Gheddou, Lynda, Orts, Julien.

NMR provides valuable analysis of the honey composition. The composition depends on many factors, such as geographical location, flowers or other feeding sources of the bees, but possibly also additives not belonging to the honey. There are therefore different reasons for being able to analyze the honey to know whether it is a frauded one or not, or to learn something about specific compounds corresponding to particular floral origin and possibly other conditions.

Here we present analysis of honey species collected across Austria, with known floral origin, geographical origin, parasite treatment and several other attributes. These all are evaluated in the statistical analysis of the measured spectra, using several different methods, such as PCA from unsupervised methods and OPLS-PLS from the supervised methods. Comparison with frauded honey samples is also presented.

Fast Diffusion-Based Metabolomics Profiling by 3D Pure Shift**HSQC-DOSY NMR with COLMARvista**

Cabrera Allpas, Rodrigo; Lee, Kyunsuh; Li, Dawei; Choo, Munki; Bruschweiler-Li, Lei;

Brüschweiler, Rafael

2D NMR-based metabolomics greatly enhances the untargeted metabolite identification in complex biological mixtures over 1D NMR due to its superior resolution. Nonetheless, for crowded spectral regions peak overlap remains a challenge. Here, we address metabolite ambiguities caused by (near-)degenerative chemical shifts by taking advantage of one of the metabolite-specific properties, namely the translational diffusion coefficient that can be measured via the DOSY method. To achieve optimal resolution and sensitivity, we present a pseudo-3D pure shift HSQC-DOSY experiment along with a novel pseudo-3D processing module implemented in the JavaScript-based COLMARvista software. The software allows the automated processing, peak picking, quantification, and visualization of pseudo-3D HSQC-DOSY datasets.

We demonstrate the effectiveness of the pseudo-3D real-time pure shift HSQC-DOSY experiment for various real-world metabolomics samples, including mouse urine, *Pseudomonas aeruginosa* biofilm extracts, and a carbohydrate mixture sample. This DOSY experiment offers significant improvement in both sensitivity and resolution over the standard pseudo-3D-DOSY experiment, yielding more precise diffusion coefficients of all cross-peaks belonging to the metabolomic mixture components even when using a minimal set of 2D planes. These tools allow the routine application of DOSY for the disambiguation of metabolites in complex metabolomics samples for the unique characterization of known and unknown metabolites.

Newly designed double BIRD filter for the Determination of ^{13}C -labeled Metabolites in Cell Culture Studies

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Inflammatory processes in humans are characterized by rapid and sustained metabolic adaptation. Peripheral blood mononuclear cells were cultivated in media containing ^{13}C -labeled glucose to trace metabolic flux through specific pathways. Cells were incubated under identical conditions with either unlabeled ^{12}C glucose or ^{13}C -labeled glucose for 96 hours in the presence of LPS to mimic inflammation.

Detection and quantification of low-concentrated ^{13}C -labeled metabolites was challenging due to the presence of unlabeled metabolites originating from the cell culture medium. 2D- ^1H - ^{13}C -HSQC spectra were measured for this purpose, however quantification of low-concentrated ^{13}C -labeled metabolites such as alanine was hindered by adjacent high-intensity peaks, particularly ^{13}C -labeled lactate, one of the major products of glucose metabolism. In addition, baseline distortion near the water frequency further complicated accurate quantification.

To address these limitations, a newly developed double BIRD-filter sequence was applied that selectively suppresses ^{12}C -bound protons. Combining the double BIRD experiment with ^{13}C decoupling during acquisition, clear baseline separation between both peaks could be achieved in substantially reduced acquisition time. Efficient water suppression was easily achieved without relevant baseline distortion. Alanine was successfully quantified even in the presence of lactate at concentrations up to 36-fold higher.

This enabled faster and more reliable high-throughput determination and quantification of alanine, thereby contributing to a better understanding of metabolic changes during inflammation and extending its applicability to broader metabolomics studies.

Determination of omega-3 fatty acids in red blood cells with ^1H -NMR spectroscopy

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In 2004, the Omega-3 Index (O3I) - defined as the sum of EPA + DHA in % of total fatty acids (FA) in red blood cells (RBC) - was first proposed as a risk factor for death from coronary heart disease. However, to date, there is no consensus as to which method is most suitable for the determination. The original developed HS-Omega-3 Index® focuses on FAs esterified in the membrane glycerophospholipids, and utilizes specific assay conditions (direct transmethylation with BF_3 -methanol at 100°C). Other methodologies focus on FAs esterified in glycerophospholipids and sphingolipids. This discrepancy underscores the need for a uniform, ideally standardized methodology for measuring the O3I.

In addition to analysis using GC-FID, ^1H -NMR represents a viable alternative and is already widely employed in the investigation of fatty acids in human plasma. The determination of fatty acids in erythrocytes using ^1H -NMR spectroscopy has been only sparsely reported in the literature due to the laborious sample preparation involved. We have therefore developed a new sample preparation method:

- instead of isolating membrane ghosts, the lipids were extracted directly after the haemolysis of erythrocytes, using a modified Bligh and Dyer method; and
- separation of cholesterol from the phospholipids with SPE.

Using this sample preparation method, DHA and the total omega-3 fatty acid content in $200\mu\text{l}$ RBC can be determined easily with a Bruker-Avance III-400 MHz-NMR instrument and a standard BBO-probe head, without a sophisticated line shape analysis.

Preliminary results demonstrate a very strong correlation of total omega-3 fatty acids with the HS-Omega-3 Index. Since the entire sample preparation is performed at room temperature, requires no chemical modifications of the fatty acids, and as ^1H -NMR is generally highly accurate for quantitative determinations, the new method can be utilized for the determination of the O3I-index.

Lyophilization-based protocol for Human Plasma metabolomics by benchtop NMR spectroscopy: optimization and validation

Giraldi, Valentina, Fantato, Linda, Vignoli, Alessia, Tenori, Leonardo

Metabolomics investigates the complete set of metabolites within a biological system, providing insights on its physiological and pathological states. NMR spectroscopy is a key technique in metabolomic studies, allowing the simultaneous identification and quantification of metabolites and lipoproteins in biofluids such as urine, serum and plasma. High-field instruments (600 MHz) are considered the gold standard, but low-field or “benchtop” NMR instruments are emerging as an alternative to potentially translate metabolomics into clinical practice. Despite their decreased sensitivity and resolution, they are in fact more compact, easier to use and have low maintenance costs.

In benchtop NMR-based biofluid analysis, conventional solvent suppression sequences often fail to adequately suppress the intense water signal. Sample lyophilization followed by reconstitution in deuterated solvents is a viable option. Herein, this approach was applied to human plasma samples, with parallel analyses on both 100 MHz benchtop and 600 MHz NMR instruments. The methodological study included the optimization of lyophilization time, reconstitution protocol and parameters for NMR acquisition and spectra processing. Moreover, the use of CPMG filter to suppress protein signals was compared to physical deproteinization strategies. The integrity of the reconstituted samples comparing to the original plasma samples was checked. Finally, short- and long-term storage of lyophilized samples was evaluated under four conditions (room temperature, 5°C, -80°C, -80°C after reconstitution) by multivariate statistical analysis such as Principal Component Analysis. In conclusion, the results indicate that lyophilization preserves plasma sample integrity while enabling reliable benchtop NMR metabolomic analysis.

NMR metabolomics studies in patients with galactosemia and methylmalonic aciduria

Nicolescu Alina, Uşurelu Natalia, Cristea Mihaela, Blăniță Daniela, Deleanu Calin

Long term monitoring by NMR urinomics of patients with galactosemia (GA) and methylmalonic aciduria (MMA) are presented.

The main metabolic markers for the two diseases, together with several non-specific metabolites have been monitored in the described cases for periods of over 10 years.

Thus, the levels of galactitol have been monitored in a girl, starting from 5 months old (age of diagnosis).

The levels of methylmalonic acid, carnitine and propionyl carnitine have been monitored in a boy, starting from 2 years old (age of diagnosis). Some episodes of decompensation leading to anememia have been identified by NMR using orotic acid and orotidine as markers. Based on an extended metabolomic study of the long-term monitored patient and other MMA patients, a urine NMR metabolomic tests was introduced. The NMR test is predicting the type of genetic mutation (MMAA versus MMUT) using the levels of glycine and methylmalonic acid in urine.

The NMR experiments included ^1H -noesy water suppression (for both signals assignments and quantification), 2D J-Resolve and 2D COSY (for signal assignments), as well as ^1H -CPMG and ^1H Diffusion (for assessing the presence or confirming the absence of proteinuria). The experiments have been performed in 5 mm Wilmad 507 NMR tubes using a Bruker Avance Neo 600 MHz spectrometer, equipped with a broadband inverse detection (BBI) probe and with full IVDr capabilities, employing B.I. Methods 2.5 (Bruker Biospin) for experimental parameters and SOPs. Both in-house quantifications and B.I. QUANT-UR (Bruker Biospin) models have been used.

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A systematic variability assessment of plasma and serum NMR lipoprofiling

Deleanu Calin, Nicolescu Alina, Duduianu Cătălin, Cristea Mihaela

The study explored the variability of NMR lipoprofiling from 300 blood samples collected in 3 types of standard blood collection tubes. The tubes have been the classical K3EDTA plasma lavender coloured tube caps, no additive serum red coloured tube caps, and gel serum yellow coloured tube caps.

A panel of over 100 NMR metabolomics and lipidomics parameters have been evaluated. A good reproducibility of results for the two types of serum separation tubes, i.e. red and yellow labelled caps, has been observed. On the other hand, a systematic shift in determined concentrations for several parameters is observed in the case of plasma separation tubes, i.e. lavender labelled caps.

We have also performed a reproducibility study, using the same types of NMR tubes, several operators, and two NMR spectrometers which indicated a maximum variability of results of maximum $\pm 2\%$. This is within the IVDr methods quantification specifications of the NMR manufacturer. Thus, the identified systematic shifting of results between plasma and serum may be confidently assessed to differences of concentrations between plasma and serum and not to the NMR method.

The blood samples from 300 healthy voluntaries have been collected in tubes type Vacutest, Kima. The experiments have been performed in 5 mm Wilmad 507 NMR tubes using a Bruker Avance Neo 600 MHz spectrometer, equipped with a broadband inverse detection (BBI) probe and with full IVDr capabilities, employing B.I. Methods 2.5 (Bruker Biospin) for experimental parameters and SOPs. The B.I.LISA, B.I.QuantPS2.0 and PhenoRisk PACS processing packages (Bruker Biospin) have been used for quantitation of metabolomics and lipidomics parameters.

This study was supported by a grant from the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project PN-IV-P2-2.1-TE-2023-1710, within PNCDI IV.

Mixing designs for NMR metabolomics reconstruction

Malmodin, Daniel, Bay Nord, Anders

Hadamard-based sample mixing has recently been proposed in NMR metabolomics to reduce spectral variability while enabling reconstruction of individual. Mixing samples prior to acquisition reduces chemical-shift differences caused by pH and matrix variation, improving spectral alignment and comparability. Previous work used strict orthogonal Hadamard designs. Here, we investigate relaxed and generalized mixing matrices that retain invertibility while allowing more flexible sample combinations and further reduced chemical-shift differences between measured mixtures.

Beer is used as a proxy for urine metabolomics, providing chemically complex spectra with realistic inter-sample variation in a reproducible model system. Sample mixing is done with an Opentrons OT-2 liquid-handling robot, improving reproducibility and robustness compared to manual pipetting.

Potential advantages include overdetermined reconstruction through acquisition of more mixtures than original samples, enabling estimation of errors not accessible in conventional one-spectrum-per-sample acquisition and improve robustness to noise and artifacts. Potential disadvantages include signal loss during reconstruction by addition/subtraction and intensity distortions caused by nonlinear effects in mixed samples. Different mixing matrices and reconstruction strategies are evaluated using simulated and experimental NMR data. The work aims to extend encoded mixing approaches beyond classical Hadamard schemes and guide future experimental design in NMR metabolomics.

NMR-based metabolomic signatures of serum and adipose tissue distinguish lipedema from overweight/obesity.

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Lipedema is a chronic adipose tissue disorder characterized by disproportionate fat accumulation, pain, and edema, frequently misdiagnosed as obesity. The aim of this study was to identify metabolic alterations associated with lipedema using NMR-based metabolomics of serum and adipose tissue.

Serum samples from age-matched women with lipedema (n=55) and overweight/obesity (n=52) were analyzed by ¹H NMR spectroscopy. Adipose tissue samples were additionally analyzed by HR-MAS NMR spectroscopy in a subgroup (40 lipedema, 52 controls). Statistical analyses included adjustment for BMI, metformin use, and statin treatment.

Compared with the overweight/obesity group, lipedema patients showed lower waist circumference, WHR, triglycerides, LDL cholesterol, and higher HDL cholesterol despite increased lower limb volume and pain symptoms. Serum metabolomics revealed significantly decreased scyllo-inositol, choline, formate, adipic acid, threonine, glucose, and lipid-associated signals, together with increased lactate, glycerol, glycine, proline, ornithine, and citrate. Several metabolites remained significant after BMI and medication adjustment, indicating that these alterations were not solely obesity-related. Scyllo-inositol showed strong discriminatory performance (AUC up to 0.83).

HR-MAS NMR analysis of adipose tissue demonstrated reduced n-3 polyunsaturated fatty acid chains and eicosapentaenoic acid (EPA) in lipedema tissue, suggesting altered lipid composition and adipose tissue dysfunction.

These findings demonstrate that lipedema is associated with a distinct metabolic phenotype differentiating it from overweight and obesity. NMR-based metabolomics may support future biomarker discovery and improve understanding of lipedema pathophysiology.

Dynamic Metabolic Analysis of *in vitro* Models by microfluidic NMR

Wenxin Long, Leon Middendorf, Annamarija Raic, Rainer Glass, Marcel Utz, Sylwia J. Barker

Understanding dynamic metabolic processes in living biological systems requires analytical tools capable of repeated measurements while preserving physiological conditions. Here, we present an automated workflow that integrates microfluidic culture with nuclear magnetic resonance (NMR) spectroscopy, enabling longitudinal metabolic analysis of living *in vitro* models with high temporal resolution.

The platform combines a microfluidic culture chip with a custom stripline-based NMR microprobe optimised for microlitre-scale samples (~5 μL). To improve reproducibility and throughput, we incorporated an automated chip insertion mechanism based on a rack-and-pinion assembly driven by a stepper motor. This automated approach removes the delays and variability associated with manual handling and enables repeated measurements at intervals as short as three minutes.

We demonstrate the capabilities of the platform using patient-derived glioblastoma spheroids. The system enables continuous monitoring of extracellular metabolic profiles while maintaining the biological model within a controlled environment between measurements. Quantitative analysis of the acquired spectra resolves more than a dozen of metabolites, and measurements performed across multiple chips show consistent spectral quality without loss of resolution.

By combining automated sample handling, microfluidic culture, and non-destructive NMR analysis, this platform provides a scalable approach for high-throughput metabolic studies of living *in vitro* models. The methodology is well suited for applications requiring longitudinal monitoring of dynamic metabolic responses, including drug screening, toxicity studies, and investigations using patient-derived tumour models.

Inter-platform comparability of NMR-derived lipid biomarkers and validation against clinical chemistry

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Background

¹H-NMR lipoprotein profiling is an inexpensive approach claiming to deliver highly reproducible data aligned for clinical diagnostics. Nightingale Health is a major supplier which has generated NMR data in large population cohorts including UK-Biobank and SCAPIS, while Bruker IVDr is a competing platform provided by the Swedish NMR Center. We conducted a pilot study to assess inter-platform comparability and agreement with clinical chemistry to support decisions on platform selection for research applications.

Methods

Fifty fasting blood samples from the SCAPIS pilot study were used. Clinical chemistry analyses, including direct LDL-cholesterol, were performed on serum samples at the Sahlgrenska University Hospital laboratory. NMR-based lipoprotein profiling was performed on plasma samples at the Swedish NMR center and Nightingale Health. Agreement with clinical chemistry was assessed using Pearson correlation coefficients and Bland-Altman analysis, with bias defined as NMR platform - clinical chemistry.

Results

Lipid measures available across both NMR platforms and clinical chemistry were LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B. Despite correlations between platforms, Bland-Altman analyses revealed substantial systematic bias. Nightingale reported consistently lower lipoprotein concentrations than both clinical chemistry and Bruker IVDr. Mean absolute and relative differences for Nightingale vs clinical biochemistry were -1,96 mmol/L (-73%) for LDL cholesterol, -0,36 mmol/L (-21%) for HDL-cholesterol and -3,37 mmol/L (-84%) for total cholesterol. In contrast, Bruker IVDr demonstrated closer agreement with clinical biochemistry with mean differences of -0,06 mmol/L (-2%) for LDL-cholesterol, -0,10 mmol/L (-4%) for HDL-cholesterol and 0,25 mmol/L (4%) for total cholesterol. Triglycerides demonstrated acceptable agreement across platforms.

Conclusion

The two NMR platforms are not interchangeable. The marked underestimation of LDL-cholesterol by Nightingale indicates that results cannot be interpreted against established clinical thresholds. The systematic platform-dependent bias suggests calibration differences, with Bruker IVDr demonstrating closer agreement with conventional clinical chemistry.

Small molecules / Drugs

PAIR DISTRIBUTION FUNCTION “FEAT” SOLID-STATE NMR: A COMBINED APPROACH FOR STRUCTURE DETERMINATION OF ORGANIC MOLECULES

Bravetti, Federica, Rosso, Chiara, Chierotti, Michele R.

The properties of solid compounds are determined by their solid-state structures. This is of particular relevance in the development of new materials, i.e., drugs and organic pigments. For nanocrystalline organic compounds (ordering lengths of 5–40 nm), the standard methods for structure determination fail. In the last decades a new technique has been established for the analysis of nanocrystalline substances: the analysis of the pair distribution function (PDF). Whilst being well established for inorganic compounds, organic compounds are rarely investigated by PDF analysis. Piepenbring and co-workers developed a method called ‘Global-PDF-Fit’, which allows for the determination of crystal structures of organic compounds from scratch by a global fit to the PDF, without prior knowledge of lattice parameters and space group. Nevertheless, there are cases for which the Global-PDF-Fit method does not yield a unique solution, but several different structures, which all fit the experimental PDF data equally well. Thus, we are developing a method to determine the crystal structures of organic compounds by combining a PDF fit with solid-state nuclear magnetic resonance (SSNMR), to achieve a faster and more reliable method than the existing ones to apply to such systems. 1D and 2D SSNMR experiments can provide precise information on the local structure, such as tautomeric character, hydrogen-bond network, intra- and intermolecular atom-atom proximities and precise atom-atom distances. All this information can be used as restraints for the generation of the structures, for the PDF fit and as additional parameters structure filtering and selection. Here we are presenting the results obtained so far for nanocrystalline organic compounds of pharmaceutical interest.

Glancing the African jungle through the eyes of NMR: Towards the discovery of antimalarial compounds

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Effective utilization of medicinal plants in alleviating infectious diseases requires the combined efforts of scientists in different disciplines. The role of a phytochemist is to isolate and characterize constituents of medicinal plants and make these natural products available to biological scientists to establish the activity of these compounds against different infectious agents. In this regard, our laboratory, at the Department of Chemistry, University of Nairobi, has provided hundreds of compounds for testing. In the process, we can train over a hundred postgraduate students towards MSc and PhD degrees. In this presentation, phytochemical and biological activity results on two *Tephrosia* species will be presented.

In the first example, the CH₂Cl₂/MeOH (1:1) extract of the stems of *Tephrosia uniflora* yielded a new β -hydroxydihydrochalcone (*S*)-elatadihydrochalcone-2'-methyl ether along with three known compounds: elongatin, (*S*)-elatadihydrochalcone, and tephrosin¹. The structures were elucidated by NMR spectroscopic and mass spectrometric data analyses. Based on the comparison of literature data with that obtained experimentally, and with computationally predicted NMR data, we propose the revision of the structures of three β -hydroxydihydrochalcones to flavanones. Five new compounds, rhodimer, rhodiflavan A, rhodiflavan B, rhodiflavan C, and rhodacarpin - along with 16 known secondary metabolites- were isolated from the roots of *Tephrosia rhodesica*. They were identified by NMR spectroscopic, mass spectrometric, X-ray crystallographic, and ECD spectroscopic analyses. The crude extract and the isolated compounds showed activity (100% at 10 μ g and IC₅₀ =5–15 μ M) against the chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*². The characterization and antiplasmodial activity of some of these compounds will be discussed.

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Investigation of Side Reactions in Industrial Hydrogenation Syntheses.

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Heterogeneously catalysed hydrogenations play a crucial role in the chemical industry, both in terms of large-scale commodity processes and in the synthesis of fine chemicals. In this context, the hydrogenation of 2-methyl-3-butyne-2-ol (MBY) to 2-methyl-3-buten-2-ol (MBE) is well recognised for its importance in the production of vitamins. However, this reaction is also accompanied by the formation of undesirable side-products, which can negatively affect selectivity and downstream purification. While the main reaction has been comprehensively documented in the extant literature, the side-products are only superficially characterised as “dimers”, and detailed structural information remains scarce. It is therefore evident that both the formation pathways and the chemical structures of these species have not been adequately elucidated to date. A more rigorous characterisation is imperative, as it constitutes a prerequisite for mechanistic understanding and, ultimately, for the optimisation of the overall hydrogenation process.

To address this gap, the side-products were separated from the main reactants and subjected to advanced analytical characterisation. Specifically, 1D and 2D high-field nuclear magnetic resonance (NMR) spectroscopy employing a cryogenically cooled probe head, in combination with gas chromatography–mass spectrometry (GC–MS), was utilised. Using this approach, a minimum of six distinct dimeric species could be identified. In addition, the formation of these side-products was investigated in dependence on the main reaction, thereby providing further insight into their occurrence under hydrogenation conditions.

Our results show that NMR spectroscopy is a valuable analysis method for gaining deep insights into industrial syntheses and providing knowledge that is essential for optimising the process.

Do binary liquids demix in mesopores? NMR and MD applied to silica pores from 4 to 50 nm

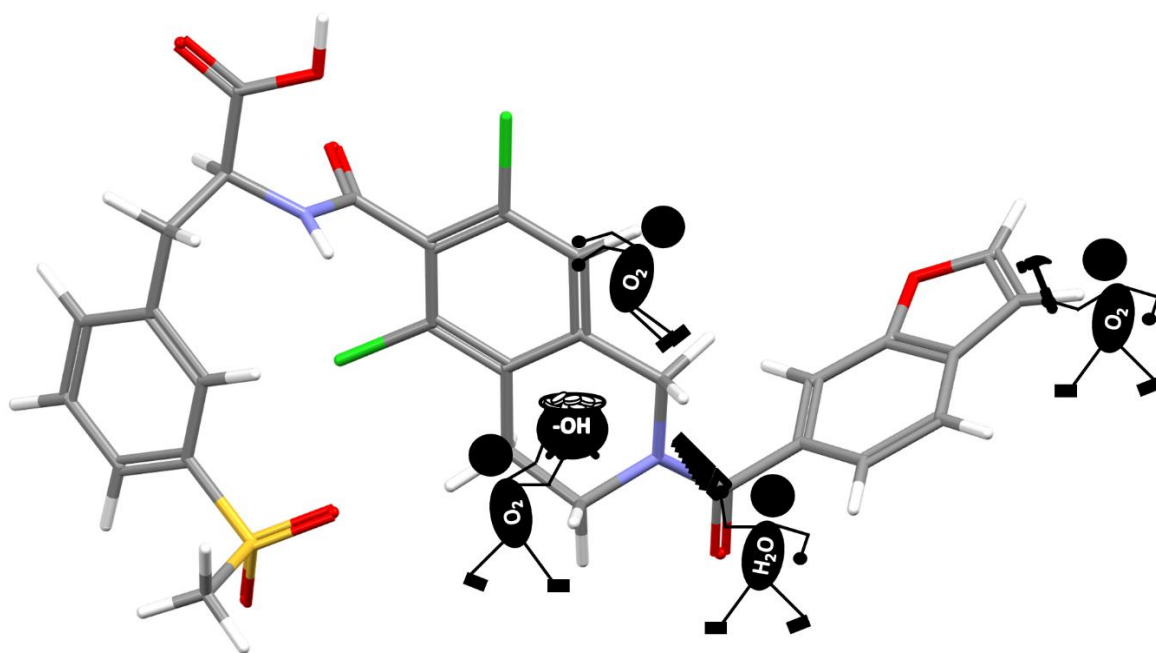
Stapf, Siegfried; Siebert, Niklas; Mattea, Carlos; Sara, Anam; Merle, Pascal; Nitsche, Felix; Dressler, Christian

Combinations of polar and non-polar liquids that are fully miscible in bulk were studied while saturating mesoporous media of different pore sizes. For the example of acetone and cyclohexane, a clear apparent phase separation was observed by MD simulations where acetone is preferably located at the polar silica wall and severely slowed down in its short-time translational dynamics; equivalent long-time diffusometry results by NMR agree in the sense that acetone diffusivity is reduced up to 1000 times compared to intraporous cyclohexane in 4 nm porous glass. The difference between adsorbed and non-adsorbed phases is much more striking in frequency-dependent T_1 relaxation where surface-mediated RMTD and bulk-like isotropic reorientation are clearly distinguished for several systems, including water and acetone. The equivalence of ^1H and ^2H relaxation properties again suggests RMTD, i.e. purely intramolecular relaxation mechanisms. Investigations in controlled-pore glass (CPG) of up to 50 nm pore diameter confirm the apparently complete phase separation for acetone and cyclohexane which is further corroborated by freezing experiments of the same system, whereas mixtures of two non-polar liquids, depending on individual molecule-surface affinity, cover the range from ideal mixtures to noticeable concentration gradients that also affect freezing behavior of the confined mixtures. Strategies are presented to quantify the degree of demixing by means of computed radial density functions and MSD as well as experimentally accessible relaxation and diffusion results.

Extreme oxidative degradation of Lifitegrast - explained

Anđela Buljan, Jadranka Buljević, Ivan Sušan, Leo Štefan, **Ana Čikoš**

Lifitegrast is relatively novel LFA-1 antagonist (approved by the FDA in 2016) provoking scientific and regulatory interest due to its outstanding targeted mechanism, safety profile and high efficacy in the treatment of dry eye disease. Owing to its high aqueous solubility, it is formulated as an ophthalmic solution for direct ocular delivery. During our studies we have extensively investigated the degradation of Lifitegrast under a range of stress conditions (from standard to extreme) and identified three structural weak spots linked to all discovered degradation products. Herein we present the overall mechanistic pathways for degradation of Lifitegrast, with special emphasis on the degradation products resulting from extreme oxidative and hydrolytic conditions (30% H_2O_2 at 50 °C and 75% RH for three months) performed aiming to discover Lifitegrast „end game“ degradation products.



Toward a New Era of Structure Elucidation in Flavor and Fragrance Molecules

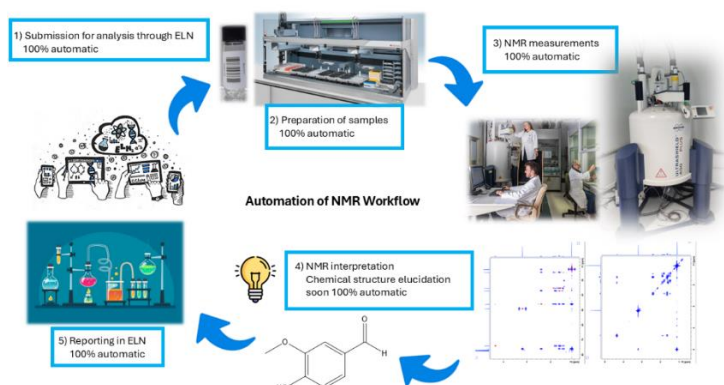
Simões de Almeida, Bruno¹

1. Analytical Sciences, Sciences & Research, dsm-firmenich, rue de la Bergère 7, 1242 Satigny, Switzerland

At dsm-firmenich, elucidating the molecular structures of flavor and fragrance compounds (usually small and volatile molecules) and their behavior in specific chemical environment are critical. It serves various purposes, from the development of new odorant ingredients to chemical mechanisms elucidation. After a highly selective process, some of the new odorant candidates we are developing will ultimately join our perfumers and/or flavorists' palette.

Our approach combines cutting-edge 1D and 2D NMR spectroscopy technology with complementary analytical methods (e.g GC-MS, LC-MS, and IR), associated with robust data analysis and computational tools. To improve our efficiency and throughput, we develop and implement new pulse sequences in liquid- and solid-state NMR (NOAH supersequences [1], multiCP [2]), automated workflows from sample preparation to processing, registration of our chemical structures in NMR database (Mnova [3]) and internally developed tools to automatically extract relevant information from NMR/MS spectra, significantly reducing the time required for interpretation. These processes not only enhance our understanding of the molecular basis of flavors and fragrances but also support the innovation and development of new compounds with desired sensory attributes.

The ongoing and combined efforts at dsm-firmenich in structure elucidation underscore our contribution to the science of flavor and fragrance, ultimately driving the creation of high-quality products that meet consumer preferences and industry standards.



1. Kupče, Ě.; Claridge, T. D. W. NOAH: NMR Supersequences for Small Molecule Analysis and Structure Elucidation. *Angew. Chem. Int. Ed.* 2017, 56, 11760-11764. <https://doi.org/10.1002/anie.201705506>.
2. Johnson, R. L.; Schmidt-Rohr, K. Quantitative Solid-State ¹³C NMR with Signal Enhancement by Multiple Cross Polarization. *J. Am. Chem. Soc.* 2014, 136, 908-912. <https://doi.org/10.1021/ja411347v>.
3. Mnova Database 15.1 from Mestrelab Research: [Mnova Software Suite - Mestrelab](#)

Deuterated Water Accelerates Phase-Separated Droplet Formation and Enables Directional Motion

Caihong Lin 1, Jingjing Yu 1‡, Dawei Qi 1,2‡, Xuncheng Shi 1, Tuomas Niemi-Aro 3, Jianwei Li 4*

Abstract:

The spatiotemporal coordination of compartment formation and directed transport is fundamental to cellular organization. However, replicating these coupled behaviors in fully aqueous synthetic systems remains a challenge. We report an isotopic-solvent signaling strategy that leverages the physicochemical differences between deuterated water (D₂O) and light water (H₂O) to control the liquid-liquid phase separation (LLPS) and motility of dynamic covalent droplets. Our system utilizes the *in situ* generation of cationic imine surfactants that complex with anionic macrocycles to form coacervate droplets. We demonstrate that D₂O significantly accelerates droplet nucleation and growth compared to H₂O by promoting early association and amplifying the hydrophobic interactions associated with imine surfactants. Furthermore, by establishing a spatial H₂O/D₂O gradient, we trigger an surface-tension imbalance that drives the directional transport of droplets from D₂O-rich to H₂O-rich regions via Marangoni flow. These motile droplets can move faster than those without isotope gradient and perform complex functions using fluorescent molecular probes. These functions include autonomous cargo transport and chemical exchange with their surroundings during migration. This work establishes isotopic substitution as a powerful and non-invasive trigger for governing supramolecular assembly and motility. It offers a new dimension for engineering adaptive, life-like soft matter.

Antibacterial macrozones: interactions with ribosome and bovine serum albumin monitored by NMR spectroscopy and molecular modeling

Jednačak Tomislav, Mikulandra Ivana, Habinovec Iva, Smokrović Kristina, Čipčić Paljetak Hana, Stepanić Višnja, Bertoša Branimir, Parlov Vuković Jelena, Novak Predrag

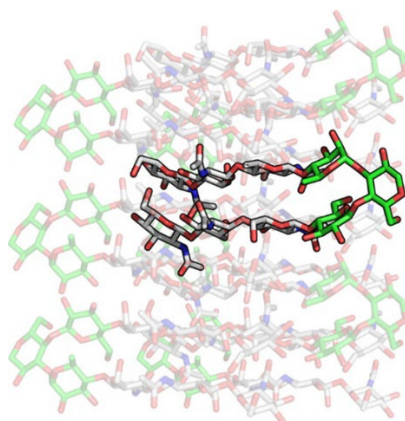
Azithromycin is a 15-membered macrolide antibiotic with broad spectrum of antibacterial activity and favorable pharmacokinetics. It inhibits the bacterial protein translation by binding to the 23S rRNA at the peptidyl transferase region of the bacterial ribosome. Despite its excellent antibacterial profile, some bacteria have acquired broad resistance, representing a global medical problem. Since the number of newly discovered macrolides is decreasing, a permanent strategy, which comprehends basic principles of interactions with bacterial ribosome, is required to ensure their efficacy. Conjugates of azithromycin and thiosemicarbazones, known as macrozones, have emerged as a promising class of macrolide derivatives with notable activity against resistant pathogens. Compared to azithromycin, the activity of 4"-macrozones has shown the greatest improvements against efflux-resistant *S. pneumoniae* and *S. aureus*, as well as very good activity against *E. faecalis*.

A combination of STD, WaterLOGSY and transferred NOESY NMR experiments and molecular docking provided insights into atomic details of interactions between macrozones and their biological receptors such as *E. coli* ribosome and bovine serum albumin. It has been demonstrated that both STD and WaterLOGSY gave consistent results unveiling atoms in close contacts with the receptors. QSAR calculations have shown that the antibacterial activity of macrozones is primarily determined by the position of the thiosemicarbazone side chain. These results can serve as a good basis to guide further medicinal chemistry efforts aimed at the discovery of more potent antibiotics.

Leveraging Glycan–Glycan interactions to tune the conformation of glycan hairpins and build rigid 3d architectures

Poveda, Ana; Yadav, Nishu; Vázquez-Mena, Yadiel; Weh, Dominik; Jiménez-Barbero, Jesús; Ogawa, Yu; Delbianco, Martina

Understanding glycan folding and aggregation remains a major challenge despite their fundamental biological and structural roles. Here, we present a reductionist approach based on a modular glycan hairpin scaffold that enables the systematic investigation of intra- and intermolecular glycan–glycan interactions. By incorporating oligomers representative of natural polysaccharides (cellulose, chitin, and xylan) as hairpin strands, we evaluated how strand composition influences conformational stability.



Molecular dynamics simulations revealed that glycan–glycan interactions strongly modulate hairpin rigidity, with chitin-based systems exhibiting highly compact and stable conformations, while xylan-based analogues remain flexible. Selected systems were synthesized via automated glycan assembly and structurally characterized in aqueous solution by NMR spectroscopy and SAXS. Experimental data confirmed the presence of interstrand hydrogen-bond networks, particularly in chitin-based hairpins, which act as noncovalent “zip-like” stabilizing elements. Overall, this work demonstrates that glycan hairpins provide a versatile platform to dissect glycan–glycan interactions and to design bioinspired supramolecular materials.

Structural revision of Thiotropocin and Tropodithietic acid in marine-like conditions

Skoven^{1,2}, Christoffer Durieux; Gram^{1,3}, Lone; Gottfredsen^{1,2}, Charlotte Held

¹CeMiSt, DNRF Center of Excellence, Søltofts Plads 221, Kongens Lyngby, 2800, Denmark

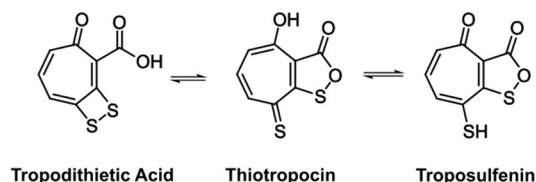
²Department of Chemistry, Technical University of Denmark, Kemitorvet 207, Kongens Lyngby, 2800, Denmark

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Marine bacteria are a significant source of natural products believed to mediate microbial interactions shaping cooperation, competition, signalling, and niche establishment. Several species from the marine bacterial genus *Phaeobacter* produces a range of important secondary metabolites. Among these, tropodithietic acid, (TDA), displays potent antibiotic activity and plays roles in both quorum sensing and biofilm formation.

TDA shares the molecular formula $C_8H_4O_3S_2$ with thiotropocin and the hypothetical troposulfenin (Fig 1). All three feature unusual heteroatom-rich chemical structures, that computational studies suggest can interconvert via tautomerism of a conjugate base. Our comparative analysis of published TDA and thiotropocin structural data shows that the reported ^{13}C chemical shifts of thiotropocin are inconsistent with related thioketones. They instead closely resemble those of TDA, differing only by minor reassignments. This finding suggests that thiotropocin may be a mischaracterised structure rather than a distinct constitutional isomer.

Figure 1:



The possible interconversion between these three species complicates efforts to correlate natural products with bacterial phenotype changes and, consequently, to assign ecological functions and roles. It remains unclear whether a single form predominates or whether multiple different structures coexist in marine environments. We aim to resolve this ambiguity and determine which tautomeric form(s) predominate under marine-like conditions using nuclear magnetic resonance (NMR) spectroscopy.

When TDA is examined under saline aqueous conditions designed to mimic marine environments, all three 1H resonances shift upfield. The signals shift unevenly and appear broadened, suggesting a change in the electronic structure. Whether this broadening comes from tautomerisation or salinity effects requires further investigation. Furthermore, these analyses reveal only a single detectable species, either due to one dominant form or rapid tautomerisation on the NMR timescale. These results show the importance of environmentally relevant conditions in structural assignments of ecologically relevant natural products.

Strategic Integration of Solution-State NMR in Commercial Drug Discovery

Theisgen, Stephan, AB, Eiso, Carneiro, Marta, Hollander, Johan

Target-driven drug discovery relies on robust data defining ligand-target interactions. While biochemical assays, biophysical methods (e.g., TR-FRET, SPR, thermophoresis), and AI modeling generate vast datasets, successful optimization requires structural context. NMR spectroscopy holds a distinct advantage over cryo-EM and X-ray crystallography by providing dynamic, solution-state insights across the early pre-clinical pipeline. It bridges data gaps through site-specific fragment screening, cryptic pocket identification, and solution-state hit validation. Furthermore, NMR rapidly validates computational models and X-ray structures while troubleshooting crystallization bottlenecks. Here, we showcase practical examples of strategic NMR integration in a commercial setting. We emphasize scalable, cost-effective, and rapid-turnaround workflows that overcome standard throughput limitations to drive small-molecule campaigns forward.

NMR structure elucidation of cyanine fluorescent dyes

Gemmecker, Gerd; Gebhardt, Christian; Lehmann, Martin; Reif, Maria M; Vanderlinden, Willem; Zacharias, Martin; Cordes, Thorben; Kolbeck, Pauline J; Lak, Aidin; Nikolaus, Thomas; Jan Lipfert

Fluorescent dyes are a widely used tool for probing and visualizing nucleic acids in a broad range of applications, from analysis and quantification of gel bands or PCR products to single-molecule and *in vivo* imaging.

Modern cyanine dyes, including the Alexa, AF and SYBR families, offer improved performance and broader optical tuneability compared to classical phenanthridinium dyes. SYBR Gold is a popular stain with very high sensitivity owing to the >1000-fold increase in fluorescence quantum yield on binding to DNA. However, their molecular structures are not known, so that their exact binding modes and effects on DNA structure and dynamics cannot be understood.

Based on 2D NMR ^1H , ^{13}C and ^{15}N measurements and MS data we could determine the structure of Alexa Fluor 555, AF 555 and SYBR Gold. The Alexa /AF family comprises a C3-linked conjugated double indole core with sulfonated sidechains. SYBR Gold has a benzoxazole-quinoline core like SYBR Green II, while the structure of SYTOX Green appears to be similar to that of PicoGreen, another known cyanine dye.

Based on the dye structures, it is possible to understand their spectroscopic and FRET properties and their interactions with nucleic acids. For SYBR Orange, magnetic tweezer studies and spectroscopic data indicate that its binding mode is based on intercalation, rather than minor-groove binding.

Chemical Biology and Fragment based screens using solution NMR

Weixiao Yuan Wahlgren, Ulrika Brath
CBCS GU node, SciLifeLab, The Swedish NMR Centre

Chemical biology comprises interdisciplinary efforts to understand and characterize interactions between biological targets and natural or synthetically produced molecules and to develop these findings in a biological context. NMR spectroscopy is well suited to studies of transient or strong interactions between specific protein targets and low molecular weight molecules such as natural products or lipids, or synthetically produced fragments, drug candidates or approved drugs. Fragment based drug discovery approaches are used for the design and development of novel lead compounds and NMR spectroscopy has emerged as a powerful method to study non-covalent protein-ligand complexes using ligand-observed techniques. Complementary, high resolution NMR spectroscopy studies of protein-ligand complexes can be made with protein target observed methodologies, for both low- and high-affinity complexes.

Fragment based screens (FBS) using NMR spectroscopy enables joint testing of a large number of fragments with low molecular weight (typically < 500 Da) against a target protein molecule or molecular complex. The Swedish NMR Centre is equipped with high field spectrometers (600-800 MHz) with cryogenically cooled probes optimized for either ^1H or ^{19}F -detection and SampleJet sample changers as well as liquid handling systems for sample preparations for FBS campaigns. With this setup, libraries of 1.000 fragments can be screened against a target protein molecule within days.

Interactions of Thioglycoside-Isochroman Derivatives with the LecA Protein Investigated by STD NMR and Molecular Docking

Tardi Zsuzsanna, Alnukari Samar, Ahmad Nawar, Tawfik Beshoy, Titz Alexander, Borbás Anikó, Timári István

LecA is a homotetrameric extracellular virulence factor of *Pseudomonas aeruginosa* that utilizes calcium-dependent carbohydrate recognition domains to selectively bind β -D-galactoside residues. This specificity facilitates key pathological processes, including adhesion, colonization, and biofilm maintenance. Consequently, LecA serves as a strategic target in drug discovery for developing inhibitors to address antibiotic resistance. To inhibit LecA, four galactose-based derivatives were investigated. The selected compounds, exhibiting the highest binding affinities in the series synthesized according to the competitive fluorescence polarization binding assays. Our purpose was to achieve an atomic-level characterization of the interactions between these ligands and LecA using NMR spectroscopy. Saturation Transfer Difference (STD) ^1H NMR experiments were performed to identify the atomic groups involved in binding to the LecA protein. The resulting epitope maps have revealed a highly similar binding mode across the compounds investigated. The glycosidic moiety consistently establishes the closest contacts with the protein in the bound state, indicating that region is likely oriented toward the core of the binding pocket. In addition, the aromatic protons also display strong STD intensities, suggesting proximity to the protein surface, which implies that the aromatic region of the isochroman scaffold contributes significantly to LecA binding. However, small differences in the overall STD epitope maps of the compounds suggest a slightly different binding mode of the α - and β -glycosides. The STD NMR results were compared with molecular docking simulations, revealing a good agreement between the experimentally determined binding epitopes and the predicted ligand binding modes.

Detection of PFAS at ultra-low concentrations using steady-state free precession ^{19}F NMR and Bayesian time-domain analysis

Andreas Erharter¹ and Daniel Häussinger¹

¹Department of Chemistry, University of Basel, Switzerland

Per- and polyfluorinated substances (PFAS) have been extensively employed across a range of industrial and consumer applications owing to their unique chemical and physical properties. However, their environmental persistence and propensity for bioaccumulation have raised significant concern for human health. Consequently, some representatives are regulated internationally through the Stockholm Convention on Persistent Organic Pollutants.

The widespread occurrence of PFAS in environmental matrices worldwide has intensified the demand for reliable analytical methodologies. In particular, discrepancies between total fluorine measurements and targeted LC-MS analyses highlight the limitations of standard mass spectroscopy methods in complex environmental samples.

In contrast, ^{19}F NMR spectroscopy enables untargeted and a virtually background-free detection of fluorinated pollutants. Although, ^{19}F is among the most sensitive spin 1/2 nuclei, NMR spectroscopy generally remains limited by comparatively low sensitivity. Recently, ^{19}F Steady State Free Precession (SSFP) techniques employing ultra-short acquisition times have demonstrated substantially improved detection limits since hundreds of acquisitions are gathered in the timespan of a single scan in traditional NMR experiments. However, data truncation in the latter requires advanced Bayesian time domain analysis, such as the complete reduction to amplitude-frequency table (CRAFT), as conventional Fourier transformation fails to preserve adequate signal-to-noise levels.

Herein, we report our efforts to detect ultra-low concentrations of PFAS in diverse sample matrices using the extraordinary combination of ^{19}F SSFP and CRAFT analysis.

Targeting Oncogenic KRAS with Natural Products:**An NMR-Based Screening Approach**

Katharina Maier, Micael S. Silva, Andreas Wasilewicz, Judith M. Rollinger & Julien Orts

Direct pharmacological inhibition of KRAS remains largely restricted to the G12C covalent mechanism, leaving a vast landscape of oncogenic variants, such as G13D, therapeutically unaddressed. We report the development of a robust NMR-based fragment screening platform designed to identify diverse chemotypes from natural product libraries that bind the native, GTP-bound active state of KRAS.

A significant challenge in characterizing active KRAS is the inherent conformational heterogeneity of the effector-binding Switch I and Switch II regions. By optimizing buffer conditions and modulating temperature to shift the chemical exchange regime, we significantly improved ^1H - ^{15}N HSQC spectral quality. This allowed for the near-complete sequence-specific backbone assignment of KRAS G13D in its active form, crucially including the residues of the Switch regions (S17, T20, and T35). Unlike traditional surrogates locked with non-hydrolyzable analogs (e.g., GMPPNP), our platform captures the physiological active-state ensemble, enabling precise, site-resolved ligand mapping.

Applying the platform to a curated set of 64 medicinal plant extracts, we identified two hit extracts that yielded five candidate binders representing flavonoid, furanocoumarin, and benzophenone chemotypes. Ligand-induced chemical shift perturbations localized engagement to the Switch I/II pocket of GTP-bound KRAS G13D, consistent with previously characterized fragments binding the same site in KRAS G12V. These outcomes validate the platform for natural-product-guided hit discovery against active KRAS and deliver promising scaffolds for subsequent optimization. It also allows the expansion beyond G12C-centric strategies to develop inhibitors effective against multiple variants.

Taming the Algebraic Instability of Molecular Conformation Analysis.

Ward, Luke; Kuprov, Ilya; Linclau, Bruno; Sinnaeve, Davy; Kieffer, Bruno; Carravetta, Marina

This work explores the application of machine learning to conformational analysis. So far, techniques for resolving conformation have relied on the Karplus equation. These however are limited by the periodic nature of the Karplus equation, wherein multiple conformational distributions may exist with identical sets of J -couplings.

To address these limitations, we used a neural network with DFT calculated energies and NMR parameters to predict conformational probability. Extensive conformational data sets were generated, after which relevant energetic and spectroscopic values were extracted and used to train the neural network to extract conformation probability distributions from experimental J -couplings.

We have used this approach to study population distributions of fluorinated proline conformers. The networks provide insight into the structural preferences of fluorinated proline residues within peptides and proteins. Such information is significant due to prolines role in modulating protein backbone geometry as well as secondary structural motifs.

Acknowledgements

This work is supported by the Leverhulme Trust via a studentship towards Luke Ward

Experimental and computational NMR of cluster C₆₀ endofullerenes

Bacanu George R., Ogino Anju, Legrady Bonifac M., Whitby Richard J., Ueno Hiroshi, Vaara Juha and Malcolm H. Levitt

The highly symmetric C₆₀ fullerene molecule has fascinated scientists ever since its discovery. Endofullerenes, in which a small atomic or molecular guest is fully encapsulated within a fullerene cage, can be accessed via distinct synthetic strategies tailored to the nature of the endohedral species. Positively charged metallic endofullerenes, such as Li⁺@C₆₀, are generated by ion implantation, wherein accelerated gas-phase metal ions are driven into solid fullerenes and become trapped within the carbon cages. Neutral molecular endofullerenes, such as HF@C₆₀, are obtained by “molecular surgery”: the C₆₀ cage is temporarily opened by a multistep chemical sequence, the guest is inserted through the aperture, and the cage is then restored to its original closed form, confining the guest. Combining these methods yields cluster endofullerenes such as Li⁺HF@C₆₀, where **both Li⁺ and HF are co-encapsulated in a single C₆₀ cage.**

We report NMR investigations of Li⁺@C₆₀, HF@C₆₀, and Li⁺HF@C₆₀, with emphasis on the chemical shifts, and the magnitude and sign of the J-couplings associated with the endohedral species. Complementary quantum-chemical calculations, employing *ab initio* and density functional theory methods, are utilised to compute the NMR parameters. Correlating the calculated and experimental NMR parameters enables characterization of spatial confinement, electronic environment, and intermolecular interactions of the endohedral species. Additionally, for Li⁺HF@C₆₀ this affords insight into the relative geometry and internal structure of the encapsulated Li⁺HF complex.

This integrated experimental-computational NMR approach elucidates the interplay between confinement and intermolecular interactions in atomic-molecular clusters encapsulated in C₆₀ fullerenes.

Structural Characterization of Polyglycerol Substructures by Multidimensional and Quantitative NMR Spectroscopy

Nahavandizadeh Nasim, Bilén Frida, Larsson Moa, Zeliouche Sami, Bordes Romain, and Evenäs Lars.

Polyglycerol is a biobased polyether derived from glycerol. Interest in polyglycerol has grown as a multifunctional alternative to polyethylene glycol in various applications. The degree of branching and molecular architecture critically influences its physicochemical properties, but detailed substructural characterization is still not well established.

To better understand the structural patterns of oligomeric polyglycerol (up to 10 units), we applied a multi-experiment NMR strategy in which DEPT-135, INADEQUATE, HSQC, and HMBC were used to assign carbon signals to specific glycerol substructures. Building on these assignments, we establish a quantitative interpretation framework that translates straightforward ^{13}C NMR spectra into detailed substructural compositions of oligomeric polyglycerol. The approach resolves the relative occurrence of terminal, linear, and dendritic units, differentiates ether linkages through primary and secondary carbons, and identifies the connecting carbon of the neighboring glycerol unit. We also explored a quantitative ^1H NMR method based on the hydroxyl proton region as a simpler option for routine compositional analysis.

By combining multidimensional assignment experiments with quantitative NMR analysis, the methodology enables unprecedented substructural resolution of oligomeric polyglycerol and establishes a practical framework for future structure–property studies.

Solids Methods

Solid-State NMR Investigation of Volatile Organic Compounds Adsorption in DUT-134(Cu)

Gao, Buyun^a, Engemann, Richard^b, Kaskel, Stefan^b, Brunner, Eike^a

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In this work, we investigate the adsorption of volatile organic compounds (VOCs) such as formaldehyde or 1,4-dioxane in DUT-134(Cu) ($[\text{Cu}_2(\text{dttc})_2]_n$, dttc = dithieno [3,2-b:2',3'-d] thiophene-2,6-dicarboxylate), a 2D metal-organic framework (MOF) that can be hierarchically assembled into well-defined, large-area carpets and tubular structures to maximize the accessibility of open metal sites.

We employed advanced solid-state nuclear magnetic resonance (NMR) spectroscopy to elucidate the molecular adsorption state within these frameworks. Cross-polarization (CP) build-up curves were measured for pristine and formaldehyde and 1,4-dioxane-loaded DUT-134(Cu) samples. Analysis of the CP build-up kinetics revealed significantly shorter time constants upon exposure to VOCs, indicating enhanced dipolar coupling between the adsorbed VOCs and the framework sites. This semi-quantitative parameter serves as a probe for adsorption strength, providing direct evidence for strong host–guest interactions at open Cu (II) sites.

Additionally, 2D ^1H – ^{13}C heteronuclear correlation (HETCOR) experiments with variable contact times were performed to resolve the spatial proximities and binding environments of VOCs within the MOF structure. The observed contact-time dependence of cross peaks confirmed localized, site-specific adsorption near the metal centers, supporting a mechanism dominated by Lewis's acid-base interactions.

This study highlights the power of solid-state NMR spectroscopy as a non-destructive, site-specific approach for quantifying adsorption in MOFs. The insights gained provide design principles for optimizing 2D MOF architectures for efficient VOC capture in real-world air filtration applications.

A Multiple-Quantum ^7Li ($I = 3/2$) NMR Study of Spin-Lattice Relaxation in SolidsStephen Wimperis,^{*,a} George E. Rudman,^b Karen E. Johnston^b^a*Department of Chemistry, Lancaster University, Lancaster LA1 4YB, UK*^b*Department of Chemistry, Durham University, Durham DH1 3LE, United Kingdom*

Spin-lattice relaxation measurements are used in ^7Li NMR studies of materials of potential use in solid-state Li-ion batteries as a probe of ion mobility on a fast (nanosecond to picosecond) time scale. The relaxation behaviour is often analysed by assuming exponential behaviour or, equivalently, a single T_1 time constant. However, the spin-lattice relaxation of spin $I = 3/2$ nuclei, such as ^7Li , is in general biexponential; this is a fundamental property of $I = 3/2$ nuclei and unrelated to any compartmentalisation within the solid. Although the possibility of biexponential ^7Li (and other $I = 3/2$ nuclei) spin-lattice relaxation in the solid state has been noted by others, it has proven difficult to observe unambiguously using conventional experimental NMR techniques, such as inversion or saturation recovery.

We have recently shown that triple-quantum filtered NMR experiments, as previously exploited in $I = 3/2$ NMR of liquids, can be used in favourable circumstances to observe and readily quantify biexponential ^7Li spin-lattice relaxation in solids with high ion mobility (J. Phys. Chem. C **128**, 5453–5460 (2024)). However, this work revealed aspects of the relaxation behaviour that were not readily explainable using theories derived from quadrupolar nuclei in liquids.

Here, we will present conventional and triple-quantum filtered saturation-recovery ^7Li NMR data from the candidate solid electrolyte material Li_2OHCl over a range of temperatures and show that invoking both quadrupolar and magnetic/dipolar relaxation mechanisms goes some way towards explaining the results, in particular the near-absence of biexponential behaviour in many conventional spin-lattice relaxation experiments. However, our results also show that in the lower part of the temperature range, just above the phase transition to a cubic form, true solid-state effects dominate and theories derived from liquid-state NMR are inadequate.

Optimal control of multispin nitrogen-proton systems: Efficient ^{15}N - ^1H and ^{14}N - ^1H correlation spectroscopy

Jan Blahut, Martin Dračinský, Zdeněk Tošner

When facing the challenge of detecting nitrogen spectra in the solid state – whether in insoluble proteins, membrane complexes, or functional materials – researchers must decide whether to focus on the abundant but experimentally challenging ^{14}N isotope, rely on the low-abundance but well-behaved ^{15}N nucleus, or invest substantial resources into ^{15}N isotopic labeling. In all cases, the central trade-off remains between resolution and sensitivity (and price). Optimal control offers new possibilities to mitigate this compromise by developing efficient experimental methods.

Here we present numerically optimized pulse sequences aimed at improving the sensitivity of indirectly detected ^{15}N experiments by preserving both components of complex signal during coherence transfer. Simultaneously, we introduce optimal-control pulses that enhance the resolution of ^1H - ^{14}N correlation spectra by selectively addressing nuclei with a desired quadrupolar coupling constant.

Although efficient ^1H - ^{14}N and ^1H - ^{15}N coherence transfers require solving fundamentally different problems due to presence of strong quadrupolar coupling for ^{14}N , in both cases the dominant loss of efficiency arises from the strongly coupled ^1H spin network. We demonstrate how this limitation can be mitigated through careful model spin-system design during the optimization process.

Our results show that the spin-system state can be reliably steered toward the desired target even in a rotating powder with a complex set of anisotropic interactions. The resulting optimal-control-based ^1H - $^{14/15}\text{N}$ correlation experiments are robust and straightforward to implement, requiring no further experimental optimization.

Supported by Czech Grant Foundation (24-10843S).

Polarization Transfers Between X-Magnetization and Anti-longitudinal ^1H Spin-States in Liquids and Rotating Solids

Jayanthi Sundaresan, Lupulescu Adonis, Grinshtein Julia and Frydman Lucio*

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

NMR relies on schemes like INEPT or cross-polarization (CP), transferring magnetization back and forth between ^1H s and heteronuclei X. In solid state, CP is also possible between multi-spin dipolar order (e.g. ^1H pairs) and X-nuclei irradiated at ca. the homonuclear dipolar coupling strength. Recently, Pang et. al reported an additional CP scheme that may arise in three-spin systems, termed as nuclear cross effect. The present study revisits such scenario, considering transfers between abundant spins (e.g. two inequivalent ^1H s) and a rare spin (e.g. ^{13}C). It is shown that ^1H s prepared in an “anti-longitudinal” state can be converted into ^{13}C polarization via a CP that can be mediated by either dipolar or J-couplings. These transfers occur in both liquids and static/spinning solids, provided that the ^{13}C (X) RF field driving this CP, matches the chemical shift difference between coupled protons. Unlike CPMAS, independence of the matching condition on MAS rate allows this kind of low RF power polarization transfer process to occur even at very high MAS rates. Conversely, a transverse X-spin magnetization that is spin-locked with an RF field matching the chemical shift difference between coupled ^1H s, can generate anti-longitudinal ^1H spin states –without ever having to pulse on the protons. A theory covering the ensuing multi-spin CP schemes is provided, in quantitative agreement with numerical simulations. Accompanying MAS and liquid-state experiments verifying the theoretical predictions will also be shown. The roles of many-body interactions and rf-inhomogeneity in the transfer process are discussed.

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Mechanochemical Conglomerate Formation or Not?

Moos, Sven; Trommelschläger, Pascal; Krüchel, Tobias, Quaranta, Calogero; Aengenvoort, Niklas; Rübartsch, Kathrin; Manzoni, Fabio; Zobel, Mirijam; Fyta, Maria; Lahtinen, Manu; Rissanen, Kari; Bolm, Carsten; Wiegand, Thomas

The emergence of homochirality in biomolecules is often linked to prebiotic symmetry breaking and chiral amplification, potentially driven by mechanochemical processes on early Earth.[1,2,3] Motivated by a report that milling racemic amino acids with ZnO in presence of MeOH yields conglomerate phases[4], we aimed to extend this research on ^{19}F -labelled valine as tracer systems for mechanochemical conglomerate formation. Vibrational ball milling and Resonant Acoustic Mixing (RAM) in the presence of MeOH or H_2O were monitored primarily by solid-state NMR spectroscopy under magic-angle spinning (MAS), complemented by powder X-ray diffraction (PXRD), single-crystal XRD, and density functional theory (DFT)-based NMR chemical-shift calculations.

Milling of specific ^{19}F -labelled and unlabeled *rac*-valine with ZnO and MeOH, followed by $^{13}\text{C}/^{19}\text{F}$ MAS-NMR analysis, revealed that in neither case a racemic conglomerate phase was formed under our conditions. Instead, unlabeled valine yielded a phase with four crystallographically distinct valine molecules, whereas ^{19}F -labelled valine gave a phase with only two. Single-crystal data identified the first material as an unprecedented racemic tetranuclear zinc complex, $\text{Zn}_4(\text{D-Val})_4(\text{L-Val})_4$, featuring deprotonated valine carboxylic acid groups in mono- and bidentate coordination modes. The same complex formed when equimolar ratios of enantiopure *D*- and *L*-valine were reacted with ZnO and MeOH. The complex is also formed upon RAM, with conversion efficiencies depending on the liquid additive and specific mixing protocol used.

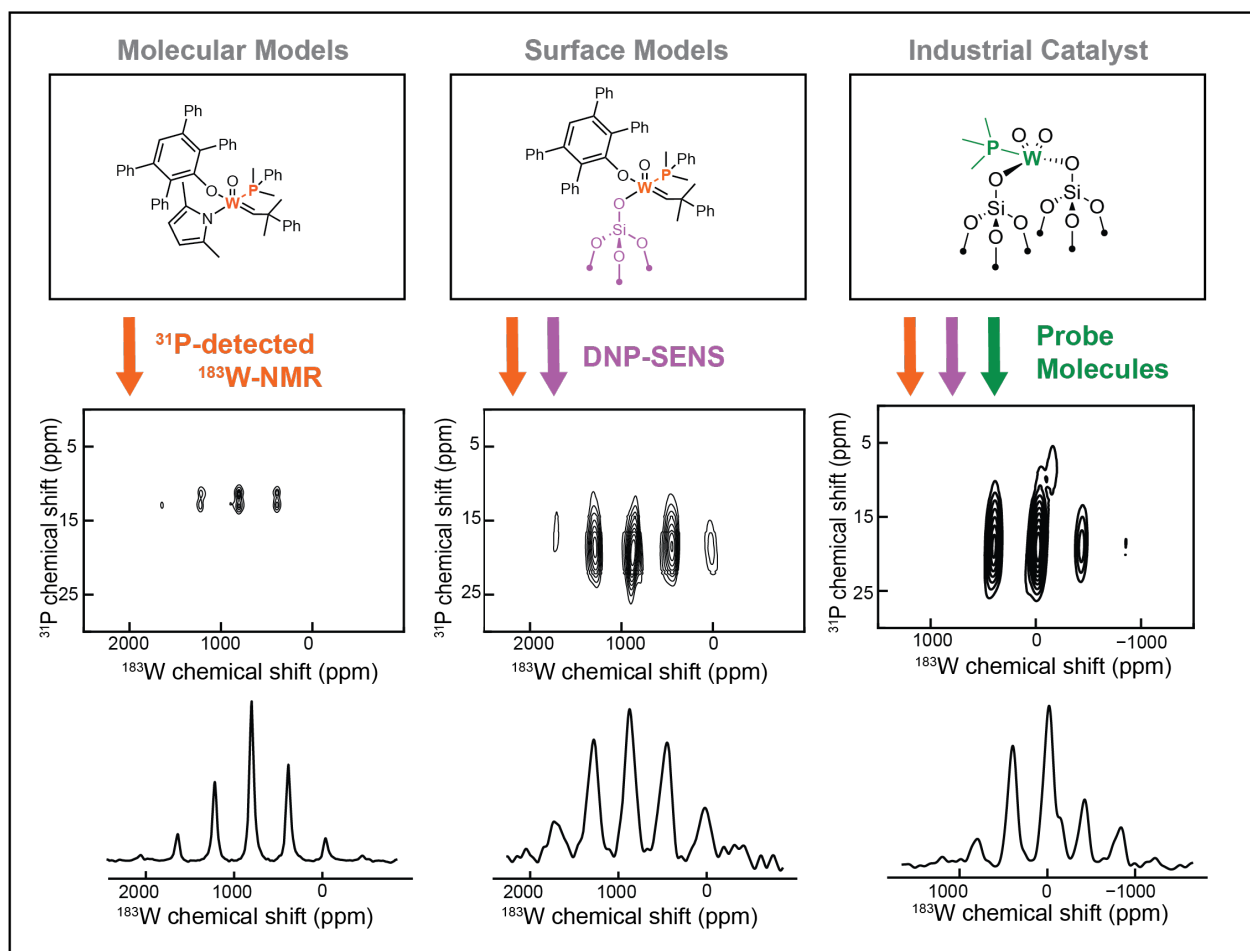
These results show that mechanochemical activation of simple amino acids with inorganic solids can give rise to previously unknown metal–amino-acid architectures. They further highlight the power of MAS-NMR in characterizing mechanochemical reaction products.

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^{31}P -detected ^{183}W -NMR Signatures of Molecules and Surface Sites

Seidel, Merlin Z.; De Zwart, Felix J.; Yakimov, Alexander, Copéret, Christophe



Solid-state NMR of ^{183}W ($I=1/2$) is extremely challenging due to its low gyromagnetic ratio (1.796 MHz/T) and 14.3% natural abundance resulting in a receptivity $\sim 90,000$ times lower than that of ^1H . Conventional approaches to boost sensitivity, such as $^1\text{H} \rightarrow ^{183}\text{W}$ Cross-Polarization (CP-MAS) are limited by the typically weak ^1H - ^{183}W dipolar couplings requiring long contact times (>20 ms), as well as by proton relaxation. These challenges are further exacerbated for surface sites that suffer from low spin concentrations ($<1 \mu\text{M}$), inhomogeneous broadening and the absence of spatially close protons.

Here, we present a strategy of utilizing indirect detection via J-coupled ^{31}P as a high- γ "spy" nucleus to enable efficient ^{183}W detection. Using a molecular system and recording 2D ^{31}P - ^{183}W -Heteronuclear Multi-Quantum Correlation (HMQC) spectra we demonstrate the validity

of the approach, which also enables us to extract the ^{183}W chemical shift anisotropy (CSA) and to thereby understand the W local environment. The application on a well-defined surface complex containing a phosphine ligand is further demonstrated, thanks to the implementation of Dynamic Nuclear Polarization Surface Enhanced NMR Spectroscopy (DNP-SENS).

Finally, we apply this approach to silica-supported tungsten oxide (WO_3/SiO_2), a key catalyst of the chemical industry, with poorly understood surface sites. Using trimethylphosphine (TMP) as a probe molecule, to introduce the required ^{31}P - ^{183}W interactions enables us to record 2D ^{31}P - ^{183}W -NMR spectra and to distinguish two surface sites. Further extracting their CSA parameters provides detailed information and enables us to determine the local environments of the W sites. Overall, this work demonstrates the first successful application of surface-sensitive ^{183}W solid-state NMR spectroscopy, thus providing a blueprint for characterization of low- γ nuclei in heterogeneous materials.

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

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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.

Sensitivity and resolution enhanced paramagnetic proton detected solid-state NMR at fast MAS regime for structural elucidation of intact pathogenic fungal samples

Lends Alons, Petkus Jana, Zvirgzdins Alvis, Carofolini Linda, Luccia Massimo, Samoson Ago

Human fungal infections and related mortality have increased dramatically over the last decade. Because the fungal cell wall is essential for virulence and antifungal resistance, its atomic-level characterization could enable the development of new antifungal therapies and diagnostic tools. Recently, proton-detected solid-state NMR (ssNMR) under fast MAS (above 60 kHz) has emerged as a unique tool to study structure and dynamics of fungal cell walls. However, broader application is limited by the need for isotopically labeled samples and by poor ^1H resolution in structurally heterogeneous intact cells. Lanthanide-based paramagnetic dopants can improve both ssNMR sensitivity and resolution. Gd^{3+} dopants induce paramagnetic relaxation enhancement (PRE), shortening ^1H T_1 relaxation times and accelerating data acquisition, while Yb^{3+} complexes improve ^1H spectral dispersion through pseudocontact shifts (PCS).

Here, we will show the prospects of (i) enhanced sensitivity via PRE; (ii) improved ^1H spectral dispersion by exploiting PCS using natural unlabeled fungal samples at 60-150 kHz MAS regimes. For PRE fungal samples were doped with Gd^{3+} complexes, which lead to a factor of 4-5 reduction of ^1H T_1 relaxation, while not compromising ^1H and ^{13}C resolution. This enabled us to acquire high-resolution for INEPT and CP based 2D hCH spectra at ^{13}C natural abundance for fungal samples within hours of measurement time. To improve ^1H spectral improvement, we doped fungal cells with $\text{Yb}(\text{DPA})$ complexes, increasing in ^1H chemical shift dispersion by 2-3 ppm. This significantly reduce proton peak overlapping, leading to more unambiguous chemical shift assignments of fungal glycans and lipids. Overall, we demonstrate that doping fungal samples with different lanthanide complexes can substantially enhance both sensitivity and chemical-shift dispersion in proton-detected ssNMR experiments.

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Experimental and computational ^{11}B NMR study of MOVPE-grown rhombohedral and bulk hexagonal boron nitride

Grochowska-Tatańczak, Magdalena, Tatańczak, Piotr, Dąbrowska, Aleksandra K., Tokarczyk, Mateusz, Binder, Johannes, Wyszomleć, Andrzej, Kazimierczuk, Krzysztof

Layered boron nitride (BN) is a representative of two-dimensional (2D) materials, whose crystal structure closely resembles graphene, with boron and nitrogen atoms arranged in a honeycomb lattice within one atomic plane. Owing to its large bandgap (~ 6 eV), BN has strong potential for deep-ultraviolet optoelectronic devices. It can host point defects that act as single-photon emitters, making it a promising material for studying quantum sensing. Although individual atomic planes are structurally identical, the stacking sequence of sp^2 -hybridized BN planes varies, giving rise to different polytypes. The arrangement of those planes in relation to one another is of key importance, as it determines the properties of the bulk materials. Particularly, the non-centrosymmetric, ABC-stacked rhombohedral boron nitride (rBN) attracts significant attention, since it exhibits ferroelectricity and nonlinear optical processes, not observed in centrosymmetric AA'-stacked hexagonal BN (hBN).

The NMR data on rBN are scarce, with only reports presenting static ^{11}B spectra of mixed rBN/hBN nanotube phases. We find it somewhat surprising, since thanks to its sensitivity to the local environment, ^{11}B magic-angle spinning solid-state NMR (^{11}B MAS ssNMR) seems to be a perfect tool for investigating this class of materials. In this contribution, we aim to address this issue by presenting the first, to our knowledge, comprehensive ssNMR study, supported with plane-wave Density Functional Theory (DFT) calculations, of two different sp^2 -hybridized BN polytypes: layered rBN grown by metalorganic vapour-phase epitaxy (MOVPE), and bulk hBN. The presence of carbon-related, most probably paramagnetic defects in the MOVPE-grown rBN prompted us to complement the NMR analysis with relaxation experiments, which revealed pronounced differences between the two polytypes. These findings, while showing the strong potential of NMR for probing the local structure of defect-containing 2D materials, highlight the need for in-depth research of other BN polytypes.

Nonsecular Resonances for Like and Unlike Spins

Jurkutat, Michael; Singh, Pooja; Safiullin, Kajum; Meier, Benno

Nonsecular resonances (NSRs) are resonant exemptions from the validity of the secular truncation of dipolar interactions and were predicted in 2012. They are expected to occur far off Larmor frequency, only for relevant RF irradiation ($B_1/B_0 \gg 0$), and for certain commensurate ratios between the frequency of the applied field and the effective Larmor precession. Under these conditions nonsecular dipole-dipole terms are recoupled that do not conserve Zeeman energy and can lead to previously unsuspected effects. Here, we recapitulate the idea behind the original prediction, cover anticipated phenomena and summarize experimental insights so far.

For like spins five NSRs are predicted to occur between one third and twice the Larmor frequency. And their irradiation leads to loss of spin polarization, for which we have shown the first experimental proof.

For unlike spins various NSRs also cover a wide range of frequencies away from the Larmor frequencies. Some of these are dissipative similar to NSRs for like spins. Other NSRs conserve overall spin polarization, if not Zeeman energy. The latter, conservative NSRs can therefore be used for cross polarization for which, unlike Hartmann-Hahn, irradiation at only one frequency is needed.

Interestingly, a set of solutions to conservative NSR conditions resembles the positive and negative solid effect in frequency and field dependence as long as $B_1/B_0 < 0.1$. We discuss the similarities, the predicted differences for very strong irradiation at low field and experimental insights thus far.

Expanding the Magnetic Resonance Toolbox: μ SR and RF- μ SR Contributions.

Louat Alex

Muon spin rotation/relaxation/resonance (μ SR) and radio-frequency μ SR (RF- μ SR) are powerful techniques that provide microscopic insight into magnetic and electronic properties of materials. Within the broader framework of magnetic resonance, these methods offer a complementary perspective to Nuclear Magnetic Resonance by exploiting the unique properties of the implanted muon as a local probe.

In this work, we present an overview of μ SR and RF- μ SR, highlighting the type of information they can access and the regimes in which they are particularly effective. μ SR is intrinsically sensitive to local magnetic fields and fluctuations over a wide range of time scales, enabling the study of static and dynamic magnetism even in systems where conventional probes face limitations. RF- μ SR extends these capabilities by allowing the investigation of coherent spin dynamics and resonance phenomena in zero or low applied magnetic fields.

We discuss how these techniques complement NMR by accessing different time windows, length scales, and sensitivity ranges, particularly in systems with dilute magnetism, strong disorder, or slow spin dynamics. Through selected examples, we illustrate how μ SR and RF- μ SR contribute to a more complete understanding of magnetic behaviour when used alongside established magnetic resonance methods.

Overall, this work emphasizes the role of μ SR and RF- μ SR as versatile additions to the magnetic resonance toolbox, broadening the scope of phenomena that can be probed at the microscopic level.

Static-gradient NMR imaging for depth-resolved molecular diffusion in poly(tetrafluoroethylene) film

Kawabata, Natsuki and Asakawa, Naoki

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Understanding spatially heterogeneous molecular diffusion in semicrystalline polymers is essential for elucidating interfacial dynamics in soft materials. In this study, static-gradient nuclear magnetic resonance (NMR) imaging is employed to resolve depth-dependent translational motion of polymer chains in a polytetrafluoroethylene (PTFE) film. By examining spin–spin relaxation behavior within amorphous regions adjacent to crystalline lamellae, multiple diffusion regimes are identified, consistent with Bloch–Torrey analysis. The results demonstrate that molecular mobility at the substrate interface—where the PTFE film is immobilized on a glass substrate using epoxy resin—is significantly restricted, likely due to interfacial pinning effects, whereas the air-facing surface exhibits enhanced mobility. These findings highlight the effectiveness of static-gradient field NMR as a powerful approach for probing dynamical heterogeneity in semicrystalline polymer systems.

Slow Magic Angle Revolution Technique: a new double magic angle spinning method for solid-state NMR

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A new method for Magic Angle Spinning (MAS) is introduced. The cylindrical sample holder (rotor) undergoing MAS is placed into a larger cylindrical stator, with both cylinders being oriented at the magic angle with respect to the external magnetic field. A single air flow is directed along the inner surface of the outer cylinder stator such that the rotor undergoes two rotations: one around its symmetry axis and a second rotation around the edge of the outer cylindrical stator. In this way the sample undergoes two rotations around the Magic Angle. The effect of the two rotations combines in removing the homo and heteronuclear dipolar interactions resulting in enhanced resolution of NMR spectra. The linewidth of experimental spectra obtained with double rotation on $^{85}\text{RbCl}$ is compared with the spectra acquired under MAS. It is demonstrated that samples undergoing double rotation will experience a reduced centrifugal acceleration as compared with conventional MAS. The reduced centrifugal forces provide the opportunity to study anisotropies created by soft hydrogen bonding which would otherwise be destroyed under fast spinning.

syncDIPSHIFT - a new method for investigating sample orientations

syncDIPSHIFT is a new method for investigating sample orientations, in which orientational moments can be estimated. In comparison to syncMAS, dipolar interactions are used instead of CSA. One important advantage is that the chemical-shift tensor is often not known with sufficient accuracy. In syncMAS analysis, which is based on chemical-shift interactions, the chemical-shift tensor and its orientation in the molecular frame must be known. In contrast, syncDIPSHIFT analysis is based on dipolar interactions, where the dipolar tensor is clearly aligned along the ^{13}C – ^1H bond.

Another advantage of syncDIPSHIFT is that higher spinning speeds can be applied, resulting in better resolution in the direct dimension. This also provides access to aliphatic carbons with low CSA values, since dipolar interactions are significantly larger than chemical-shift anisotropy, even for CH_3 groups.

In this study, the syncDIPSHIFT method was successfully applied to a series of stretched polycarbonate (PC) samples to investigate the effect of deformation level on the segmental orientational moments. PC samples with higher deformation levels exhibit larger oscillation amplitudes, resulting in relatively higher $\langle P_2 \rangle$ values.

Quantum Optimal Control Approaches for Solid-State NMR of Quadrupolar Nuclei

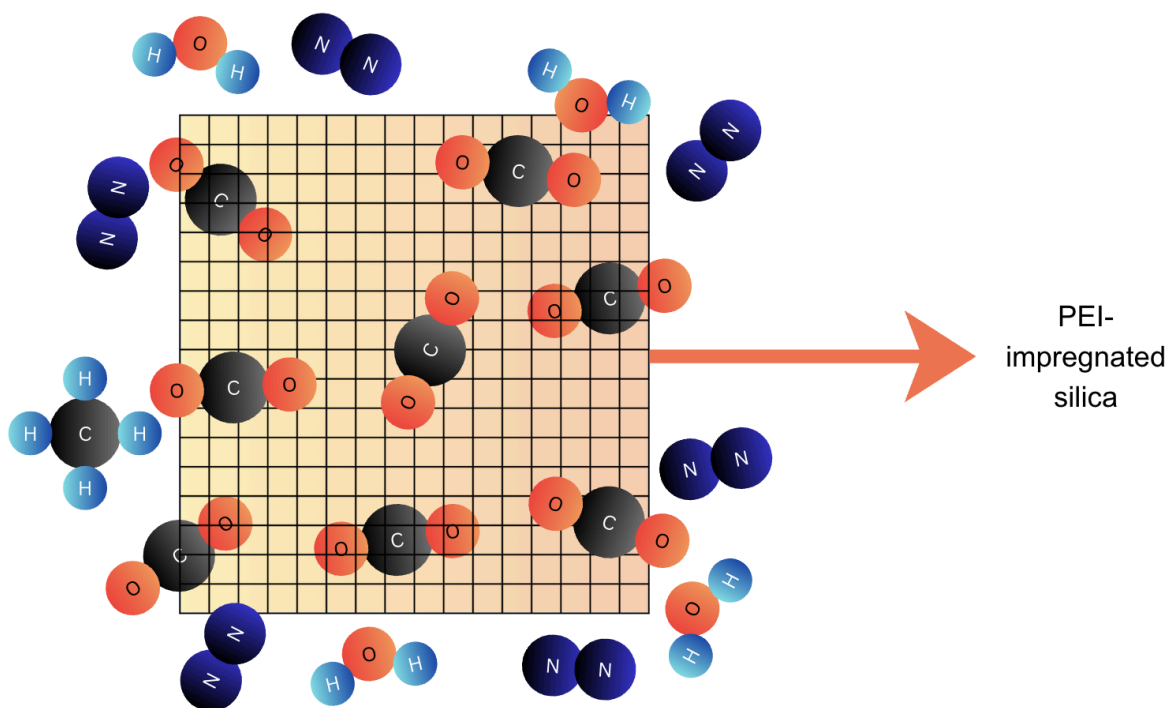
Waldock, Jenna, Kuprov, Ilya, Potter, Matthew, Carravetta, Marina.

Quadrupolar nuclei are important probes of local structure in solid-state NMR, but low receptivity, broad orientation-dependent lineshapes, and restricted excitation bandwidth often limit their experimental observation.

Quantum optimal control can be used to numerically design radiofrequency pulses that account for multiple experimental constraints simultaneously. In this work, shaped pulses were generated using GRAPE-based optimal-control simulations in *Spinach* with both amplitude and phase modulation. A long dead time was included directly in the simulations, so that the optimised pulse acts as an echo pulse with refocusing behaviour built into the waveform. Static conditions have been used as an initial test case to reduce the computational cost of the optimisation.

The pulses were designed to transfer the longitudinal magnetisation, L_z , including the satellite contribution to the transverse central transition, CT_x , leading to an enhancement in signal intensity relative to conventional echo experiments. However, some orientation-dependent lineshape distortions have been observed. Initial experiments were performed on ^{87}Rb ($I = 3/2$) in RbNO_3 , a well-characterised and high- γ model system. The method was then applied to ^{25}Mg NMR of $\text{Mg}(\text{OH})_2$, a lower- γ spin-5/2 case at 20 T, where ringing after the rf-excitation is much more significant due to its low resonance frequency.

Carbon dioxide (CO_2) is a significant greenhouse gas (GHG) that contributes most to the greenhouse effect (GHE), amongst other GHGs. It is realistically difficult to reduce the emission of CO_2 due to global population growth. However, CO_2 can be captured and used to meet the global energy demands. One method of capturing gas is through the use of adsorbent materials. Amongst these adsorbents, polyethylenimine (PEI)-impregnated silica composite materials are well studied. However, the adsorption mechanism and molecular interactions that occur within this material are yet to be researched. The different mechanisms for this adsorbent affect the adsorption capacities and specificities for the wanted gas. To study these materials, they were first synthesized, verified for proper synthesis, and then passed with CO_2 . Different analytical techniques were employed to observe the changes before and after the passing of CO_2 . Specifically, solid-state nuclear magnetic resonance (NMR) was utilized to gain insights into the molecular-level interactions between the composite material and CO_2 . This research aims to elucidate the interactions between the two adsorbent materials and CO_2 .



Origins of ^{19}F coherence lifetimes and linewidths in fluorinated amino acids studied by solid-state NMR

Zvirgzdins, Alvis, Paluch, Piotr, Lends, Alons

The ^{19}F nucleus is highly attractive for biomolecular solid-state NMR because of its high sensitivity, 100% natural abundance, and absence in most native biological systems.

However, compared to more conventionally used nuclei - ^{13}C or ^1H , in comparison, ^{19}F exhibits relatively broad linewidths, which remain a major limitation for more applications using ^{19}F -detected MAS NMR. In this work, we systematically investigated the origins of ^{19}F linewidths and coherence lifetimes in fluorinated amino acids containing either CF_3 or CH_2F groups. Measurements were performed on alanine and valine derivatives at magnetic fields corresponding to ^1H Larmor frequencies of 600, 700, and 1200 MHz and at MAS frequencies between 5 and 60 kHz, with and without ^1H decoupling.

The results demonstrate distinct relaxation and linewidth behavior for CF_3 and CH_2F moieties. In a case of CF_3 -alanine, ^{19}F linewidths are predominantly governed by ^{19}F - ^{19}F interactions, while in CF_3 -valine, ^1H - ^{19}F interactions are more dominant. CH_2F -alanine exhibits substantial contributions from strong ^{19}F - ^1H dipolar couplings and its cross-relaxation products with ^{19}F CSA. Increasing MAS frequency and applying ^1H decoupling efficiently suppresses first-order heteronuclear interactions, leading to narrower ^{19}F linewidths and extended coherence lifetimes. In contrast, higher order dipolar and CSA mediated interactions remain significant, particularly at high magnetic fields.

Experimental findings were supported by numerical simulations using SPINACH and SIMPSON, which enabled the separation of CSA, homonuclear, and heteronuclear dipolar contributions to the observed spectra. In summary, here we have demonstrated combined experimental and simulation analysis, providing detailed insight into the mechanisms governing ^{19}F spectral resolution under fast MAS conditions. The results in this work will further help expand ^{19}F -detected solid-state MAS applications to a wide variety of $-\text{CHF}_2$ and $-\text{CF}_3$ -labeled groups.

M=CR₂ (M = U, Y) Diphosphonioalkylidene and U≡O Terminal Oxo Multiple Bond Interactions Encoded in ¹³C and ¹⁷O Nuclear Magnetic Resonance Chemical Shift Anisotropies: Correlations of M=C Bond Order to Chemical Shift Tensors

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Diphosphonioalkylidene ligands provide a unique platform for probing f-element multiple bonding, yet their highly variable carbene ¹³C NMR chemical shifts have complicated interpretation of M=CR₂ bonding. Here, is demonstrated how advanced solid-state ¹³C and ¹⁷O NMR spectroscopy, combined with chemical shift anisotropy (CSA) analysis, can directly probe the electronic structure of actinide and rare-earth alkylidene complexes.

The complexes [U(BIPM^{TMS})(O)(Cl)₂] and [Y(BIPM^{TMS})(I)(THF)₂] where BIPM^{TMS} = {C(PPh₂NSiMe₃)₂}²⁻ are investigated^{1, 2}, which exhibit dramatically different solution/solid-state carbene ¹³C isotropic chemical shifts δ_{iso}. Measurement of the full chemical shift tensors reveals distinct spectroscopic signatures for U=C, U≡O, and Y=C bonding environments. In the uranium complex, exceptionally large ¹³C δ₁₁ and span (Ω) values, together with a highly deshielded solution ¹⁷O resonance, indicate strong σ ↔ π* and π ↔ σ* magnetic shielding interactions and significant 5f-orbital participation in bonding. These tensor features also provide evidence for an inverse *trans* influence across the C=U≡O linkage.

By contrast, the yttrium complex displays ¹³C tensor parameters consistent with weaker Y=C bonding and increased ylidic C=P character. Comparison across transition-metal, lanthanide, and actinide alkylidene complexes reveals strong correlations between bond order and key NMR observables, including δ_{iso} (σ_{iso}), δ₁₁ (σ₁₁), Ω, and the paramagnetic shielding contribution (σ^p).

This work highlights CSA analysis as a sensitive NMR probe of covalency and electronic structure in metal–ligand multiple bonds, extending the scope of multinuclear solid-state NMR spectroscopy for studying actinide chemical bonding.

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Broadband Refocusing of Central Transition of Quadrupolar Nuclei for Reliable D-HMQC

Šmelko, Andrej, Blahut, Jan, Nishiyama, Yusuke, Tošner, Zdeněk

Dipolar Heteronuclear Multiple Quantum Coherence (D-HMQC) is used to detect spatial proximities between spin-1/2 and half-integer-spin quadrupolar nuclei in solid-state NMR. However, the central-transition-selective 180° refocusing pulse at the quadrupolar channel fails to uniformly refocus the full spectral window, particularly at high B_0 fields (>18.8 T). Signals at the sides of the spectral window are refocused with roughly half the efficiency of those in the center. This is especially problematic for nuclei with large chemical shift dispersion.

To address this, we replaced the conventional refocusing pulse with a numerically optimized Optimal Control (OC) shaped pulse. We obtained a low-power, $150\ \mu\text{s}$ long, central-transition-selective pulse, which uniformly refocuses a $20\ \text{kHz}$ wide spectral window, corresponding approximately to the ^{27}Al zeolite chemical shift range ($0\text{--}60\ \text{ppm}$) at $B_0 = 28.2\ \text{T}$ (^1H Larmor frequency of $1.2\ \text{GHz}$). Experimentally, we demonstrated a uniform performance of $^1\text{H}\text{--}^{27}\text{Al}$ D-HMQC across a $>20\ \text{kHz}$ range in AlPO-14 with an isopropylammonium template. Moreover, we observed sensitivity enhancement by a factor of 1.3 in 2D D-HMQC spectra. Taken together, the OC 180° pulse enables more reliable D-HMQC measurements.

Additionally, although the OC 180° pulse was optimized for ^{27}Al , it shows a high degree of universality. It performs well in both liquid and solid state, for all half-integer-spin quadrupolar nuclei ($I = 3/2, 5/2, 7/2, 9/2$), at any MAS rate (including static), and across a wide range of magnetic field strengths, dipole-dipole couplings, quadrupolar couplings, and electric field gradient asymmetries.

This work was supported by the Grant Agency of Charles University (grant No. 150824)

Proton multiple-quantum NMR spectroscopy for the structural investigation of surface complexes

Duong Tuan Nghia, Balodis Martins, Gajan David, Camp Clément, Thieuleux Chloé, Lesage Anne

Two-dimensional double-quantum (DQ) and triple-quantum (TQ) ^1H – ^1H NMR correlation experiments are widely used to investigate the structure of solid materials. Beyond revealing proton connectivity and spatial proximities, these experiments can also be used to estimate the number of ^1H spins within a dipolar-coupled network. We have recently shown that proton DQ and TQ experiments could be implemented to get insight into the number of hydrides attached to supported organometallic complexes. For more complex metal hydrides, where a larger number of ^1H spins are present, higher order multiple-quantum (MQ) experiments are desirable. While such experiments are challenging due to the low efficiency of MQ excitation and reconversion, they were recently successfully implemented to characterize supported zirconium metallocene.

In this study, we use a RN-symmetry-based recoupling sequence to achieve proton MQ excitation and reconversion. Up to 8Q coherences are created. Efficiencies are compared to conventional back-to-back sequence, using powdered isoleucine as a model sample. Strategies to excite both even- and odd-order MQ coherences will be discussed together with application on organometallic complexes containing a large number of hydrides.

Inter-platform comparability of NMR-derived lipid biomarkers and validation against clinical chemistry

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Background

¹H-NMR lipoprotein profiling is an inexpensive approach claiming to deliver highly reproducible data aligned for clinical diagnostics. Nightingale Health is a major supplier which has generated NMR data in large population cohorts including UK-Biobank and SCAPIS, while Bruker IVDr is a competing platform provided by the Swedish NMR Center. We conducted a pilot study to assess inter-platform comparability and agreement with clinical chemistry to support decisions on platform selection for research applications.

Methods

Fifty fasting blood samples from the SCAPIS pilot study were used. Clinical chemistry analyses, including direct LDL-cholesterol, were performed on serum samples at the Sahlgrenska University Hospital laboratory. NMR-based lipoprotein profiling was performed on plasma samples at the Swedish NMR center and Nightingale Health. Agreement with clinical chemistry was assessed using Pearson correlation coefficients and Bland-Altman analysis, with bias defined as NMR platform - clinical chemistry.

Results

Lipid measures available across both NMR platforms and clinical chemistry were LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B. Despite correlations between platforms, Bland-Altman analyses revealed substantial systematic bias. Nightingale reported consistently lower lipoprotein concentrations than both clinical chemistry and Bruker IVDr. Mean absolute and relative differences for Nightingale vs clinical biochemistry were -1,96 mmol/L (-73%) for LDL cholesterol, -0,36 mmol/L (-21%) for HDL-cholesterol and -3,37 mmol/L (-84%) for total cholesterol. In contrast, Bruker IVDr demonstrated closer agreement with clinical biochemistry with mean differences of -0,06 mmol/L (-2%) for LDL-cholesterol, -0,10 mmol/L (-4%) for HDL-cholesterol and 0,25 mmol/L (4%) for total cholesterol. Triglycerides demonstrated acceptable agreement across platforms.

Conclusion

The two NMR platforms are not interchangeable. The marked underestimation of LDL-cholesterol by Nightingale indicates that results cannot be interpreted against established clinical thresholds. The systematic platform-dependent bias suggests calibration differences, with Bruker IVDr demonstrating closer agreement with conventional clinical chemistry.