

EUROMAR

July

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BOOK OF ABSTRACTS

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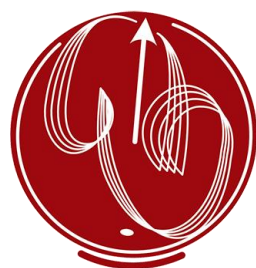
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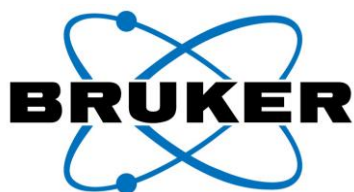


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Fractals and Polymer Upcycling: Imaging, Spectroscopy, Relaxometry, Diffusometry

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MR imaging, liquid and solid-state NMR, relaxometry and diffusometry were all deployed to examine the chemical kinetics of the decomposition of a certain class of polymers. The results were surprising: the chemical kinetics are non-ideal and non-linear, an unreactive intermediate limits decomposition, and reaction order parameters are governed by local dynamics. The work I present was accomplished with Drs. Sophia Fricke (presently at UC Berkeley) and Shira Haber (presently at Ben Gurion University).



Magnetic Resonance and Integrative Structural Biology, Outside and Inside Mammalian Cells

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I will present our work on next-generation magnetic resonance methods for atomic-level characterization of large biological assemblies and proteins in their natural environments. I will demonstrate the integration of magic angle spinning (MAS) NMR with medium-resolution cryo-EM and data-driven MD simulations to gain insights into the structure and dynamics of HIV-1 protein assemblies and their functions. I will introduce ^{19}F dynamic nuclear polarization (DNP)-enhanced MAS NMR in protein assemblies and proteins in mammalian cells. With over 35-fold signal enhancements, high signal-to-noise ratio MAS NMR spectra can be recorded on nanomole quantities of proteins in only minutes, and two-dimensional dipolar correlation spectra with remarkable resolution are obtained. This work paves the way for ^{19}F DNP-enhanced MAS NMR applications in cellular environments to probe protein structure, dynamics, and ligand interactions. I will discuss challenges and opportunities for magnetic resonance in integrative structural biology.



Animal Detected Magnetic Resonance

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Small migratory songbirds are extraordinary navigators. Weighing less than 50 g, they fly thousands of kilometres between their breeding and wintering grounds, alone and at night, ultimately with centimetre precision. How do they find their way? Well, they can use the sun and the stars, olfaction and landmarks, but they can also detect the direction of the Earth's magnetic field. Despite more than 50 years of research, the biophysical mechanism of this remarkable magnetic sense remains obscure. In this lecture, I will discuss the mechanistic insights available from behavioural studies of birds subjected to radiofrequency magnetic fields. Such findings arguably offer the most convincing evidence to date that the geomagnetic compass relies on the quantum spin dynamics of light-induced radical pairs in cryptochrome flavoproteins located in photoreceptor cells in the birds' eyes.



RNA dynamics and its impact on function

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Many RNA functions depend on rearrangements in secondary structure that can be triggered by external factors, such as protein or small molecule binding. These transitions can feature, on one hand, localized structural changes in base pairs(1) or can be presented by a change in the chemical identity of e.g. nucleobase tautomers(2). We use and develop R1 ρ -relaxation-dispersion NMR methods(3) for characterizing transient structures of RNA that exist in low abundance (populations <10%) and that are sampled on timescales spanning three orders of magnitude. Furthermore, other complementary methods are used in the lab to measure nucleic acid excited states.

The talk will be on nucleic acid biological systems, such as miR-34a targeting mRNA of Sirt1(4) and HNF4 α , in both liquid and DNP-enhanced solid-state NMR(5) and ribosomal RNA dynamics.

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From NMR of tissue samples to Metabolic MRI in patients

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NMR of tissue samples provides a wealth of information on metabolism that can be used as biomarkers for diagnosing diseases, monitoring treatments or basic understanding of human biology. However, tissue samples often require invasive biopsies, are no longer intact, lack an overview of heterogeneity, and complicates longitudinal investigations.

This presentation will provide an overview of translating the NMR into a metabolic MRI system to obtain non-invasively a large grid of NMR spectra throughout the human body of patients during the course of chemotherapy. Challenges of facing magnetic field non-uniformities, motion induced artefacts, long scan times and quantifications are addressed. Moreover, hardware innovations are shown that include whole body 14T system designs, ultrasonic gradients and large volume RF resonators to facilitate the metabolic MRI.



New applications of magnetic resonance and DNP NMR methods to study interfaces and complex oxides in lithium batteries

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Rechargeable batteries have been an integral part of the portable electronics revolution and are now playing a critical role in transport and grid applications to help mitigate climate change. However, these applications come with different sets of challenges. New technologies are being investigated and fundamental science is key to producing non-incremental advances and to develop new strategies for energy storage and conversion.

This talk will discuss NMR and methods that allow devices to be probed while they are operating, from the local, to particle and then cell level. This allows transformations of the various cell components to be followed under realistic conditions without having to disassemble and take apart the cell. For example, ^6Li and ^{33}S NMR studies allow both solid and solution components to be tracked in a lithium - sulfur battery allowing various hypotheses for battery operation to be tested. Work on the application of electron spin resonance and dynamic nuclear polarisation (DNP) NMR to graphitic anode materials and lithium metal batteries, to understand battery function and degradation will be described. NMR methods can be used to quantify impurities in solid state battery electrolytes, to correlate impurities with transport and dendrite formation. Finally, extensions of new magnetic resonance metrologies to study a wider range of electrochemical systems will be described.



Magnetic resonance at nanoscale enabled by diamond spin qubits

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Spin qubits in diamond have recently emerged as a candidate material for a range of quantum applications, quantum sensing. In this talk, I will show how single nitrogen-vacancy (NV) colour centres can be created near the diamond surface and used as nanoscale sensors of electric and magnetic fields. I will also demonstrate novel dynamical decoupling techniques to improve the sensitivity of diamond quantum sensors and discuss applications of small-scale quantum registers for nanoscale NMR. I will also show how nuclear spin quantum memories can be used to improve the spectral resolution of NV centre quantum sensors. Nanodiamonds based NV quantum sensing will be presented.



Exploring the properties of photogenerated multi-spin systems by transient EPR spectroscopy

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Photogenerated organic triplet–doublet systems hold great promise for a range of technological applications in the emerging field of molecular spintronics, including quantum sensing and optoelectronic devices. Typically, they consist of an organic chromophore covalently attached to a stable radical. Upon photoexcitation, the chromophore triplet state is rapidly generated by a process referred to as radical-enhanced intersystem crossing. If the exchange coupling between the chromophore triplet state and the radical spin centres is strong, compared to other magnetic interactions active in the system, quartet states may be formed by spin mixing.

For any applications of such photogenerated high-spin systems in molecular spintronic devices, their optical and magnetic properties are of major interest. In this contribution, we present our recent work focussed at exploring how to control the properties of photogenerated quartet states by rational design. Combining the experimental results with insight from ab-initio calculations, we shed light on the factors influencing (i) the rate and yield of triplet state formation, (ii) the magnitude of the triplet–radical exchange interaction, as well as (iii) the quartet state spin coherence times.



Reinventing dissolution Dynamic Nuclear Polarization (by accident and ignorance)

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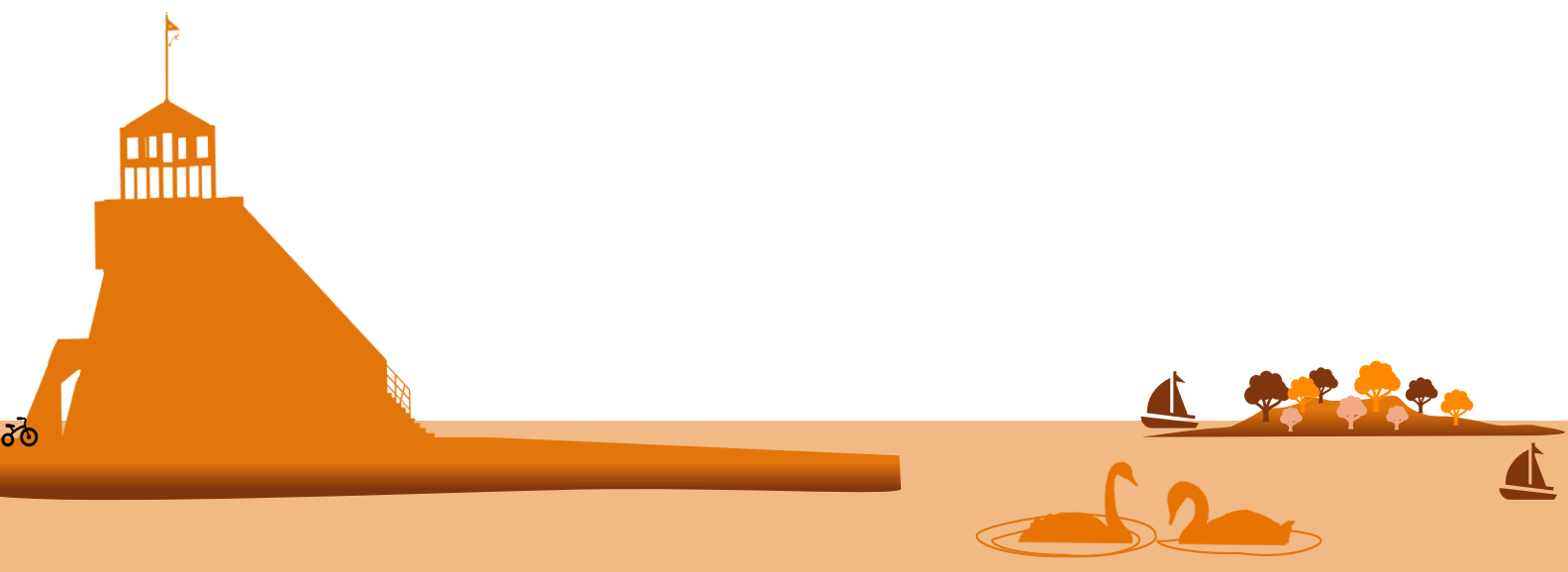
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Since the invention of dissolution dynamic nuclear polarization (dDNP) more than twenty years ago, the method has continued to evolve, progress, and transform into unexpected directions. In our group, we have worked over the past years to understand [1] and improve the efficiency [2], compatibility [3], and repeatability of dDNP. Our candid objective was to enhance the method and enable its applications [4,5]. Nonetheless, serendipity and ignorance offered us surprising discoveries, which led us to:

- i) extending hyperpolarization lifetimes from minutes to days and enabling transport to distant sites [6,7];
- ii) performing hyperpolarization transfers through zero field passages [8];
- iii) radically shifting our DNP conditions to benchtop and liquid nitrogen temperature [9];
- iv) and more...

During the plenary lecture, I will describe this scientific journey, driven by highly objective-driven projects yet enriched and enlivened by serendipitous encounters. - - -

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Solid-state NMR of viral (membrane) proteins

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The present study focuses on viral proteins, a topic of long-standing interest to the research group. In this context, we use protein solid-state NMR to address the structure of biological targets that are difficult to access by other more conventional approaches. A key issue is how to identify these targets, bearing in mind that they are often difficult to produce and place in a near-native environment.

While numerous high-resolution images of highly ordered viral particles have been documented, viral proteins often adopt irregular or dynamic structures to protect their genomes in ribonucleoproteins or to build the viral factories at the heart of progeny production. In addition, the formation of as yet uncharacterized superstructures by virulence factors has been observed to facilitate more efficient reproduction in viruses. We are therefore developing structural approaches to address this type of assembly in assemblies from hepatitis B, C and D and, more recently, bunyaviruses.

The presentation will discuss the use of proton-detected fast MAS solid-state NMR in the study of these entities, and how cell-free approaches to protein production can provide the necessary synergy for successful analysis.



From Hyperpolarization Chemistry to Quantum Oscillators: Rethinking What Nuclear Magnetic Resonance Can Be

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Over the past five years, guided by pioneers in the field, my group has explored whether nuclear magnetic resonance signals measured in magnetic fields lower than Earth's (the so-called hypogeomagnetic regime) could open new analytical and conceptual frontiers. Could we rethink the paradigms of high-field NMR and derive insights that are both practically useful and conceptually unifying?

Pursuing this vision, we combined a range of in situ hyperpolarization techniques (PHIP, SABRE, CIDNP) with sensing platforms such as benchtop NMR spectrometers and optically pumped magnetometers. We investigated robotic automation to enhance reproducibility and examined how relaxation and chemical exchange processes shape spectral linewidths and peak positions in near-zero-field NMR. Most recently, we demonstrated how programmable digital feedback can transform molecular spin ensembles into "quantum J-oscillators" — systems capable of sustaining ultranarrow (<1 mHz) resonances over extended periods. This talk will reflect on how these developments may help reimagine what nuclear magnetic resonance can become, especially when freed from conventional constraints.



Structural basis of the indirect inhibition of apoptosis by Bcl-xL

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Apoptosis, a form of programmed cell death, is a key mechanism in multicellular organisms that requires strict regulation. Cells that cannot perform apoptosis can grow unhindered, leading to tumors, while cells that perform apoptosis unnecessarily contribute to degenerative diseases, such as Alzheimer's disease. [1]

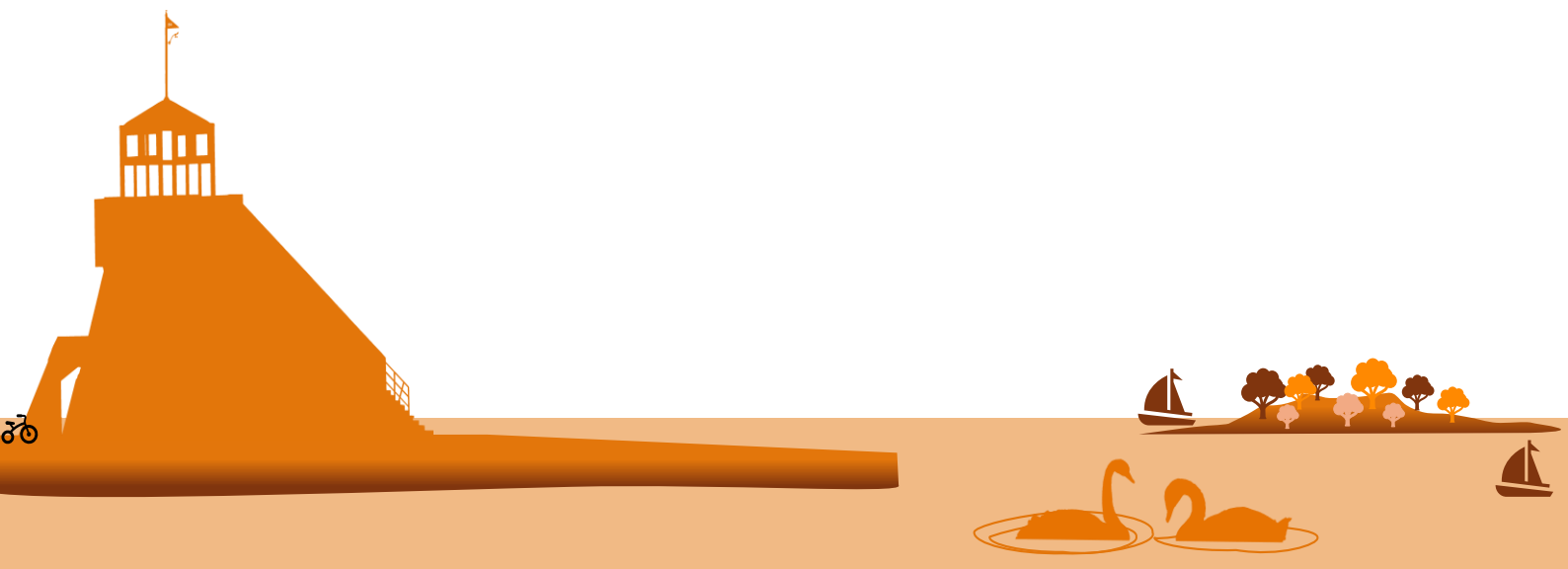
The mitochondrial pathway of apoptosis in cells is performed and regulated by the Bcl-2 protein family, consisting of the proapoptotic effector proteins (e.g. Bax) which form pores at the outer mitochondrial membrane, the proapoptotic activator proteins (e.g. Bid) which trigger the effectors' activation, and the antiapoptotic proteins (e.g. Bcl-xL) which inhibit the effectors and/or the activators. The inhibitory heterocomplexes between pro- and anti-apoptotic partners are not yet structurally characterized.

Here we present the inhibitory Bcl-xL/tBid complex at the outer mitochondrial membrane based on molecular modeling and DEER data acquired on spin-labeled Bcl-2 proteins in liposomes and mitochondria. The inhibitory complex is characterized by a unique combination of structured and dynamic domains, which provides new insights in the mechanism of indirect inhibition of apoptosis[2,3].

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Mobile MRI and TD-NMR of plants: keep it simple, stupid!

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In this contribution we present the development of mobile NMR sensors and MR imagers for measuring plants. A prerequisite was that they be based on permanent magnets, to minimize power consumption, and that the air gap be wide and accessible. This allows measurements of branches and stems of field-grown plants, but also enables illumination of leaves and cereal spikes inside the magnet. Ultimately an open C-shaped magnet design was chosen, as these are affordable, scalable, and relatively easy to build. To permit operation outdoors, both temperature-stable and temperature stabilized versions were constructed. Pole gap width and resilience against handling were prioritized over homogeneity and field strength.

To overcome the adverse effects of short $T2^*$ that may result from these choices, multi-spin echo imaging strategies with short echo times and high spectral widths were employed. Microscopic resolution requires fast-switching gradients, driven by strong and fast amplifiers. While small-scale spectrometers and RF amplifiers are readily available, suitable small-scale gradient amplifiers are not. We therefore constructed a small, 3-channel gradient amplifier based on a current-controlled AB amplifier design. Tailored to low-impedance gradient coils, it remained compact, battery-operable, and met demands for speed, power, and duty cycle. The finished imaging setup, mounted on an aluminum trolley, weighed 45 kg and fit into a car.

By abandoning imaging, and instead aiming for non-spatially resolved time domain relaxometry, the system can become simpler, smaller, and more affordable still. Key to allow operation by non-experts was to tailor the relaxometry method to a single, well-defined application: to estimate total proton density (fresh weight) and solid proton density (solid matter content) in living plant samples with 15–100% moisture. Mobile relaxometry thus becomes a valuable tool for monitoring drought response and yield development in trees and crops.



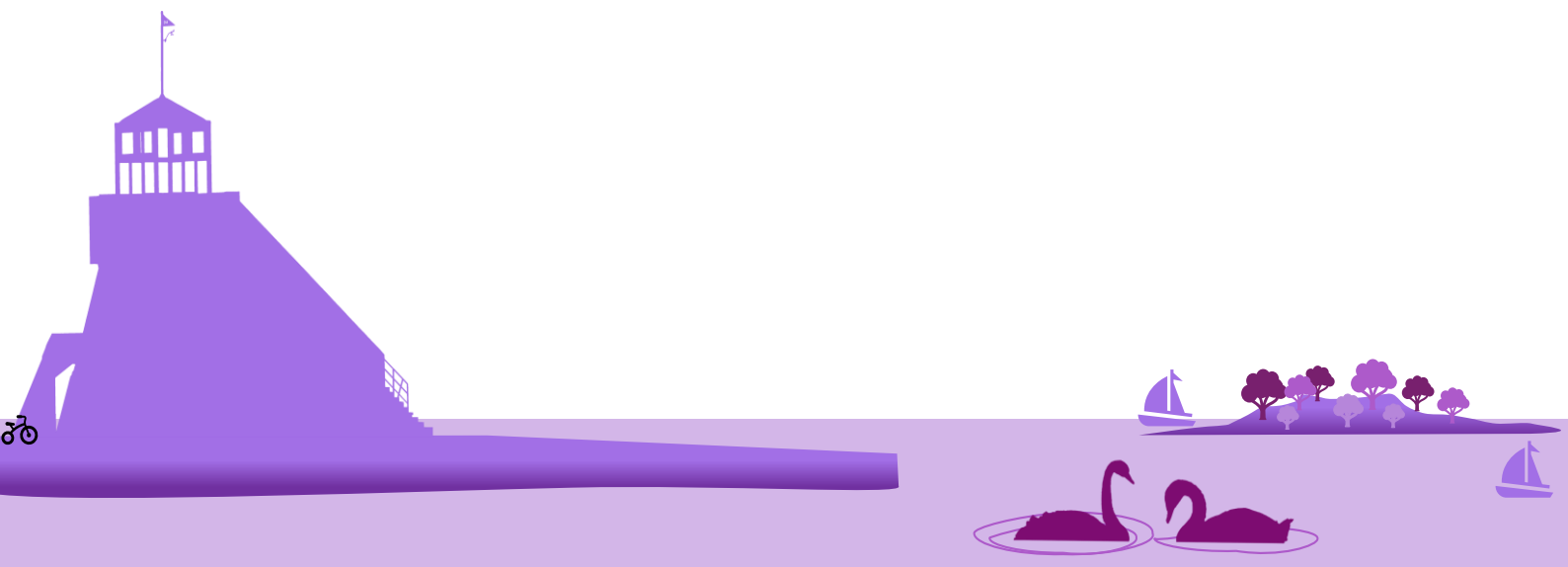
Solid-State NMR Techniques for Efficient Protein Structure Determination

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Solid-state NMR based structure determination of disordered and insoluble proteins such as membrane proteins requires many structural constraints, including conformation-dependent chemical shifts, internuclear distances, and molecular orientations. Modern 2D and 3D correlation magic-angle spinning (MAS) NMR experiments for resolving and assigning chemical shifts are now well established. In contrast, obtaining a sufficient number of distance restraints remains a major bottleneck in structure determination. This limitation results from the short distance reach of ^{13}C and ^{15}N nuclei, the semi-quantitative nature of the distances from cross peak intensities, and the difficulty to resolve weak long-range cross peaks from many strong short-range cross peaks in dense 2D and 3D spectra. In this tutorial I will present a suite of ^{19}F -based homonuclear and heteronuclear dipolar recoupling techniques that address these problems. These ^{19}F solid-state NMR techniques measure distances up to ~ 2 nm, yield quantitative distances, and produce simple spectra because of the small number of fluorines that are incorporated into the protein. We have combined these ^{19}F distance NMR techniques with fast MAS, ^1H detection, and semi-automated computational analysis to increase the throughput of distance extraction. These ^{19}F NMR distance experiments critically enabled and accelerated the de novo structure determination of several membrane proteins, including the influenza BM2 proton channel, the SARS and MERS E cation channels, and the bacterial multidrug resistance transporter EmrE. Multiple conformational states were determined for these proteins, giving insights into their ion-conduction or transport mechanisms.



Multidimensional diffusion-relaxation correlation NMR and MRI

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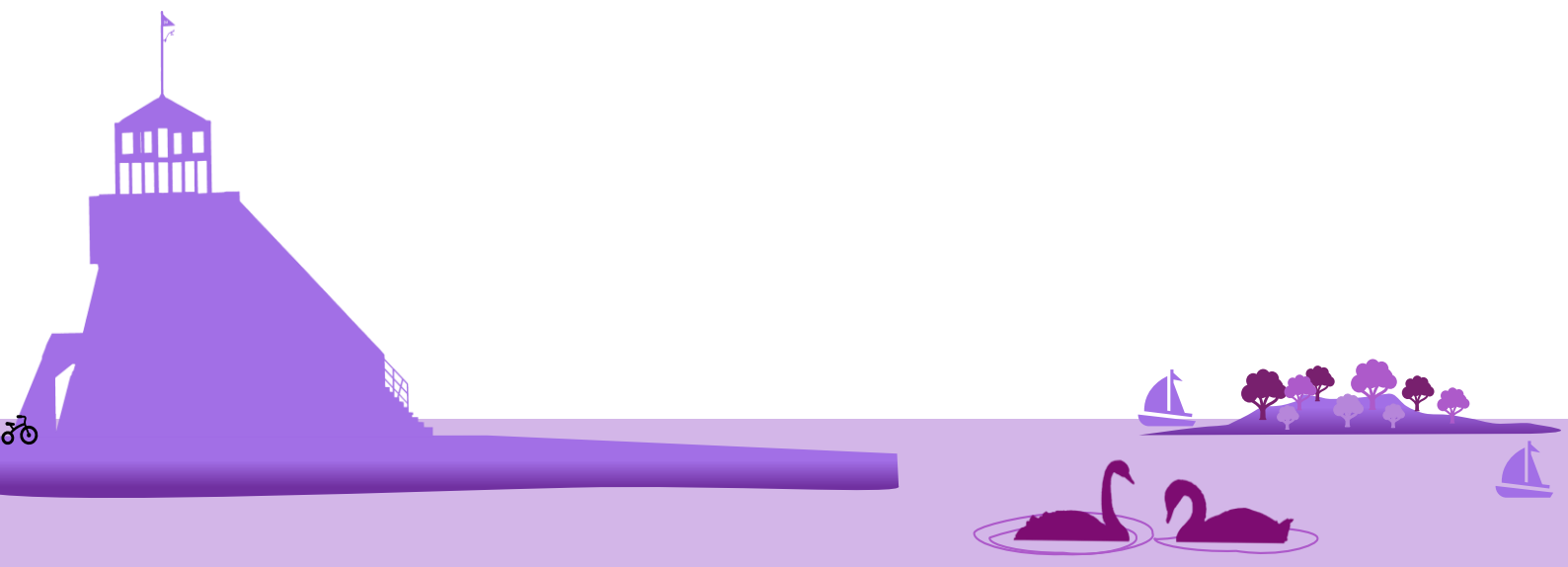
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Low or inhomogeneous magnetic fields prevent using the chemical shift to resolve different molecular species, sites, or environments. Under such circumstances, resolution may instead be provided by diffusion coefficients or relaxation rates, in particular when combined in multidimensional correlation measurements. Over the last decades, diffusion-relaxation correlation has become standard for applications in, for instance, porous media and food science [1,2]. More recently, the approach has also been translated to in vivo human MRI [3,4]. This tutorial will include an overview of how diffusion and relaxation are influenced by molecular and microstructural properties, pulse sequences and acquisition protocols [5,6] for time-efficient correlation studies, and data inversion algorithms [7,8] able to tackle the challenging Laplace relation between the measured data and the multidimensional relaxation-diffusion distributions in which resolution of distinct component may be achieved.

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Intrinsically disordered regulators of endocytosis - an integrated NMR/single molecule fluorescence approach

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Intrinsically disordered proteins (IDPs) lack clearly defined structure and are therefore highly flexible and easily adaptable to different binding partners. This makes them important players in many biological processes, often with vital regulatory functions. Their dynamic features and broad range of interaction modes, however, render them difficult to study and analyzing their complexes often requires integrated approaches. Integrating complementary parameters from of nuclear magnetic resonance (NMR) and single molecule fluorescence approaches allowed us to describe the conformational landscape of IDPs at molecular resolution and promises to shed new light onto various biological processes.

Among those counts clathrin mediated endocytosis. The early phases of clathrin mediated endocytosis are organized through a highly complex interaction network mediated by clathrin associated sorting proteins (CLASPs) that comprise long intrinsically disordered regions (IDRs). We characterize the IDRs of those CLASPs in their entirety and at molecular resolution, using NMR titrations, spin relaxation and relaxation dispersion techniques, and uncover a plethora of interactions of various strengths and dynamic features with their endocytic interaction partners. This allowed us to propose a rationale for how first interactions and dynamic rearrangement of partners take place during the uptake of a coated vesicle.



NMR sheds light on the DNA binding mechanism of multi-zinc finger proteins

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BCL11A is the master regulator of the switch between fetal and adult hemoglobin that happens around birth. It acts as a transcription silencer of the fetal gamma-globin gene, allowing expression of the adult beta-globin gene. BCL11A is a prototypical multi-zinc finger transcription factor and primarily binds DNA through a repeat of three zinc fingers at its C-terminus, termed ZnF456. We reported a comprehensive structural investigation of ZnF456, both in the presence and absence of DNA. X-ray crystallography was used to determine the co-structure of ZnF456 and DNA, whereas NMR was necessary to obtain the structural ensemble of apo ZnF456. We then delved into the dynamics and mode of interaction with DNA and discovered a critical role of inter-domain interactions in the apo conformational ensemble. The last zinc finger of BCL11A (ZnF6) plays a special role. It is constrained in the conformational ensemble and would clash with DNA upon binding; however, it provides a positive entropic contribution to the interaction of ZnF456 and DNA. These findings explain the biological importance of ZnF6, despite its moderate contribution to DNA affinity. Multi-zinc finger domains are widely used by nature as high affinity DNA binders, but the specifics of these interactions are still poorly known. As a matter of fact, BCL11A is the first transcription factor where both apo and DNA-bound structures have been determined.

Ref: T Viennet, M Yin, A Jayaraj, W Kim, ZJ Sun, Y Fujiwara, K Zhang, D Seruggia, HS Seo, S Dhe-Paganon, SH Orkin, H Arthanari. Structural Insights into the DNA-binding Mechanism of BCL11A: The Integral Role of ZnF6. Structure 32, 2276-2286 (2024).



New directions in ultralow-field NMR

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Over the past decade, ultralow-field magnetic resonance (ULF-MR) has undergone a revival, due to game-changing advances in signal detection and hyperpolarization techniques. Once limited by bulky, expensive equipment, ULF-MR is now powered by compact, highly sensitive atomic magnetometers—offering unprecedented precision from dc to MHz frequencies. At the same time, hyperpolarization methods like SABRE-SHEATH are boosting signal strength, often relying on strong-coupling dynamics below earth's field.

Our lab's research leverages both ULF magnetometer and hyperpolarization techniques, and the following advances within these may be discussed in the talk: - - Rethinking ULF MR using imaging information –

We have developed ways to spatially map the sensitive region around an atomic magnetometer vapor cell, as well as image contrast in <10 microliter volumes at low field. The larger goal is to integrate atomic magnetometers operating at ambient temperature (30 to 40 celsius) into a monolithic, chip-scale analytical platform for magnetometry studies of biosamples and/or with microfluidics. - - Profiting from ULF MR as a passive measurement tool –

Longitudinal magnetization dynamics can be measured in real time without polarization destruction, such as to probe para-H₂ hyperpolarization buildup and oscillations, or for continuous quality control monitoring of polarized-¹³C MRI tracer agents. - - Developing new methods for coherent control –

A new formalism to design “composite pulses” has been developed, based on regular shapes such as the regular polygons and polyhedra. This greatly expands options for error-tolerant spin control in low magnetic fields, or generally any 3D system where rotations are performed about arbitrary axes.



Rethinking Brownstein and Tarr in Biological Porous Media

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Over forty years have passed since Brownstein and Tarr published their seminal paper on relaxation theory in porous media. In the following decades, the work has been a foundation for multitudes of publications, billion-dollar industries, and a cornerstone of NMR in geological materials. However, despite Brownstein and Tarr having developed their theory studying rat muscle cells, porous media theory has failed to find a similar foothold in biological tissues. Studies are plagued by repeatability issues and robustness problems, limiting the possibility of large scale application. In their paper, Brownstein and Tarr proposed both surface sink and volume sinks as relaxation mechanisms but stated that the effect of volume sinks could be neglected. In this lecture, traditional nuclear magnetic resonance porous media theory is reviewed, and the effect and influence of volume sinks is presented. The case is made that for biological tissue, the volume sink is a better representation of how relaxation occurs and quite possibly the dominant mechanism.



Exploring Xe Exchange Kinetics for Molecular Host Systems in HyperCEST NMR

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Dissolved Xe-129 is a very sensitive probe for exploring molecular cavities and designing targeted MRI reporters. It can combine features of hyperpolarization and saturation transfer techniques (CEST) to reveal low-populated states of host occupation or to visualize molecular targets at low concentrations where the uptake requires much longer times than the typical hyperpolarization lifetime. This talk will show how different host systems provide different CEST signatures and how these require different saturation transfer conditions to reveal the full range of exchange kinetics and to design ultra-sensitive MR imaging agents.



SABRE of pyruvate: Linking catalyst design to spin and exchange dynamics

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Hyperpolarized pyruvate is among the most promising probes for metabolic imaging, yet SABRE-based ^{13}C enhancement remains challenging due to the complex interplay of binding dynamics and relaxation processes. In this talk, I will present recent progress in catalyst design aimed at improving SABRE performance for pyruvate. We systematically investigated a series of Ir–NHC complexes spanning several carbene ligand families, including both classical and previously unexplored variants.

Our results confirm IMes as a robust benchmark, delivering high ^{13}C polarization in methanol- d_4 . Structurally distinct ligands such as IPr and SIPr provide only modestly lower performance, and enable single-scan detection at 1.4 T. We further introduce two new fluorinated NHC ligands, IPrF1 and IMesF1, which retain the unsaturation in the NHC ring and match the hyperpolarization levels of IMes, while offering increased hydrophobicity—an advantage for future purification strategies.

Temperature-dependent studies and a mechanistic kinetic model support a polarization mechanism governed primarily by exchange dynamics, rather than spin–spin couplings. Building on these insights, we developed a temperature-jump SABRE protocol that selectively enhances the free pyruvate signal, achieving ~30% higher polarization and opening new avenues for in vivo applications.



Stability of MOF in presence of water: New insights from high-field solid-state nmr

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The stability of MOFs in the presence of water is a key factor for their performance in applications such as freshwater production, dessication, humidity control and gas capture. Solid-state nuclear magnetic resonance (ssNMR) with its atomic-level resolution, is a powerful local probe for exploring the structural defects of MOFs under such conditions. In particular, quadrupolar nuclei like ^{17}O and ^{91}Zr offer unique insight into the inorganic cluster of MOFs, such as the Zr-based UiO-66. However, ssNMR studies of these nuclei are challenging due to the low natural abundance of ^{17}O (0.037%) and the ultrawide line of ^{91}Zr spectra.

To address this challenge, we explore isotopic enrichment strategies for ^{17}O using either H_2^{17}O or a ^{17}O -enriched terephthalate ligand synthesized by mechanochemistry[1]. ^{17}O ssNMR reveals distinct isotope distributions among the $\mu_3\text{-OH}$, $\mu_3\text{-O}_2$, and carboxylate (COO^-) oxygen sites, depending on the enrichment route. Furthermore, hydrothermal treatments reveal dynamic oxygen exchange between these sites, highlighting the lability of Zr–O bonds even in structurally intact frameworks.

We also demonstrate the possibility of acquiring ^{91}Zr ssNMR spectra on UiO-66 and UiO-67-NH₂, in presence of steam at different temperatures, using advanced NMR pulse sequences, high magnetic field (18.8 T) and cryogenic conditions (140 K).[2] ^{91}Zr ssNMR provides valuable information in both framework integrity and guest molecule adsorption. We notably show that UiO-66(Zr) remains stable under steam exposure at various temperatures for 7 days. In contrast, UiO-67-NH₂ containing a 2-amino-[1,10-biphenyl]-4,4'-dicarboxylate linker exhibits structural degradation between 80-150 °C but surprisingly remains intact at 200 °C. The lower stability of UiO-67-NH₂ stems from its larger pores and its weaker Zr–O coordination bonds, whereas its preservation at 200 °C results from a more limited condensation of water in the pores.

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[2] DOI: 10.1039/d4sc04589k



The Return of Symmetry-Based Pulse Sequences

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Symmetry-based pulse sequences were originally designed for use in magic-angle-spinning solid-state NMR. Trains of radiofrequency pulses with an internal structure which conforms to special symmetry constraints are synchronised with the sample rotation. The symmetrical construction procedures are defined by their class (either C or R), and by the values of three integers, which are called symmetry numbers. These symmetry numbers define selection rules on the average Hamiltonian, which govern the nuclear spin dynamics. For example, sequences of the C class, and with symmetry numbers 7, 2 and 1, generate double-quantum recoupling of dipole-dipole interactions in solids, and may be used for defining structural connectivities and for estimating internuclear distances. Different choices of symmetry numbers allow the estimation of chemical shift anisotropies, in the presence of dipole-dipole couplings.

Recently we have also used symmetry-based sequences to drive singlet-triplet transitions in solution NMR, and to probe the dynamics of nuclear spin populations in dissolution-DNP experiments. We are currently exploring the use of symmetry-based pulse sequences for double-quantum excitation, in spin systems that are not amenable to the conventional INADEQUATE method.



Effects of methyl groups in proteins on electron spin echo decay

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Methyl protons mix with nearby electron spins through three mechanisms. First, the component of the hyperfine coupling perpendicular to the magnetic field is comparable to the nuclear Zeeman interaction at moderate magnetic fields. Second, for proton pairs at distances between about 5 and 15 Å, the difference between the dipolar hyperfine couplings can match the nuclear-pair dipole-dipole coupling. Third, differences between hyperfine couplings of protons in the same methyl group can match the quantum-rotor tunnel splitting of the methyl group. The last two mechanisms are field-independent and thus relevant even under high-field DNP conditions. Here, we focus on these two mechanisms for methyl groups in proteins. We estimate the contribution of the methyl groups to Hahn echo decay by numerical simulations.

As a test case, we consider a CH₃ group of an amino acid side chain in a matrix that is devoid of protons. We show that tunnel frequencies computed by density functional theory vary by orders of magnitude between different amino acids and may vary substantially between different rotamers of the same amino acid sidechain. The amino group of lysine, which is fully protonated at physiological pH of 7.4, behaves as a quantum rotor analogous to methyl groups. By studying the geometry dependence of the mixing, we find that the quantum-rotor contribution is typically shorter range than the nuclear-pair contribution.



Exploring Organic Photovoltaics from the Spin Perspective with EPR and EDMR

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Organic photovoltaics hold promise as a renewable energy technology, largely due to the high degree of tailorability of the organic molecules at the basis of the conversion of solar energy to electricity. While recent advances in the design of donor and acceptor molecules have pushed power conversion efficiencies beyond 20%, a clear understanding of the photophysical mechanisms underlying device function is still missing. The crucial role of paramagnetic states in the photovoltaic mechanism provides the opportunity to gain additional insights by investigating organic photovoltaics from the spin perspective using Electron Paramagnetic Resonance (EPR) and Electrically Detected Magnetic Resonance (EDMR).

We have investigated a series of donor:acceptor blends for organic photovoltaics across three generations of acceptor molecules. Following a multifrequency EPR characterisation of photoinduced charged states on donor and acceptor molecules, we focused on the investigation of their role in the photovoltaic mechanism. Transient EPR experiments combined with theoretical modelling enabled us to follow the initial photoinduced charge separation process. The spin polarisation of the detected states and its evolution following photoexcitation revealed spin-correlated pairs of charges on donor and acceptor and free charges, the latter mostly arising from ultrafast charge separation in higher performing blends. In order to investigate spin-dependent charge transport and charge recombination processes, we turned to EDMR on fully functional miniature devices to selectively detect spin states directly involved in current generation. Measurement of the transient current response following coherent spin manipulation in EDMR-detected Rabi experiments provided evidence for recombination after re-encounter of charged states on donor and acceptor molecules and for contributions from spin-dependent processes involving triplet states.



Shining light on polyaspartates: Novel photoresponsive alignment media for structure elucidation

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Anisotropic NMR parameter become increasingly important in organic structure elucidation for the determination of conformation and relative configuration of natural products, synthesized compounds and catalysts.[1] To obtain such anisotropic NMR parameter, suitable alignment media are necessary. We use lyotropic liquid crystals based on helical polymers for that purpose.

To achieve tailored and switchable properties for a certain application, stimuli-responsive moieties can be incorporated into polymers. Among the many conceivable stimuli, light is the most intriguing one due to its abundant availability and non-invasiveness.

Herein, we present the design, synthesis and application of novel photoresponsive polyaspartates as alignment media for structure elucidation with NMR spectroscopy. These helical (co-)polymers with ortho-fluorinated azobenzenes[2,3] in the sidechain undergo a reversible helical inversion upon irradiation with green or UV light respectively.[4] These features can be used for the measurement of anisotropic NMR observables like residual dipolar couplings (RDCs). The light-responsiveness enables the measurement of several sets of RDCs enhancing the content of structural information.[5] Therefore, the presented photoresponsive alignment media are very promising for the determination of the relative configuration of unknown and flexible compounds.

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Ultrahigh-resolution NMR spectroscopy

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The strength of NMR spectroscopy derives from its ability to distinguish chemical sites, providing detailed insights into molecular structure and dynamics. However, as the number of chemical sites increases, spectral complexity grows, leading to significant signal overlap that complicates interpretation.

In ^1H NMR spectroscopy, the primary source of signal overlap is the multiplet structure arising from homonuclear scalar (J) coupling. Several methods, including new targeted approaches, will be introduced that either suppress spin-spin coupling to generate 'pure shift' NMR spectra—where each chemical environment is represented by a single, sharp resonance instead of a multiplet—or extract a single multiplet from a region of overlap.

The benefits of these recent advances in pure shift and ultra-selective NMR approaches will be demonstrated for applications in complex mixture analysis. Applications in 1D and 2D NMR will be presented, showcasing the advantages of pure shift techniques in resolving spectral congestion and simplifying mixture spectrum deconvolution.



NMR transport studies in battery electrolytes – ion transport mechanisms and ion-ion correlations

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The development of advanced electrolyte materials for battery applications requires a detailed understanding of transport mechanisms. While electrochemical methods monitor overall charge transport, it is often relevant to clarify the role of distinct electrolyte constituents. Pulsed Field Gradient (PFG)-NMR, as a rather common method, also known as DOSY, provides diffusion coefficients. A specialized application of PFG-NMR is the monitoring of molecular migration in an electric field by electrophoretic NMR (eNMR), yielding drift velocities and electrophoretic mobilities of molecular species. In a multinuclear approach (e.g. ^1H , ^7Li and ^{19}F) often a full characterization of all constituents is feasible.

This lecture describes applications to battery electrolytes, including ionic liquid- based electrolytes, concentrated liquid electrolytes and polymer electrolytes. By determination of the electrophoretic mobility of all molecular constituents, conclusions on speciation, i.e. the existence of transport-relevant ionic clusters, are drawn and transport mechanisms (e.g. structural vs. vehicular) can be identified. Transference numbers T_+ , which describe the fraction of current carried by the active ion, are a crucial parameter to assess the electrolyte performance. They can be easily derived from ion mobilities, which we show for various classes of electrolytes, even including post-Li electrolytes based on Mg, K or Na, where electrochemical experiments become challenging. Moreover, mobility data aid the quantification of ion-ion correlations (Onsager coefficients) in liquid electrolytes, which often pose a limitation to charge transport, thus generating knowledge for optimization of electrolyte composition.



Shining the light on Diffusion NMR. The Time-Resolved Diffusion NMR for studying photochemical reactions

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Time-resolved diffusion NMR (TRD-NMR) is an acquisition technique that allows the measurement of PGSTE experiments with excellent time resolution. The methodology's main application is for reaction monitoring, where the change in mass or diffusion coefficient takes place.

From a vast area of photochemical reactions, two types seem to be a perfect fit with TRD-NMR: photopolymerization and photodegradation. We decided to showcase the method based on three interesting systems:

- Open-air green-light-driven ATRP with conversion control.
- Retinyl Acetate photodegradation (TRD-NMR interleaved with TR-NUS HSQC)
- PET (from plastic bottle) degradation from UV light.

The method, in contrast to other fast diffusion methods, allows us to do this without the sensitivity loss. The TRD-NMR is an adaptation of the Time-Resolved Non-Uniform-Sampling to the world of diffusometry. Thanks to the similar acquisition concept, the two methods can be used in an interleaved way.

The TRD-NMR method allows the study of time-dependent processes where mass change occurs, providing a powerful tool for chemists and material scientists.



Following Conformational Changes in Cas13a with PELDOR

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The type VI CRISPR effector protein Cas13a is an RNA nuclease, catalyzing two RNA cleavage events, the maturation of pre-crRNA and the cleavage of target RNA, activating a non-specific, collateral cleavage. Although all four structures of Cas13a on route from apo, via the pre-crRNA bound complex (Cas13a/precr), the crRNA bound complex (Cas13a/cr), and the crRNA-target RNA bound complex (Cas13a/cr/target) are known, they origin from proteins of different organisms and contain different extends of truncation. To identify and follow conformational changes, structures of Cas13a in all states from one organism are needed. We aim to follow conformational changes by site-directed spin labeling in combination with Pulsed Electron-Electron Double Resonance (PELDOR or DEER) spectroscopy, which enables measuring distance distributions between spin labels in e.g., biomolecules in the range of 1.6-16 nm. We choose *Leptotrichia buccalis* (lbu) Cas13a for which the structures in complex with crRNA and crRNA/targetRNA are known, whereas the structures of the apo and precrRNA bound complexes are unknown. We labelled lbuCas13a with pairs of nitroxide spin labels at different positions and compared the resulting distance distributions to in-silico generated distributions. These were calculated with the software mtsslWizard using experimental and AlphaFold2 structures as input. We show that the apo structure of lbuCas13a has a very flexible, and wide-open recognition lobe (REC-lobe). In contrast, the nuclease lobe (NUC-lobe) is preorganized. Upon pre-crRNA addition, a rigidification and conformational selection of the Helical-2 domain in the NUC-lobe occurs. The structure of the Cas13a/precr complex is very similar to the known structure. The conformation, and conformational changes between Cas13a/cr and Cas13a/cr/target fit to the known structures. In addition, our data suggest Cas13a dimerization in the presence of RNA.



Advances in oscillator-based magnetic resonance

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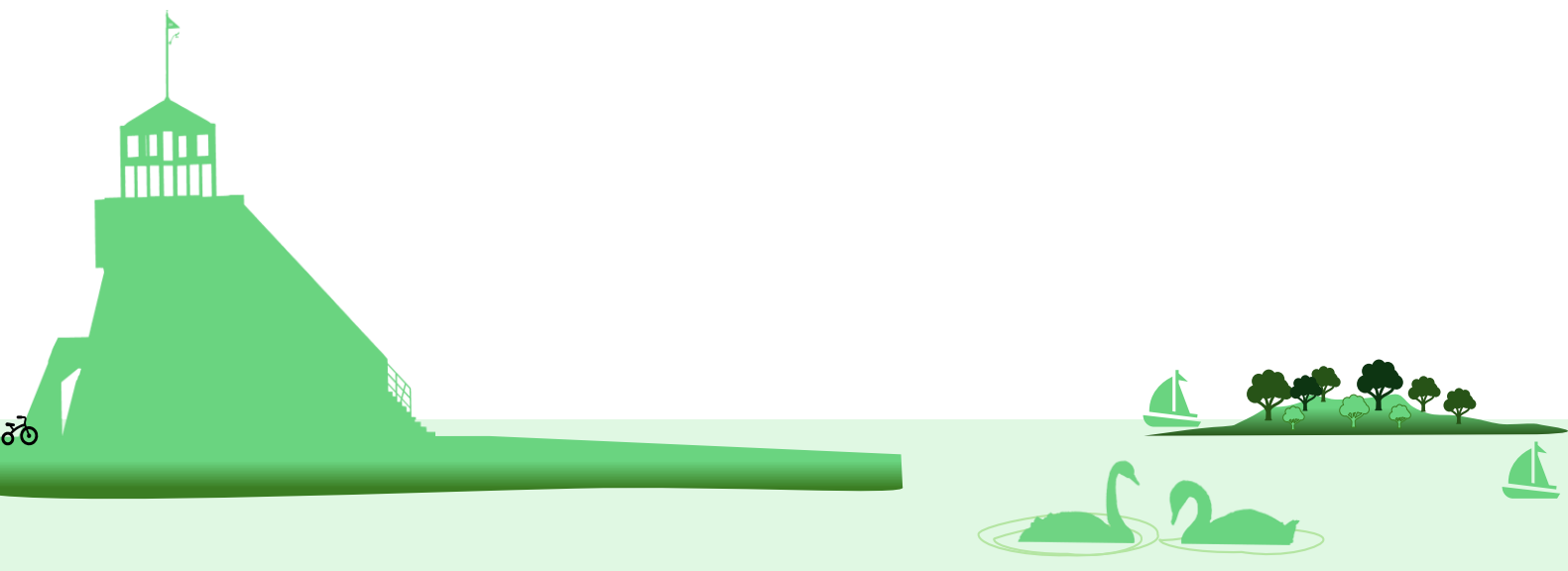
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Oscillator-based magnetic resonance detection has emerged as a powerful alternative to conventional receiver architectures, offering distinct advantages in sensitivity, noise performance, and integration potential. The talk will start with a concise overview of the fundamental detection principle underlying oscillator-based magnetic resonance, highlighting how the unique features of self-oscillating circuits can be harnessed to create detectors for magnetic resonance with outstanding performance.

Following this foundational recap, the talk will survey the current state-of-the-art in oscillator-based detection, delineating key technological milestones and application areas. The talk will then present recent breakthroughs from his research group, including the development of dead-time free NMR detection techniques that enable continuous signal acquisition without sensitivity gaps. In addition, we will discuss advances in phase-coherent detection schemes that were previously missing in the context of oscillator-based magnetic resonance.

These developments pave the way for more compact, robust, and versatile magnetic resonance systems, with implications across biomedical imaging, chemical analysis, and portable NMR and EPR technologies. The talk will conclude with an outlook on future research directions and application opportunities.



Fast magic-angle spinning rates and deuteration facilitate proton detected solid-state NMR spectroscopy to study the cell walls of pathogenic fungi

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The cell wall plays a crucial role in the fungal life cycle. Therefore, characterization at the atomic level of intact fungal cell wall would allow to gain new insights into fungal life cycle and developed novel antifungal therapeutics. In recent years, the solid-state NMR spectroscopy has emerged as non-destructive technique for characterization of intact cell walls in biological systems. Previously, most of the solid-state NMR studies of fungal samples relied on ^{13}C detection methods at slow (<20 kHz) MAS rates. By applying ^1H detection methods, additional gains in sensitivity could be achieved. However, the applications of ^1H detected solid-state NMR is limited due to the high structural heterogeneity and molecular complexity of intact cell walls, which result in broad ^1H resonances. To facilitate ^1H detected ssNMR application for such systems, drastically faster MAS rates and/or additional deuteration are required. Here, we will present a detailed study of ^1H line widths, coherent life-times, chemical shift assignments and dynamics of cell walls using fully protonated and partially deuterated *Candida albicans* fungus at 60-150 kHz MAS rates.

The increased MAS rates drastically improved ^1H line widths. As the result, at 150 kHz MAS, we manage to acquire highly resolved 2D and 3D spectra for chemical shift assignment. We also acquired site-specific ^{13}C T1 and T1rho relaxation measurements, which revealed increased local dynamics of the outer shell molecules. To study ^2H effects on ^1H line widths for intact cell walls, we managed to adapt the fungus to conditions that promote ^2H incorporation. Remarkably, various partial deuteration techniques enabled to decrease the ^1H line widths by a factor of 3, even at 60 kHz MAS. This opens new possibilities to study structure, dynamics and interactions of intact cell walls at moderately fast MAS regimes, while gaining additional sensitivity.

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Development of MAS solid-state NMR methods and applications to the structural characterization of amyloid fibrils

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Multidimensional solid-state NMR experiments suffer from low sensitivity due to inefficient magnetization transfer dynamics. Low efficiency is due to powder averaging and experimental imperfections such as radio-frequency inhomogeneity. We have shown that in addition to the static distribution of amplitudes along the coil axis, dynamic radial RF inhomogeneities are induced by sample rotation. During magic angle spinning (MAS), a spin package travels through areas of different RF fields and experiences periodical modulations of both the RF amplitude and the phase. We present optimal control derived pulse sequences which allow to overcome these instrumental limitations. At the same time, multidimensional pulse schemes exploit PEP (Preservation of Equivalent Pathways) or COS (Coherence Order Selective) transfer concepts to increase the sensitivity of an experiment by more than the expected factor of $\sqrt{2}$ for each indirect evolution period.

Applications of these solid-state NMR techniques are presented for the structural characterization of amyloid fibrils. Protein deposits consist primarily of the amyloid protein, but can contain other factors such as lipids, proteoglycans, and chaperones. So far, it is unclear how the cellular environment modulates fibril polymorphism. In the past, we investigated the structural properties of Alzheimer's disease A β and Diabetes type II hIAPP (human islet amyloid polypeptide) fibrils. Both fibril structures contain unstructured regions which are potential interaction sites for chaperones but might as well be important for seeding involving secondary nucleation.

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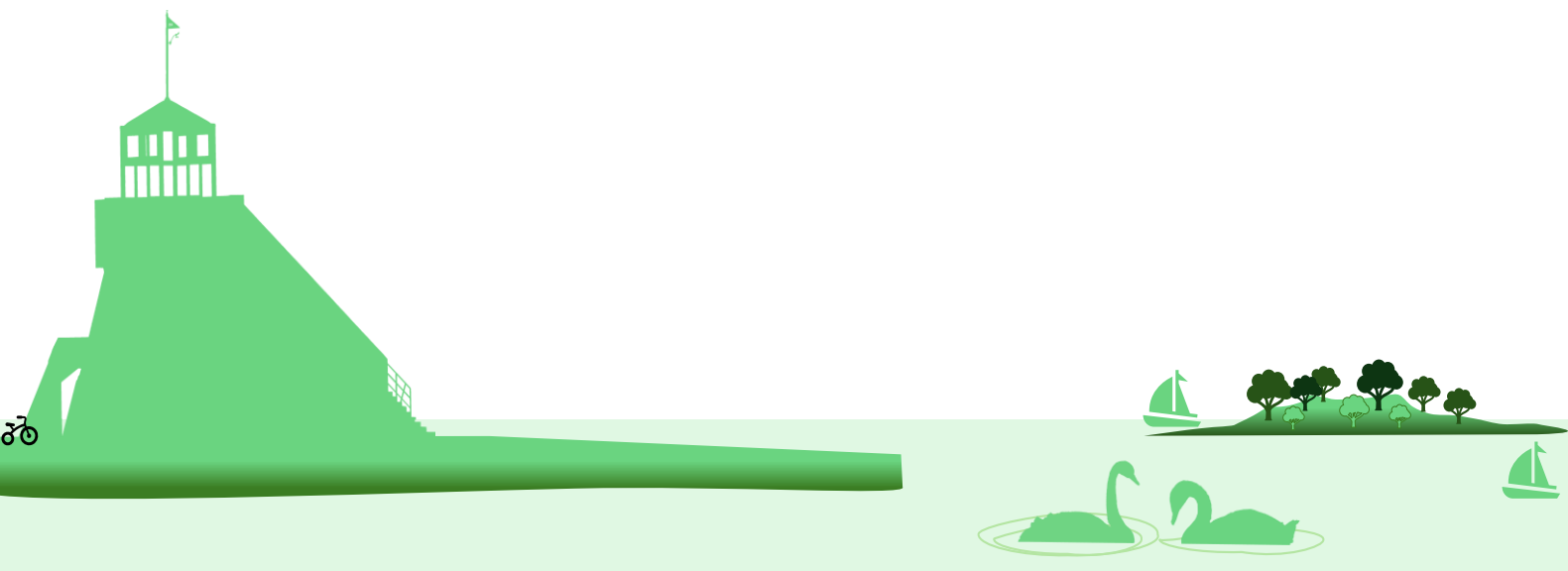
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The dynamic chaperone network in the endoplasmic reticulum

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The endoplasmic reticulum (ER) serves as the major protein folding compartment of the eukaryotic cell. Efficient ER functioning relies on a network of chaperones, calcium level and redox state, whereas variation in these cellular parameters can lead to ER stress and diseases. Here, we present solution NMR studies to resolve key mechanisms underlying the dynamic chaperone network at atomic resolution.

In a first step, we introduce in cyclo NMR to resolve the complete functional cycle of an ATP-driven molecular machine at atomic level. We create a non-equilibrium steady-state under turnover conditions inside the NMR tube, resolving all significantly populated states, their structures, mechanisms and kinetics connecting them, and the dynamic interplay with co-chaperones. The data resolve that the Hsp70 chaperone BiP undergoes a branched functional cycle comprised of seven states that is regulated by two autoinhibition switches.

In a second step, we characterize the disulfide isomerase PDIA6. We find that PDIA6 forms biomolecular condensates, both in vitro and in the ER lumen during protein folding homeostasis. Two specific interfaces in PDIA6 create the multivalency required for the condensate formation, which are dynamically regulated by calcium concentration. PDIA6 condensates recruit Hsp70 BiP and a number of further chaperones specifically into the condensates to form functional “folding factories” inside the ER. Together, our data establish the existence of a functional chaperone condensate that subcompartmentalizes the ER.



Cross-correlated relaxation experiments for studying protein structure and dynamics

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For decades, proteins were regarded as molecules with a rigid spatial structure. At the turn of the twentieth century, this view was broken down, with the identification of a class of intrinsically disordered proteins [1]. These proteins, despite their high mobility and only temporary adoption of secondary structure elements, were found to play important functions in living organisms. It is now widely accepted that a continuum of states of protein order exists [2], ranging from complete disorder to fully structured proteins. However, in each case, the structural inclinations of a protein are related to its function and for this reason are worth investigating. A technique that allows such studies is the measurement of cross-correlated relaxation (CCR) constants.

Here we present a set of CCR experiments which allow to determine local correlation times [3] as well as dihedral angles (ϕ , ψ) distributions [4], for individual amino-acid residues. The experiments were designed for the purpose of studying intrinsically disordered proteins.

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Optical detection of single defect spins in diamond

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Diamond has emerged as a unique material for a variety of applications, both because it is very robust and because it has atomic crystal defects with interesting properties. One of these defects, the nitrogen-vacancy (NV) center, shows quantum behavior up to above room temperature. Our group is exploring single NV centers as a platform for realizing nanoscale sensors with exquisite sensitivities and new capabilities. In this talk, I will discuss recent efforts at integrating NV centers into diamond photonic structures, optical and microwave control of their spin and charge state, and selected applications in molecular sensing.



Quantum sensing with diamond nitrogen vacancy centers

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Nitrogen vacancy (NV) centers in diamond are powerful quantum sensors that are shedding light on various phenomena in condensed matter, biological and chemical targets. I will discuss our work in NV-based scanning probe microscopy of material systems, focusing on novel probes of dynamics. Then I will discuss ongoing improvements to NV-based quantum sensors, focusing on how entanglement can be leveraged for metrological advantage. To this end, gaining control over the diamond material environment is key and I will describe how we use NV-based spin resonance spectroscopy to reveal the static and dynamic properties of its local environment.



Exploiting NMR Crystallography for the Study of Disorder in Phosphate-Based Frameworks

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The structural characterisation of microporous phosphate-based frameworks can be challenging, owing to the high levels and different types of disorder that are often present. NMR spectroscopy, with its sensitivity to the local atomic-scale structure and to motion over a wide range of timescales offers an ideal and complementary probe to diffraction-based methods for the study of these materials. In recent years, there has been growing interest in the emerging field of “NMR crystallography” [1], where information from multinuclear and multidimensional NMR experiments is combined with basic information from XRD, typically using computational approaches, to provide as complete a picture of possible of the short-range and long-range structure.

Our recent work uses an NMR crystallographic approach to characterise disordered aluminophosphate and gallophosphate frameworks, often revealing surprising structural complexity. Here we will focus on the chabazite-type frameworks, demonstrating (i) the unusual dehydrofluorination of GaPO-34 prepared with the 1-methylimidazolium template [2], (ii) the effect of varying the gel aging time in the synthesis of GaPO-34 to produce GaPO-34A (which exhibits compositional, positional and temporal disorder, and is one of the most complex problems tackled by NMR crystallography) [3], (iii) the preparation of a new set of AlGaPO-34 materials [4] which exhibit a non-random distribution of cations in the framework and (iv) some preliminary work on new AlGaPO-14 and AlGaPO-53 materials. This work demonstrates the detailed information that can be obtained when combining XRD with NMR spectroscopy, ensemble modelling and periodic planewave DFT calculations.

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Fast and Highly Resolved NMR: Photo-CIDNP and super resolution NMR

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Resolution and low signal to noise are bottle necks in NMR.

With a dynamic scaling of the number of scans in the indirect dimension of multi-dimensional NMR experiments (usually 2D) the resolution in homonuclear NMR spectra can be increased by a factor of two by fighting the transverse relaxation decay in the FID while in the ^{13}C assignment dimension of a HNCA-type spectra the resolution can be enhanced by a factor of 5 by pseudo decoupling the 1JCACB coupling. The implementation of these experiments to ARTINA enables high throughput assignment of double labeled or unlabeled proteins with minimal measurement time. It is also explored how automated assignment strategies allow for new experimental designs not suitable for man made assignments.

Photo-CIDNP (chemical induced nuclear polarization) to small molecules is a low cost approach to boost NMR signals because only a laser is needed. Photo-CIDNP based drug screening optimizes the measurement time by several orders due to the combined effect of polarization and selective T1 relaxation of the ligand due to protein binding yielding a high contrast also at low field. Photo-CIDNP is demonstrated also to mixtures of small molecules and also to ^{19}F detection. Further photo-CIDNP applications currently under investigations are also elaborated on.



High-field optically induced NMR hyperpolarization in solids

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Poor sensitivity is still one of the main barriers in cutting-edge applications of NMR spectroscopy for structural and chemical analysis. In this regard, hyperpolarization alleviates the sensitivity issue by enhancing the population imbalance between the nuclear spin states, and thus the NMR signal.

For solid-state NMR, optical hyperpolarization methods would be particularly enticing. They exploit transient electronic excited states as the polarization source, which can in principle induce very large nuclear spin polarizations. One such optical technique is solid-state photochemically induced DNP (photo-CIDNP), where light is used to excite a donor–acceptor system, generating a spin correlated radical pair whose evolution and decay build nuclear spin hyperpolarization. We will report on progress following our recent demonstration that bulk optical ^1H hyperpolarization can be obtained at 0.3 T by polarization relay from a synthetic donor–acceptor molecules.[1] Here, we extend the concept of ^1H and ^{13}C photo-CIDNP from synthetic donor–acceptor molecules to the high magnetic fields required for high-resolution NMR spectroscopy, namely, 9.4 T and 21.1 T, and explore the mechanisms in play that generate and transfer polarization. [2,3,4]

[1] J. Am. Chem. Soc. 145, 14874 (2023)

[2] J. Phys. Chem. Lett. 15, 5488 (2024)

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[4] J. Phys. Chem. Lett. 15, 11097 (2024)



Elucidating Biomolecular Interactions using Hyperpolarization by Parahydrogen and Dynamic Nuclear Polarization

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Introduction

Biomolecular interactions among proteins, ligands and water as solvent are responsible for cellular functions related to metabolism, signal recognition, and others.

Aims

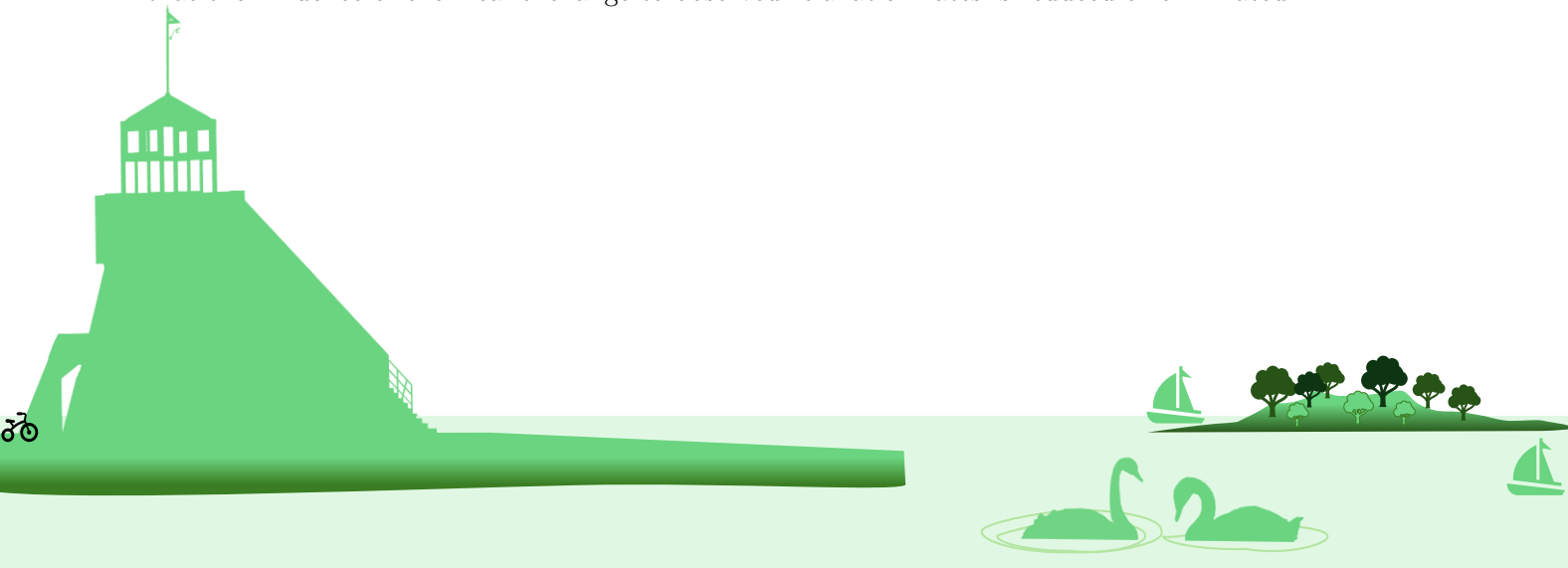
We aim to demonstrate the hyperpolarization of biological ligands to improve their detection by NMR, contrasting the capabilities of dissolution dynamic nuclear polarization (D-DNP) and parahydrogen polarization.

Methods

Spins of ^{19}F and ^1H in small molecule ligands and in water are hyperpolarized by D-DNP. Parahydrogen polarization is produced using non-hydrogenative polarization in homogeneous solution, in a water/organic phase mixture comprising reverse micelles, and using a heterogeneous catalyst embedded in a metal-organic framework.

Results

DNP enhances the initial spin polarization by $>10^3$ -fold compared to high-field NMR at 9.4 T but is usable only in single-shot experiments where structure and interactions are determined by measuring transient polarization transfer or spin relaxation. Hyperpolarization of water restores the ability for multi-scan NMR experiments when polarization is transferred incrementally to a target molecule. In the experiment described, a ^{15}N spin of benzamidine is hyperpolarized by cross-polarization in the liquid state and subsequently used to measure the interaction of the molecule with the protein trypsin. Although signal enhancements at high field are lower than for DNP, parahydrogen polarization is repeatable. We show the measurement of a ligand interaction with a protein using a parahydrogen polarized metal complex acting as a sensor in the reverse micelle medium. Additionally, parahydrogen polarization is compatible with NMR at low magnetic field. At a milli-Tesla measurement field, the nominal signal enhancement is $>10^6$. Measuring biological ligands becomes possible using this hyperpolarization, with the added feature that the influence of chemical exchange to observed relaxation rates is reduced or eliminated.



Adapting parahydrogen hyperpolarization for metabolomics applications

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NMR is one of the go-to tools for metabolomics – the process of chemical analysis of small molecule metabolites in living organisms. Usually, we applaud NMR in metabolomics due to its robustness, reliable quantitation and relatively easy to interpret data. However, NMR is also a relatively low-sensitivity technique, limited to the higher concentration metabolome. This leaves a sizeable portion of metabolites inaccessible for NMR, forcing potential users to adopt other analytical methods whenever analysis of dilute metabolites (e.g., low micromolar or below) in a biological sample is desired.

This has stimulated efforts in adopting nuclear hyperpolarization to increase sensitivity of NMR metabolomics. Out of several available hyperpolarization techniques, dissolution-DNP, photo-CIDNP and parahydrogen hyperpolarization have been shown to be compatible with biological samples, including human biofluids. We are working with non-hydrogenative parahydrogen-based hyperpolarization methods that can provide 1000-fold sensitivity enhancements for NMR detection of metabolites in biological samples.

This talk will give an overview of our work in adapting parahydrogen based nh-PHIP and SABRE hyperpolarization methods to biological samples. These are chemoselective techniques that enhance detection of specific metabolite classes at a time. We will discuss the currently known application scope and describe the strategy for directing the signal enhancement process towards different analyte classes like N-heterocyclic metabolites, amino acids or oligopeptides. We will then show the necessary processes and principles for achieving compatibility of nh-PHIP and SABRE with different biological sample matrices like urine, blood or tissue extracts. The last part of the talk will outline our efforts in adopting parahydrogen hyperpolarized metabolite detection on more affordable lower field spectrometers, where hyperpolarization compensates for the lower sensitivity of such machines.



New frontiers in NMR metabolomics: integrating more sensitive and resolved NMR into the metabolomics workflow

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No ideal analytical platform exists to answer all the specific criteria of the metabolomics workflow: NMR and MS presents both distinct advantages and limitations that are largely complementary. The future of metabolomics lies on building on their respective strengths while overcoming the weakness of both analytical platforms across the entire workflow - from sample preparation, to data acquisition, processing and interpretation. In the case of NMR metabolomics, our team has carried out several methodological developments to enhance the sensitivity and resolution of NMR methods and to facilitate their integration with MS-based metabolomics.

Among these developments, we recently showed that a better sensitivity can be obtained thanks to NMR methods based on dissolution Dynamic Nuclear Polarization. We recently illustrated the potential of this approach on the first clinical metabolomics study of urine at natural ^{13}C abundance, allowing the detection and quantification of two additional metabolites of chronic kidney disease compared to classical 1D NMR metabolomics. Although this approach relies on complex instrumentation, it opens many development and application perspectives.

To address the issue of signal overlapping in 1D ^1H NMR, 2D NMR acquisitions can be redeeming. For compatibility with the high-throughput demand of metabolomics, we implemented time-saving strategies (such as ultrafast 2D NMR and non-uniform sampling) in metabolomics workflows, showing great promises for targeted and untargeted metabolomics. While challenges remain in signal integration of heavy datasets, our recent results demonstrate that fast 2D NMR improves statistical performances and metabolite identification rates.

Finally, preliminary work to combine the strengths of classical 1D ^1H NMR metabolomics to MS metabolomics and perspectives to combine those afore-mentioned novel NMR methods into metabolomics, are explored through correlation, multiblock integration and metabolome coverage.



Approaches for operando Nuclear Magnetic Resonance of rechargeable batteries

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Operando NMR of battery cells is a powerful technique to analyze charge and degradation mechanisms in batteries in real time, while the cell is charging or discharging. Battery cells are however challenging to measure by NMR, due to the technical issues associated with their multicomponent nature and their metallic components - namely overlaps, distortions and strong broadenings leading to low sensitivity and resolution for solids and liquids within the cell.

In this talk we will present our work towards the improvement of in situ and operando NMR using several experimental setups, always with the goal to transition towards the measurement of commercial cells with excellent electrochemical performance for statistically relevant measurements.

We explored the experimental challenges and limitations with spinning electrochemical cells and with using metallic pouch cells. We also worked on the improvement of the experimental setup and methodology to increase spatial resolution and follow each electrode independently [1] or to increase sensitivity (hence time resolution) for Li-plating or state-of-charge monitoring in NMC622//graphite Li-ion cells [2,3]. We will finally report on our recent results using pilot-line-assembled NMC811//Si aluminized Li-ion pouch cells that can be charged in as fast as 15 minutes [4].

[1] M. Tang, V. Sarou-Kanian, P. Melin, J.-B. Leriche, M. Ménétrier, J.-M. Tarascon, M. Deschamps, E. Salager, Nat. Commun. 7 (2016) 13284. <https://doi.org/10.1038/ncomms13284>

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[3] K. Bagheri et al., manuscript in preparation

[4] R. Praud et al., E. Salager, manuscript in preparation



Solution-State NMR and Biomineralization: Watching Minerals Form in Real Time with Hyperpolarization, AI, and Fractals

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Biomineralization is one of nature's best material design strategies—organisms build complex, functional structures like bones, shells, and silica skeletons using simple organic templates. But how do these soft, disordered biomolecules assemble minerals with precision?

One challenge is that biomineralization happens in solution, at low concentrations, and through transient, weak interactions—conditions that are difficult for conventional NMR. We need methods that are faster, more sensitive, and capable of capturing these fleeting structures in real-time.

Recent advances in dissolution Dynamic Nuclear Polarization (dDNP) NMR, ML-driven spectral deconvolution, and fractal-cluster molecular dynamics (MD) are changing the game.

dDNP boosts NMR signals by four orders of magnitude, allowing us to detect biomineralization intermediates in solution that were previously invisible.

MLI-based deconvolution compensates for the resolution loss in dDNP, using neural networks to remove spectral distortions and recover high-resolution signals.

Fractal-cluster MD simulations bridge the gap between atomic-scale NMR data and large-scale mineral assemblies, revealing how peptides self-assemble into templates that dictate mineral morphology.

A concrete example: silica-precipitating peptides, like R5 from diatoms, form supramolecular templates in phosphate buffer. These templates guide silica deposition, controlling particle size and shape. dDNP-NMR, combined with real-time 2D NMR, captures these early-stage peptide self-assemblies at residue resolution. AI-assisted spectral processing then extracts structural details hidden by hyperpolarization artifacts. Finally, fractal-cluster MD explains how these peptide assemblies nucleate silica and translate molecular-level order into macroscopic structures.

This talk will discuss how these new tools let us “watch” biominerals form—not just statically, but dynamically, as a function of time, sequence, and environment.



SimpNMR – a tool for ab initio assisted paramagnetic NMR analysis

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Assignment and analysis of seemingly simple one-dimensional ^1H and/or ^{13}C NMR spectra of paramagnetic metal complexes often turns into a challenging task that requires an input from quantum chemistry calculations.

Here, we present a Python-based tool SimpNMR that helps to

- 1) Simulate pNMR spectra using outputs from quantum chemistry calculations (Gaussian/ORCA)
- 2) Assign and fit experiential peaks data to extract full magnetic susceptibility tensor

The observed shift is simulated as a sum of diamagnetic and paramagnetic components, where paramagnetic part is calculated as a product of spin-only hyperfine coupling tensor and the magnetic susceptibility tensor.

Practical consideration, pitfalls and successes of pNMR analysis of solution ^1H and ^{13}C NMR spectra of iron(II) complexes will be shown as examples.



Protein NMR assignment by isotope pattern recognition

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The current standard method for amino acid signal identification in protein NMR spectra is sequential assignment using triple resonance experiments. Good software and elaborate heuristics exist to facilitate the process, but it remains laboriously manual – the task is essentially of repeated visual identification and matching. Predictably, artificial intelligence helps; it is expected to be particularly efficient for NMR signals with residue-specific shapes, for example when selectively labelled pyruvate biosynthesis is used.

However, a hard problem emerges on the computer science side: training databases must contain millions of samples that capture every corner of related physics and every kind of instrumental artefact. Such databases can in principle be generated by high-fidelity simulations, but cannot actually be stored on any realistic computer because they are too big.

We recently found a solution to this problem (<https://www.science.org/doi/abs/10.1126/sciadv.ado0403>) that uses polyadic decompositions to store millions of synthetic three-dimensional NMR spectra, on-the-fly simulation of instrumental artefacts during training, and a probabilistic way of incorporating prior and posterior information.

The resulting neural nets take $[^1\text{H}, ^{13}\text{C}]$ dimension slices of HNCA spectra and return an amino acid probability table for each signal, taking into account amino acid sequence information and selectively labelled pyruvate biosynthesis signal shape information. When both are available, backbones of common proteins (GB1, MBP, INMT) are rapidly assigned from just the HNCA spectrum.



Methods and Instruments for High-Field MAS DNP toward Intracellular Structural Biology

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We have been implementing DNP at ultra-low temperatures (ULT, $T \sim 30$ K) using helium gas that greatly improved the DNP efficiency at high field ($B_0 = 16.4$ T). A key technological breakthrough therein comprises the closed-cycle helium gas circulation (CHC) MAS system and dedicated DNP probe [1], that maintains stable MAS for weeks without consuming any helium. The long-term stability and its seamless temperature controlling ability between 20 and 200 K has enabled a number of applications in biological [2] and material sciences [3], and also contributed to understanding the mechanistic origin of the Overhauser DNP [4]. The system is also combined with a cryogenic signal detection system for the thermal noise suppression, being cooled "for free" using the cold (~ 40 K) return helium gas on its way back to the CHC compressor [5]. Recently, the same long-term ULT MAS facility was combined with a solid-state MW source, proving its enhanced utility [6]

Beyond the sensitivity gain, we have been exploring new DNP methods e.g., for the nano space-selective observation [7] and background signal suppression [8], looking to target selective DNP within unpurified mixture samples. We also demonstrate nanodiamond is a promising polarizing agent for MAS DNP on proteins at 16.4 T within biological cells [9]. These basic technical advances combined should open up a new avenue toward high-sensitivity intracellular structural biology. In the presentation, we will discuss the latest results on such methods, instrument and applications.

- [1] Y. Matsuki et al., eMagRes 7, 9- (2018)
- [2] M. Takamuku et al., Neurochem. Int. 157, 105345 (2022)
- [3] Y. Matsuki et al., PCCP 23, 4919- (2021)
- [4] F. A. Perras et al., JCP 158, 154208 (2023)
- [5] Y. Matsuki et al., JMR 335, 107139 (2021)
- [6] F. Hobo et al., JMR 373, 107842 (2025)
- [7] Y. Matsuki et al., JPC C 124, 18609- (2020)
- [8] Z. Zhang et al., PCCP 26, 9880 (2024)
- [9] K. Kato et al., DOI: 10.26434/chemrxiv-2023-x9m17-v2 (2023)



Increasing NMR sample throughput

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Vastly accelerated substance screening via NMR, from large sample collection sets, has potential applications in material science, drug development, and forensic tasks, potentially with important societal consequences [Kupvce2021], for example, when responding to national health crises. The main benefits of NMR would potentially be leveraged for these collections, including the avoidance of false positives and negatives, and achieve a vast increase in informational density through the use of multiple pulse sequences applied to the same sample. However, it is challenging to do so. Solving this challenge is a hardware and software task. For one, the hardware must permit rapid transfer of sample through a probehead and support parallel RF and gradient excitation and acquisition of multiple sample streams, and the software must compensate for all non-idealities, mainly resulting from space restrictions inside the magnet bore.

In this contribution we will review the Karlsruhe group's contribution to date to establish a variety of bespoke hardware and software concepts for high throughput NMR substance screening. Our focus will be mainly on the digital twinning, design, and micro-manufacturing of these concepts, and simple demonstrations of function.

[1] Kupce, E.; Frydman, L.; Webb, A. G.; Yong, J. R.; Claridge, T. D. Nature Reviews Methods Primers 2021, 1, 27



Optimizing Pulse Sequences Based on Effective Hamiltonians

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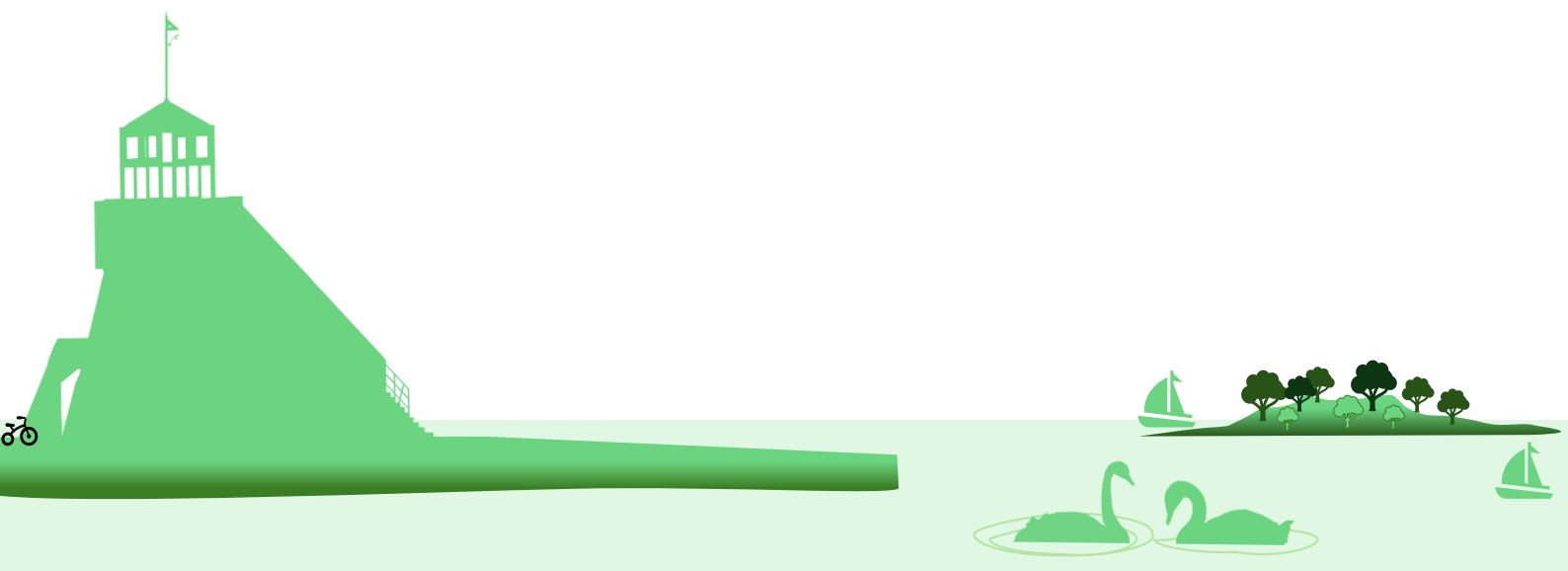
Computer optimization of pulse sequences in NMR and EPR has been mostly based on optimal-control methods [1] where the sequence is optimized as a state-to-state transfer of the density operator. Such sequences have a fixed length and cannot be concatenated. Analytical pulse sequence design is mostly based on effective Hamiltonians calculated for short repetitive sequences where the length of the mixing time can be adjusted to the magnitude of the interaction.

Using continuous Floquet theory [2,3] we have implemented pulse-sequence optimization based on analytically calculated effective Hamiltonians. Such calculations only require single-spin interaction-frame trajectories as a function of the chemical shift and pairwise convolutions of these trajectories to calculate the effective two-spin Hamiltonians that include resonant and non-resonant contributions. We show as a proof of concept that it is possible to numerically optimize periodic repetitive pulse sequences for MAS NMR and DNP. This framework allows us to include pulse-sequence imperfections like rf-field inhomogeneity or pulse transients into the effective Hamiltonian calculation to generate stable sequences. The sequences are shown to work as predicted by numerical simulations and experiments on test substances. While we have included only first-order effective Hamiltonians so far, the method can be extended to include higher orders of the effective Hamiltonian.

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Molecular dynamics, sparse isotope labeling, and ^{19}F : Towards site-specificity in DNP-enhanced biomolecular MAS NMR

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Solid-state NMR under magic-angle spinning (MAS) is an important tool in many scientific fields, including structural biology and materials science. However, NMR's inherently low sensitivity requires oftentimes long acquisition times or even renders many experiments unfeasible. Dynamic nuclear polarization can overcome this limitation by transferring much larger electron spin polarization to neighboring nuclei, thus enhancing their magnetization by up to several orders of magnitude. Typically, fast spin diffusion within the hyperpolarized ^1H bath is taken advantage of in order to widely spread polarization over relatively large distances before transferring the ^1H polarization to heteronuclei for detection or further evolution. This results in a fast and uniform enhancement of the whole NMR spectrum.

However, spectral crowding becomes a serious problem for large biomolecular assemblies or complexes. DNP can feature several approaches to also introduce spectral selectivity—besides sensitivity—through site-specificity. One such approach is the use of molecular dynamics such as methyl group reorientation or ring inversion dynamics which introduce the possibility of specific cross-relaxation enhancement by active motions under DNP (SCREAM-DNP). By this method, in combination with DNP-enhanced ^1H - ^2H CPMAS NMR, we have investigated how different biomolecular environments affect the dynamical properties and thus the transfer efficiency of this process and how sparse isotope labeling as well as frequency-selective recoupling can further isolate specific transfer pathways. Furthermore, it will be shown how direct ^{19}F DNP can be optimized by tuning of the spin diffusion of enhanced polarization from the polarizing agent to the ^{19}F -carrying analyte through the sample matrix. This will open up new possibilities for spectral selection of sought-after regions in large biomolecular assemblies by DNP.



Decoding Order and Disorder in Proteins by NMR Spectroscopy

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Proteins often exhibit complex architectures composed of both structured domains and intrinsically disordered regions (IDRs). These heterogeneous features pose significant challenges for traditional structural biology. A primary difficulty arises from the dynamic and flexible nature of IDRs, which lack stable three-dimensional structures, thereby complicating the application of conventional structural techniques. As a result, structural studies have frequently focused on isolated domains, providing insights into specific regions but often neglecting the interactions and regulatory dynamics between domains.

To fully understand the structural and functional complexity of multidomain proteins, it is essential to investigate larger constructs that preserve domain-domain interactions. Nuclear magnetic resonance (NMR) spectroscopy offers powerful tools to address this need. We have developed tailored NMR experiments that exploit the dynamic differences between domains, selectively detecting signals based on their nuclear relaxation properties. This approach enables the acquisition of high-quality 2D and 3D spectra, facilitating the sequence-specific assignment of complex constructs. We provide examples illustrating how these methodologies uncover valuable insights in heterogeneous proteins.



A Preclinical Low-Field MRI scanner for Multimodal Imaging: Photon detected-MRI and beyond

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Low-field magnetic resonance has gained renewed attention due to advancements in permanent magnet-based systems, which offer advantages such as portability, affordability in both purchase and upkeep, greater environmental sustainability, and enhanced flexibility for innovative, tailored designs. This flexibility is particularly attractive when MRI is integrated with other imaging technologies like PET or CT, or with alternative detection and hyperpolarization methods. The GAMMA-MRI initiative aims to investigate the feasibility of using MR techniques to spatially encode gamma photons emitted by hyperpolarized xenon isomers. To capture these photons, gamma detectors are positioned around the subject being imaged. This detection strategy holds promise for pushing the sensitivity limits (down to 10^{13} spins) while preserving the adaptability and spatial resolution typical of conventional high-field MRI. For this purpose, a new custom-designed low-field MRI system has been developed to meet multiple constraints without compromising image quality.

In this work, we present core principles and examples of custom-built MR systems, with a particular emphasis on a low-field preclinical device for small animal MRI developed within the GAMMA-MRI project.



The revolving power of fast magic-angle spinning

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Magic-angle spinning (MAS) solid-state NMR has undergone a quiet revolution, with spinning rates reaching 100 kHz and beyond. While such speeds have transformed biomolecular NMR, their application to paramagnetic systems has remained limited—despite the potential to extract rich structural and electronic information. Here, we show that ultra-fast MAS at 100 kHz dramatically enhances spectral resolution and coherence lifetimes in highly paramagnetic materials, enabling site-specific assignments even at sub-milligram scale.

We demonstrate that the expected sensitivity losses from reduced rotor volume are mitigated in paramagnetic solids by accelerated relaxation, and that the resolution gains allow direct access to local structure and spin delocalization effects. Examples include lanthanide complexes, iron catalysts, mixed-metal battery cathodes, and cobalt and copper-containing enzymes.

We also present the first paramagnetic NMR spectra acquired at 160 kHz MAS using Bruker's new 0.4 mm probe, pushing resolution and sensitivity even further. These results confirm that ultra-fast MAS overcomes key bottlenecks in the study of paramagnetic systems, opening new perspectives for the routine atomic-level characterization of functional materials and redox-active biomolecules.



Lanthanoid tags for biomolecular NMR

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Pseudocontact shift (PCS) NMR based on paramagnetic lanthanoid chelating tags (LCTs) is a very useful tool in biomolecular NMR spectroscopy. PCS have the unique ability to localise spin positions in space using only a simple chemical shift difference as input. Combined with a very long range, exceeding 100 Å, this technique allows for structure refinement, localization of ligands within a biomolecule, monitoring protein-protein interactions, characterizing transient high-energy states of proteins and assigning of delicate membrane proteins like G-protein coupled receptors. We are currently looking into applications for RNA, design new scaffolds for LCTs and use our LCTs also for UV-Vis emission spectroscopy.



Flow characteristics of periodic porous media using double rotation-modulated gradient diffusion NMR

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Pulsed field gradient (PFG) NMR is a mature method for flow measurement in complex systems ranging from laminar flow in biological tissues and geophysical porous media to turbulent flows encountered in industrial processes. However, PFG lacks the specificity to isolate the anisotropy and frequency dependence characteristic to flow in such systems.

The double rotation-modulated gradient (DOR-MG) diffusion encoding sequence [1] measures the diffusion spectrum tensor, which provides detailed insights into restricted diffusion, curvilinear motion, and flow around obstacles. In this talk, we explore the application of this method to simulated flow through a periodic porous medium [2].

From the diffusion spectrum tensor, we can ascertain features such as the primary direction of flow, characteristic length of the pores, degree of turbulence anisotropy, and energy spectrum of turbulent eddies [3]. These findings illustrate the utility of the DOR-MG approach and inform experiment design to better understand flow phenomena.

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Compression-Induced Microstructure in Osteoarthritic Cartilage: Insights from micro-MRI and Sodium MQF Spectroscopy

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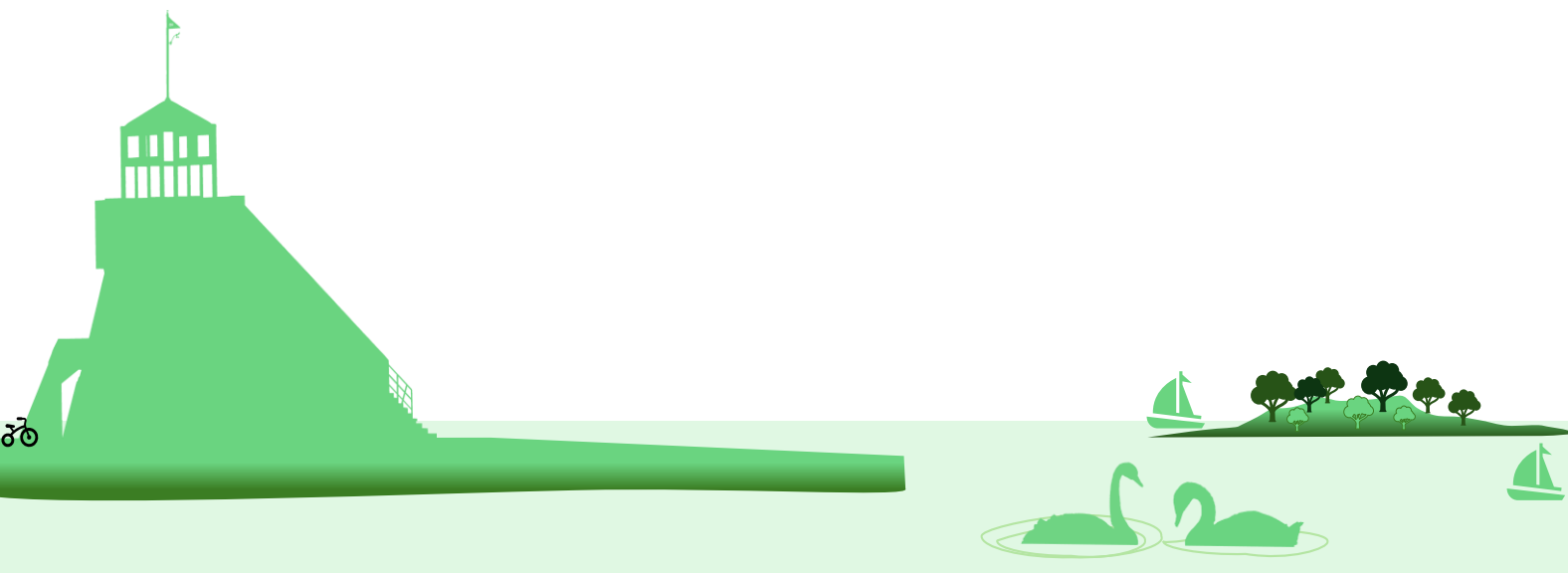
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Osteoarthritis (OA) is a degenerative joint disease characterized by the failure of multiple tissues within diarthrodial joints. It is the most prevalent chronic rheumatic condition and a leading cause of disability worldwide. The clinical application of OA magnetic resonance imaging (MRI) relies on correlating cartilage compression and relaxation contrast with histological staining. This enables the refinement of OA MRI protocols to target key histological markers, such as collagen fiber remodeling (^1H MRI) and glycosaminoglycan (GAG) depletion (^{23}Na MQF spectroscopy). These advancements allow for non-invasive disease monitoring and assessment of therapies aimed at improving cartilage response to mechanical loading.

Porcine cartilage specimens were sourced, cut into twelve 1 cm^3 cubes, and divided into four groups: control, collagenase-treated, and two trypsin-treated (trace and excess). Samples were soaked for 48 hours to remove excess sodium, enzymatically digested, vacuum-sealed, and subjected to MRI and spectroscopic analysis under compression and relaxation cycles.

Experiments were conducted using a 9.4 T Bruker Avance III Microimaging system with a dual-tuned $^1\text{H}/^{23}\text{Na}$ coil. Imaging protocols included multi-gradient echo (MGE) and multi-spin multi-echo (MSME) sequences (Paravision 6.01). Sodium MQF spectroscopy employed triple quantum filtered (TQF) and 2D TQ-TPPI techniques (TopSpin 3.6) to analyze sodium storage changes in cartilage. Collagen fiber remodeling was visualized at $\text{TE} = 8\text{ ms}$ using MGE sequences under compression/relaxation.

Cartilage sodium storage capacity (CSSC) was quantified from free-to-bound sodium ratios in 2D TQ-TPPI spectra. Results revealed mechanical changes distinguishing OA models from controls, with collagen depletion impacting stress response—hallmarks of OA progression. This study supports MRI and sodium MQF spectroscopy as valuable tools for non-invasive OA diagnosis and treatment assessment.



Long-lived coherences in the direct dimension to follow redox kinetics of antioxidants in cells

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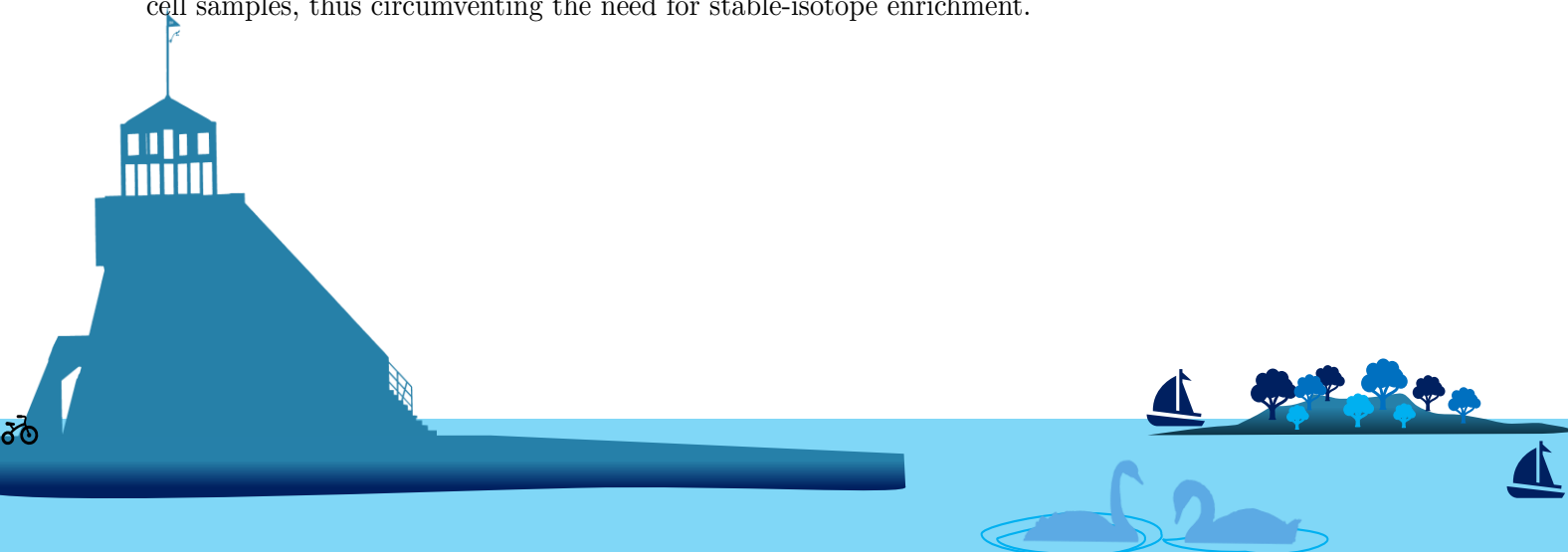
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Introduction: For in-cell ^1H NMR studies, resolution is limited by the coherent and incoherent relaxation mechanisms. Signal overlaps and line broadening hurdle biomarker assignment. Long-lived coherences (LLCs), i.e., coherent superpositions of nuclear 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 direct dimension for improved detection of biomarkers in cells without recurring to isotope enrichment. This is demonstrated to follow oxido-reduction kinetics on different times scales for glutathione (GSH), the most abundant endogenous antioxidant in cells.

Methods: Burkitt lymphoma RAJI B cells have been induced to overexpress GSH upon simulation of Nrf2, a transcription activator. RAJI cells (5×10^6) were harvested, diluted in 550 μL D $_2\text{O}$ and gene activation was triggered. We have adapted the LLC pulse sequence to alternate sustaining intervals ($\nu_1 = 2$ kHz) with direct detection via small-angle extraction pulses. The experiment was repeated to ensure reproducibility.

Results: We show the first in-cell application of direct-dimension detected LLC's. We distinctly observe the presence oxidized / reduced glutathione signals (GSH/GSSG) in RAJI cells. This new method enabled the follow-up of GSH kinetics on timescale of seconds (fast oxidation) and hours (slow oxidation) using LLC-based resolution improvements.

Conclusions: This demonstrated detection of biomarkers in cell using LLC's can enable LLC-based Magnetic Resonance Spectroscopy in vivo. Chemical shift dispersion can now be improved using ^1H LLC's in cell samples, thus circumventing the need for stable-isotope enrichment.



Combining NMR and AlphaFold to identify new partner protein binding motifs in intrinsically disordered proteins

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Intrinsically disordered proteins (IDPs) constitute approximately 40% of the human proteome and play essential roles in various biological processes. Many IDPs engage with their partner proteins through short linear motifs, typically exhibiting moderate binding affinity. Recent advances in artificial intelligence-driven structure prediction have revolutionized structural biology. However, prediction of complexes involving IDPs remains a major challenge due to the lower quality of their multiple sequence alignments and the underrepresentation of IDP complexes in the Protein Data Bank (PDB) [1].

In this study, we investigate two scaffold proteins, JIP1 and JIP2, from the JNK signaling pathway, both of which possess long intrinsically disordered tails (450 and 510 amino acids, respectively). Using NMR spectroscopy, we obtain an atomic resolution description of these tails and study their interactions with the kinase JNK1 through a combination of chemical shift titrations, $R1\rho$ relaxation rates and CPMG relaxation dispersion. Our data reveal previously unrecognized kinase-binding motifs within the scaffold proteins, and we determine high-resolution crystal structures of JNK1 in complex with these motifs [2]. Using the experimental NMR and X-ray crystallography data, we evaluate the ability of AlphaFold to predict these interactions. While some motifs are accurately predicted, others are not captured by AlphaFold. To improve prediction accuracy, we introduce a sliding-window approach, which outperforms predictions based on the full-length disordered sequences.

Our work not only demonstrates the power of NMR spectroscopy in identifying novel binding motifs within long disordered regions but also highlights both the strength and limitations of AlphaFold in modeling complexes involving IDPs, even when using adapted prediction strategies.

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Live magnetic observation of parahydrogen hyperpolarization dynamics

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Hyperpolarized molecules exhibit dramatically enhanced NMR signals, enabling novel applications in spectroscopy and imaging. However, conventional NMR detection requires a magnetic field pulse to perturb the system's quantum state, followed by signal detection. This process destroys the hyperpolarized state and provides low-frame-rate observations when tracking dynamic processes (e.g., with trains of small-tip-angle pulses). We show that optical magnetometers can be used for continuous, nondestructive tracking of hyperpolarized nuclear spin dynamics in real time. We observe ^1H and ^{13}C magnetization in parahydrogen-polarized molecules, including real-time adiabatic transitions at low-field avoided crossings and chemical-exchange-driven ^{13}C hyperpolarization. By providing uninterrupted monitoring of hyperpolarization processes even in the presence of time-dependent fields, this technique opens new possibilities for real-time quantum state control and precision measurement. This high-resolution view of nuclear spin dynamics is analogous to switching from still-image film to video.



Toward a more accessible NMR metabolomics with ultra-resolved methods to enhance the performance of compact NMR

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The analysis of complex biological mixtures is challenging due to their numerous molecules of different nature, size and concentration. Despite these difficulties, their analysis remains essential in metabolomics for the diagnosis of disease and in food science for the control of adulterations. In this field, liquid-state NMR spectroscopy is recognized as a powerful analytical tool capable to provide valuable information such as identity and quantity in a mixture.

Cryogen-free and less expensive benchtop NMR spectrometers are limited by their weaker magnetic fields, reducing their sensitivity and ability to disperse signals. Manufacturers have sought to minimise these drawbacks by developing more powerful magnetic fields above 80 MHz. Moreover, gradient coils on benchtop NMR spectrometers have led to a number of method developments. Thus, benchtop NMR apparatus have become attractive for complex mixtures and the new applications offered by their growing capabilities motivate the need to develop methods to outperform such instruments.

In this context, we have implemented solvent-suppressed pure shift (SSPS) NMR strategies on an 80 MHz benchtop instrument to eliminate the signal of the water as well as J couplings to simplify the spectral information. First, several SSPS methods (Zangger-Sterk, PSYCHE and J-resolved) were evaluated on a model mixture of metabolites in pure water. Their performance was compared considering analytical criteria: sensitivity, resolution, spectral purity and repeatability. 1D projections of 2D J-resolved double-echo spectra (J-RES DE) gave the most promising results: 27 % of 1D 1H sensitivity, 4 Hz average linewidth at 10 % height, CV = 4 % for repeatability and minimum of artefacts. Finally, J-RES DE was showing its superior signal recognition capabilities with an easy annotation of a commercial fish feed formulation. We expect that the J-RES DE strategy will pave the way for metabolic / lipidic profiling on compact NMR spectrometers.



Diamond DNP at 3.4 T and 7 T

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Defects in diamonds are revolutionizing fields of quantum computing/sensing and more. The nitrogen vacancy (NV) centers and their precursors, P1 centers (substitutional nitrogen centers), at the forefront of this progress. P1 centers play a critical, yet often less appreciated, role in NV centers. P1 centers have long coherence times at room temperature, and are key contributors to NV center relaxation dynamics, often limiting NV center performance in sensing applications. Moreover, they can serve as sources of polarization for nearby carbon nuclei during dynamic nuclear polarization (DNP).

In previous work at 3.4 T, we reported that the diamond powder under investigation exhibited remarkable heterogeneity in P1 center environments, spanning from isolated centers to dense P1 clusters. [1] This heterogeneity gave rise to multiple DNP mechanisms occurring simultaneously, including the solid effect, cross effect, truncated cross effect, and Overhauser effect. We further explored how the frequency modulation (FM) of microwave (MW) irradiation affects the DNP process. [2] We showed that it is possible to control which mechanisms are most dominant by changing the FM parameters.

Building on this foundation, we now present an expanded exploration of diamond DNP at room temperature, incorporating new results at 7 T and comparing them with our earlier findings at 3.4 T. Using electron-electron double resonance (ELDOR) techniques, we probe the critical role of electrons in the DNP process. By comparing the effects at 3.4 T and 7 T, we show how changes in the magnetic field work with the FM to alter the behavior of the active DNP mechanisms.

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Symmetry-Based Pulsed DNP and EPR Sequences

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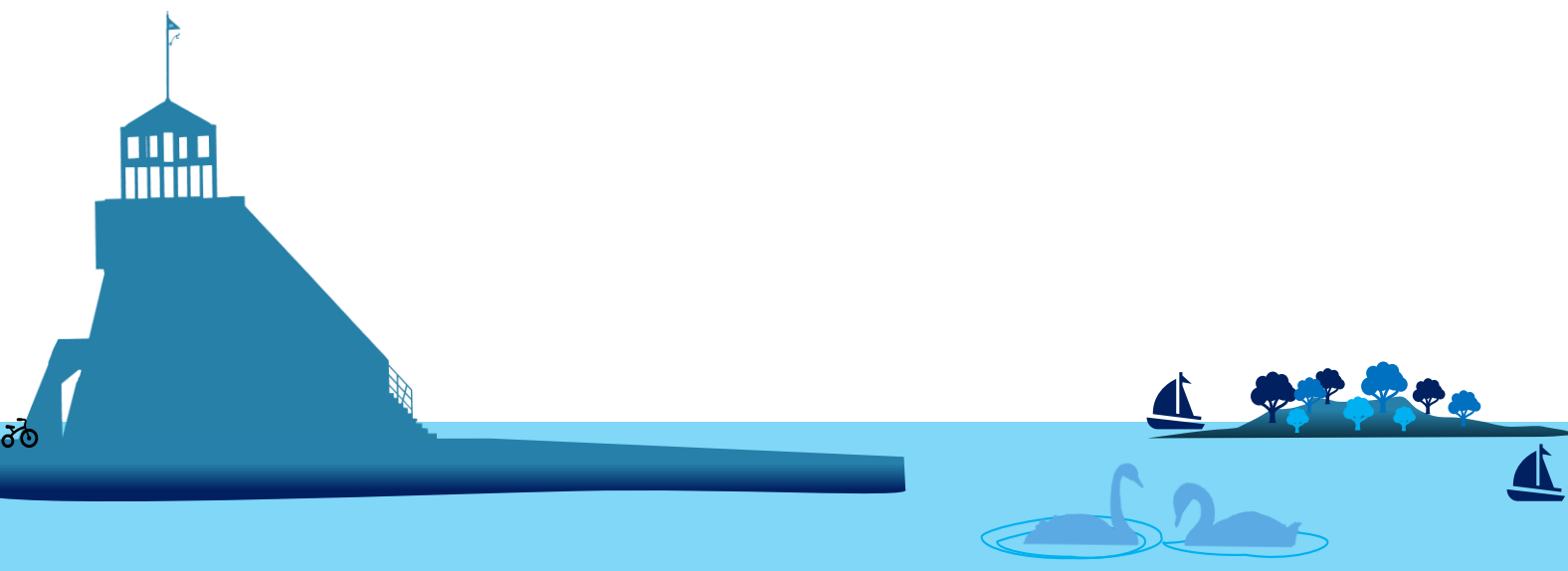
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Dynamic nuclear polarization (DNP) enhances NMR signals by transferring polarization from electrons to nuclei. Compared to continuous-wave DNP, pulsed DNP is more sophisticated but should offer higher efficiency and robustness against offsets and various experimental artifacts. Inspired by the symmetry-based dipolar recoupling sequences (CN or RN) used in MAS solid-state NMR, we hypothesize that electron-nuclear dipolar couplings, which are averaged by the nuclear Larmor interaction, can be recoupled for DNP using similar symmetry-based sequences. To adapt symmetry-based sequences for pulsed DNP, we synchronize the C/R sequences to the nuclear Larmor period instead of the rotor period used in conventional MAS experiments. We realized DNP using symmetry-based sequences with trityl OX071 in DNP juice on a 0.35 T X-band Bruker machine equipped with a 1 kW TWT amplifier and an arbitrary waveform generator. In addition to conventional CN and RN sequences, which provide similar DNP enhancements as NOVEL, the riffled RN sequence exhibits significantly higher tolerance to microwave power inhomogeneity. We also compared the performance of PulsePol with other riffled RN sequences, suggesting that more alternative symmetry-based pulses may outperform PulsePol.

Beyond DNP, we explored the application of symmetry-based sequences in pulsed EPR experiments. We measured the evolution of the EPR signal during the DNP buildup process using a constant-time experiment. Both DNP and EPR initial buildup curves show some correlations that align with numerical simulations. Pulsed DNP methods incorporating symmetry-based pulses offer a new suite of high-performance, quantitative, and controllable DNP sequences. These advancements would enhance DNP performance and provide novel strategies for characterizing paramagnetic spin systems.



NMR of endofullerenes and endofullerides

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The highly symmetric C60 fullerene molecule has fascinated scientists ever since its discovery.[1] Endofullerenes are supramolecular complexes where one small endohedral species (atom/molecule) is completely confined within a bigger fullerene molecule.[2,3] “Molecular surgery” was developed for their production: empty C60 is opened through a series of chemical reactions, endohedral species is inserted through the opening and then C60 is closed back to its original form with the endohedral species enclosed.[2,3]

Endofullerenes offer ideal “particle in a box” nano-laboratories to observe quantum mechanical phenomena. A selection of topics related to the NMR of endofullerenes and endofullerides will be presented.

NMR investigations of endofullerenes, performed at ambient and cryogenic conditions will be presented. Experiments have been performed in isotropic & anisotropic liquid crystal solutions and in the solid state. These consist of ^1H , ^{13}C and ^3He experiments on various endofullerenes: $^3\text{He}@\text{C}_{60}$, $\text{CH}_2\text{O}@\text{C}_{60}$, $\text{CO}@\text{C}_{60}$, $\text{H}_2@\text{C}_{70}$, $2(\text{H}_2)@\text{C}_{70}$, etc.

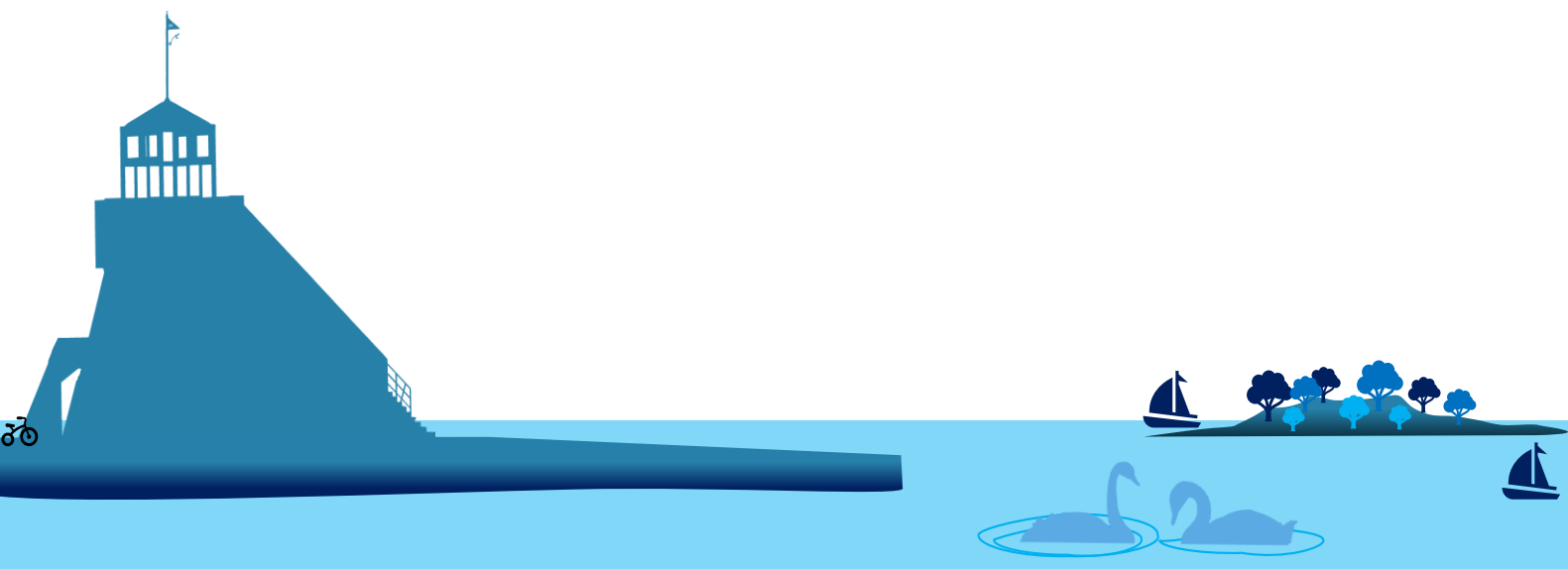
The fullerene family extends to fullerides = ionic salts of negatively charged C60 cages counterbalanced by positively charged metal ions.[4] If the C60 cages are filled with an endohedral species, this leads to endofullerides.[4] Solid state NMR investigations, from room temperature down to cryogenic temperatures, will be presented for endofullerides of the form $\text{M}_x(\text{A}@\text{C}_{60})$: M is an alkali metal (K or Rb), x is the stoichiometry of the metal with respect to C60 and A is the endohedral species ($\text{A} = \text{H}_2$, HD, ^3He). The material properties depend on stoichiometry, if x is equal to: 3 = conductor/superconductor, 4 = semiconductor and 6 = insulator. Endohedral species are shown to act as probes for the material properties of (endo)fullerides.

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Time-dependent diffusion power spectra and correlated exchange in entangled polymers

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Operando measurements of diffusion power spectra provide fresh perspective on molecular interactions in complex environments, which are central to the design of advanced materials with tailored macromolecular behavior and reactivity. In these systems, confined diffusion governs transport at the interface of structure and dynamics. However, experimental limitations have historically hindered the study of time- and frequency-dependent diffusion in heterogeneous materials. Here, we present a multidimensional extension of the modulated gradient spin echo (MGSE) experiment that detects frequency-correlated exchange, using a unilateral magnet setup as a low-cost, portable sensor.[1,2] This experiment directly links proximally diffusing molecular moieties in operando settings and resolves multiple components in complex mixtures, an aspect often overlooked in traditional approaches. We validate the method using simulated data and controlled samples of linear and crosslinked polydiketoenamines (PDKs) swelled with dimethoxyethane (DME).[3,4] The observed exchange between frequencies indicates molecular diffusion across regimes of motional averaging, porous confinement, network localization, and conformational exchange. Applying this experiment to study polymer deconstruction, we assess the influence and extent of crosslinking in reacting material networks. This approach advances the measurement of heterogeneous reactions and provides deeper insights into network dynamics in complex materials. Its relevance extends to energy dissipation, aggregation/fragmentation, biological processes, and information scrambling, broadening its utility across multiple disciplines.

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Pulsed EPR and DNP at 94 GHz

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The National High Magnetic Field Laboratory (Maglab) in Tallahassee, acquired in 2010 a High-Power EPR spectrometer (HiPER) developed by the group of G. Smith (St Andrews). HiPER is based on a high-power amplifier (1 kW) operating at 94 GHz with Quasi-Optics enabling the utmost EPR sensitivity at high field. To make the most out of the combined very high sensitivity and high-resolution, the Maglab HiPER is being upgraded with a variety of new elements to enable advanced EPR and DNP experiments. We present here newly installed capabilities as well as upcoming developments.

HiPER has been upgraded with an Arbitrary Waveform Generator (AWG) which, combined with NMR detection, enables pulsed DNP. The NMR system can be used for DNP mechanism studies or for high sensitivity Electron Nuclear Double Resonance (ENDOR). In addition, the AWG, thanks to its ability to rapidly chirp microwave signals, increases the quality of traditional EPR relaxation measurements. T1 can now be measured on all types of samples without spin diffusion artifacts thanks to complete saturation of the EPR lines. Additionally, an optional 10 W solid-state amplifier can be used instead of the 1 kW short pulse amplifier. This new solid-state amplifier allows long pulse sequences ($> 20 \mu\text{s}$) without a duty cycle limit, therefore permitting high accuracy T1 measurements in the tens to hundred μs where the 1kW amplifier is blind due to its duty cycle limit. While on the software side, Specman4EPR is now routinely used to generate complex sequences, such as Gaussian shaped pulses, and collect EPR data over a GHz of bandwidth. Other improvements include a new 9 T magnet delivered with a 15-ppm homogeneity, which was improved by a factor 5 thanks to the addition of custom ferroschims. The implementation of these enhancements have resulted in a truly unique instrument leading to several high impact publications. HiPER is accessible via the Maglab user program. nationalmaglab.org



Tracing host-pathogen interactions through pulse dipolar EPR spectroscopy – pathogen surface protein interaction with host tissue

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Protein structure prediction has reached a remarkable accuracy heralding an age where the routine production of experimental high-resolution structures becomes obsolete. Protein domains, individual folds, and short-range structural correlations are being predicted with ever better precision. However, disordered regions, long-range structural arrangements and interactions remain problematic, often eluding traditional methods such as x-ray crystallography, cryo-EM or NMR. Computational predictions (e.g., using AlphaFold3) and sparse spectroscopic methods are transforming the way we investigate structure and function of proteins, nucleic acids and their complexes.

Pulse dipolar EPR spectroscopy (PDS) is ideally suited to validate structural models and structural heterogeneity on the nanometre scale by measuring distance distributions between selected sites within the structural model or hypothesis. Crucially, the main limitations of complexity and size for NMR and disorder for crystallography and cryo-EM do not apply to PDS.

In this contribution we will be first demonstrating the value of a combined approach of PDS and structure predictions to understand new structures and functions of proteins.[1,2] We will further demonstrate that a few well-chosen spin labelling sites allow validating the highly unusual fold of a dimeric pathogen surface protein of unknown full-length structure. We further explore the interaction between host tissue and pathogen surface protein. First results hint at a structural transition that could underpin both – immune system evasion and the host's autoimmune response.[3]

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19F MAS DNP Enhanced NMR Spectroscopy of Drug Molecules

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Dynamic nuclear polarization (DNP) enhanced solid-state NMR is a powerful technique for structural studies of low-concentration systems, such as active pharmaceutical ingredients (APIs) in drug formulations and cellular components.

When combined with fluorine NMR, it offers significant potential for pharmaceutical applications, allowing selective detection and analysis of fluorinated APIs, which are widely used in drug development, yet absent in most common excipients.

A notable challenge in 19F DNP NMR is hardware limitations that prevent simultaneous pulsing on both 1H and 19F frequencies, restricting the transfer of hyperpolarization from protons to fluorine via cross-polarization (CP), a process known as indirect DNP. Although some progress has been made with specialized probes, a more accessible approach uses probes tuned separately to either nucleus, preventing magnetization transfer between 1H and 19F. Recent studies have demonstrated significant direct 19F DNP enhancements using such probe setups, leveraging radicals and sample formulations originally optimized for 1H DNP in proteins, small molecules, and materials.

In this work, we investigate fluorinated derivatives of established DNP polarizing agents, TEKPol and bCTbK, for 19F DNP NMR. We evaluate the performance of these fluorinated radicals in enhancing 19F NMR signals of fluorinated drug molecules and demonstrate that fluorinated dinitroxides lead to significantly higher enhancements, shorter build up times and overall improved sensitivity compared to their protonated counterpart. This will be illustrated on various 19F-containing samples, including APIs in frozen solutions, solid-state formulations, and commercial over-the-counter pharmaceutical tablets.



Enantiomeric discrimination of pharmaceutical ingredients by NMR in chiral weakly orienting media

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Differentiating the enantiomers of a chiral molecule is of paramount importance in organic and pharmaceutical chemistry. Chiral-agent (CA)-assisted NMR enables spectral enantiodifferentiation of a chiral solute by creating stable diastereomers or transient diastereomeric adducts resulting in a variation in chemical shifts (1). This approach often involves the use of tailored CAs (often not commercially available), while the possible small deviation in chemical shifts is poorly suited to moderate- or low-field spectrometers.

We propose an alternative NMR method using a liquid crystal based on a commercial polymer (PBLG) dissolved in an organic solvent. In such a chiral orienting medium, enantiomers are discriminated through anisotropic interactions (residual chemical shift anisotropy (RCSA) and dipolar coupling (RDC)) (2). The analytical value of this approach has recently been demonstrated by the enantiomeric analysis of principal active ingredients (APIs) involving different chemical functions by variation of ^{19}F -RCSA and ^{19}F -RDC (3).

Interestingly, the enantiomeric differentiation achieved by interaction like RDC is independent of magnetic field strength, making this method usable even with low-field benchtop spectrometers. We demonstrate the potential of this approach at both high and low fields on a scope of chiral solutes, and the first enantiomeric discrimination of an API (fluoxetine) by ^{19}F NMR with benchtop spectrometers operating at 1 and 2 T.

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NMR relaxation and diffusion study of liquid phase separations in confinement

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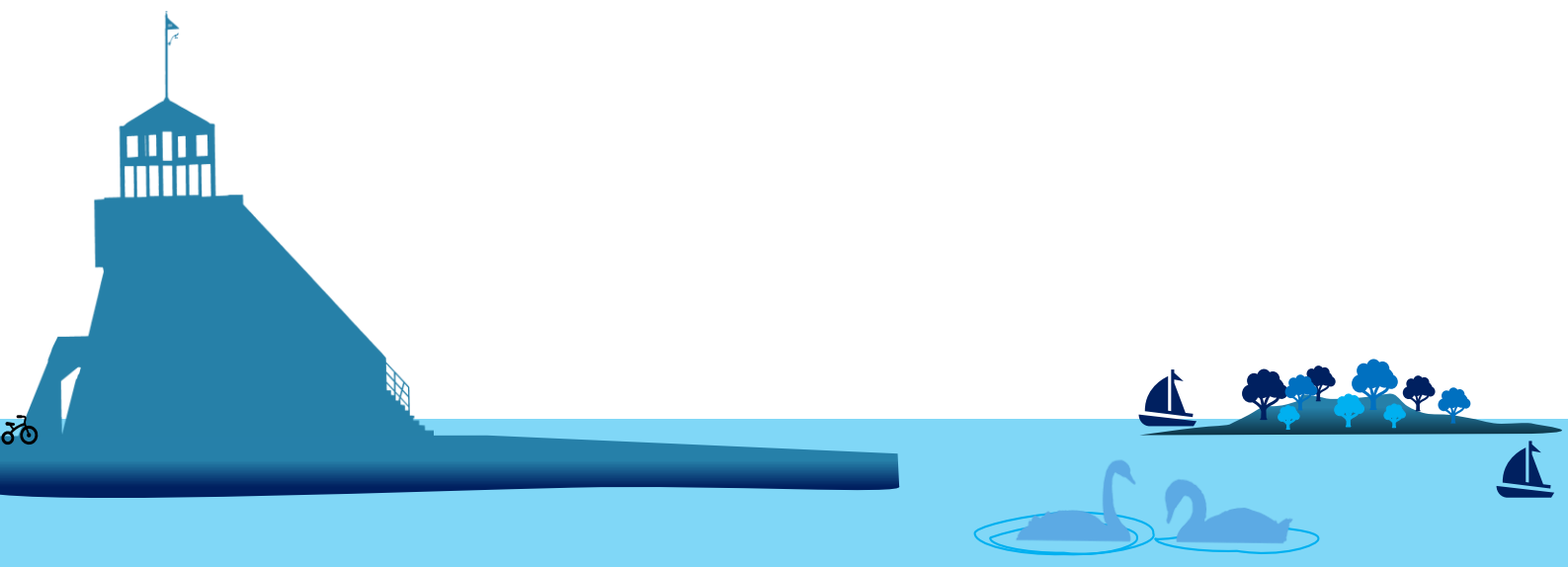
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Bulk miscible liquids of low viscosity feature typical liquid properties in NMR experiments such as an absence of frequency dependence of relaxation times, $T_1=T_2$ and normal Gaussian diffusion with mobilities that are linked to the molecules' hydrodynamic size. Confining the same mixtures in mesoporous materials alter these properties in currently rather unpredictable ways that appear dominated by polarity as the main factor affecting NMR response: on a polar surface, polar molecules tend to be preferentially adsorbed and shield the surface from contact with an unpolar species such as in the case of acetone and cyclohexane. As a consequence, the unpolar species has bulk-like relaxation properties while the polar molecule is strongly affected by surface interactions, leading to a strong T_1 dispersion. On larger scales, confinement to a thin surface layer reduces the self-diffusion coefficient of the polar species beyond the geometrical tortuosity of the confining matrix. If both liquids are polar (acetone/water, acetone/THF), these features appear less pronounced but the proticity of water gives rise to additional strong surface interaction which again suggests phase separation.

In this study, liquid mixtures over a wide range of concentrations, using different matrix materials such as glass and silica with pore diameters of 4 nm and above, were studied experimentally and by MD simulations where density profiles and surface contact frequencies are used to quantify the occurrence and temporal dependence of microscopic phase separations. MD simulations in combination with defined surface modifications (deuteration, silanization, addition of radicals) allows verifying the molecular dynamics close to the solid interface and the statistics of rotational and translational motions.



Ultrafast Laplace NMR for biomaterials

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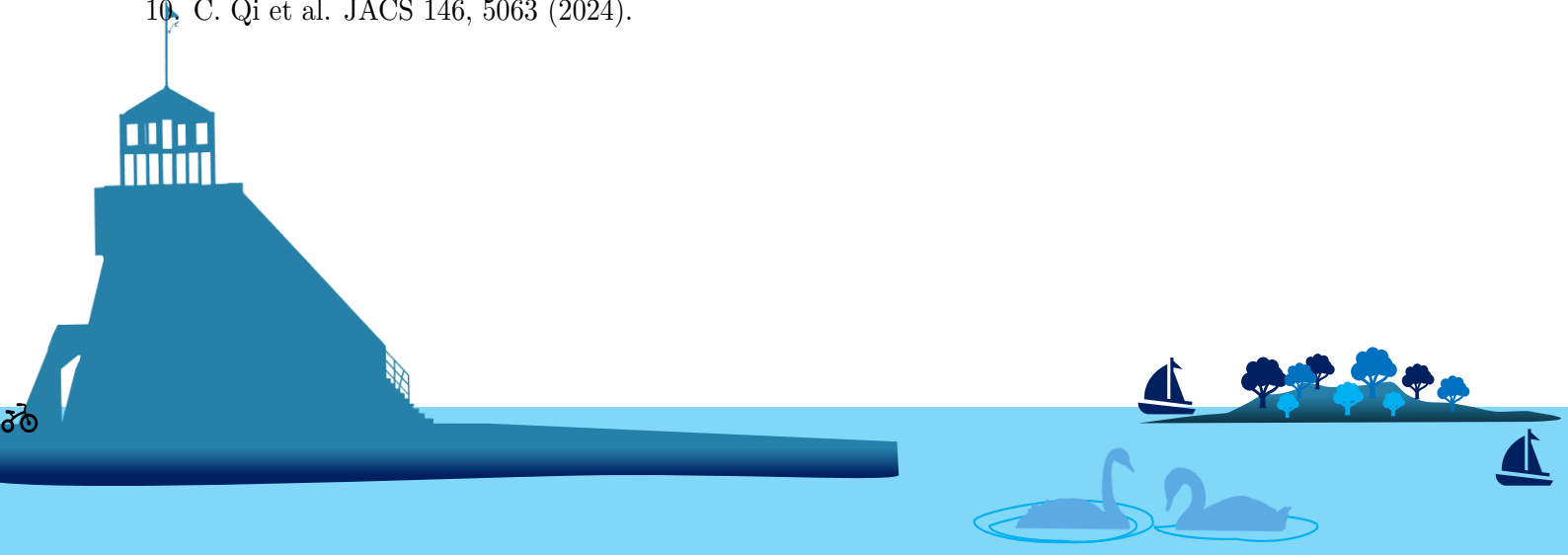
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NMR relaxation and diffusion measurements provide versatile information about dynamics and structures of e.g. porous materials and reveal interactions of nuclei within their environment. Since this data comprise exponentially decaying components, the processing requires an inverse Laplace transformation (ILT) to extract the studied distributions. Thus, these methods are referred to as Laplace NMR (LNMR).[1]

Multidimensional approach increases the chemical resolution of NMR experiments. Multidimensional and some 1D experiments are time consuming since the need of several repetitions with varying evolution delay or gradient strength to measure multidimensional data. This also restricts the applicability of LNMR and is considered general problem of multidimensional NMR. Also, in many cases it prevents the use of hyperpolarization for signal amplification. These problems can be tackled by introducing spatial encoding of two-dimensional data, as was originally done in ultrafast NMR spectroscopy [2,3] and later in ultrafast LNMR [4-8]. The price to pay is reduced sensitivity. However, the single-scan nature enables the use of hyperpolarization, which provides higher boost than the loss due to spatial encoding.

In this presentation we focus on recent progress in ultrafast LNMR and showcase different applications related to biomaterials. Furthermore, these methodologies are applicable with mobile NMR devices, thus widening the applicability and opening avenues towards industrial applications.[4-10]

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NV Centers Based Hyperpolarization of Local and Bulk ^{13}C Nuclei in Diamond at 7 and 14 T

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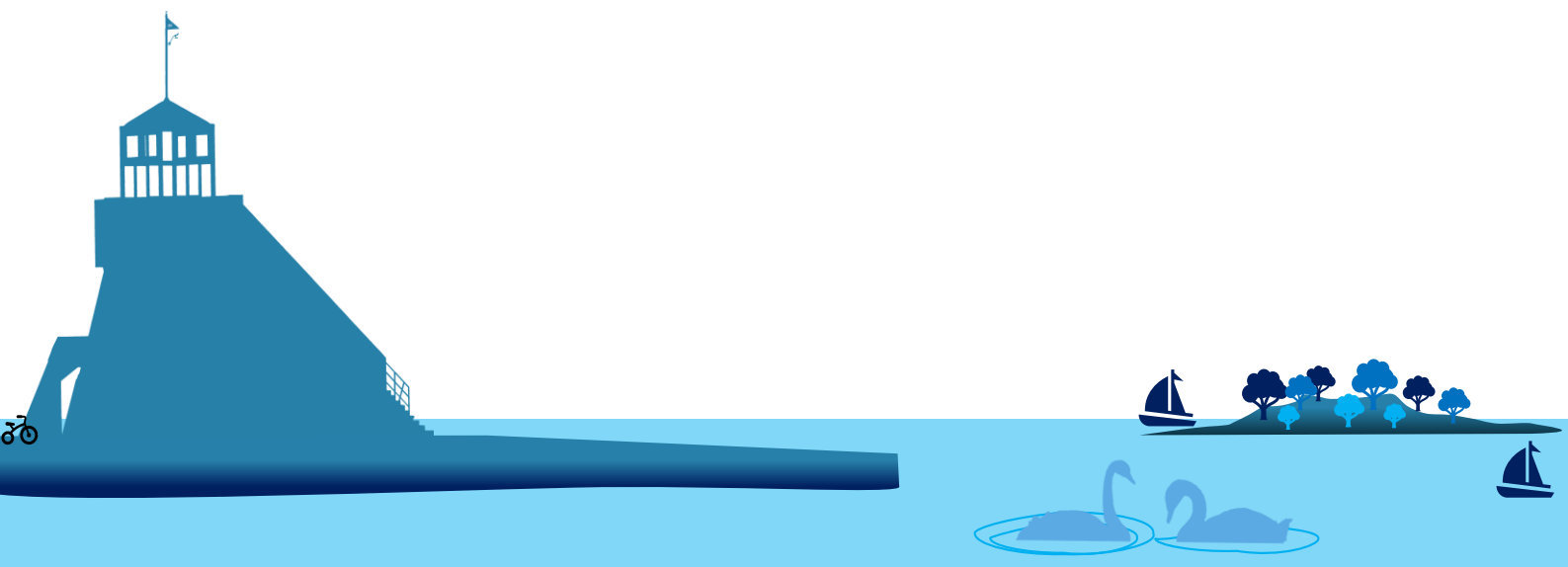
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The negatively charged nitrogen-vacancy (NV) center is a paramagnetic defect in diamonds, well known for its unique coupling between optical and electron spin properties, allowing to hyperpolarize its electron spins by green light illumination, and long coherence times at room temperature. NV centers proved to be efficient dynamic nuclear polarization (DNP) agents, efficiently enhancing NMR signals by transferring their electron spin polarization to nuclei. The NV centers are mostly studied at low magnetic fields (≤ 0.3 "T"), where their high DNP enhancements are partially negated by the need to transfer the sample to a high field for high-resolution NMR experiments. Therefore, it is beneficial to perform NV-center based DNP at the high magnetic fields where the high-resolution experiments can be performed, dispensing with the transfer step and allowing for signal averaging.

In this work we present high-field NV-based DNP, as well as the first pulsed EPR data of NV centers at 14 "T" . We find that while light illumination alone does not lead to the hyperpolarization of strongly hyperfine coupled ^{13}C nuclei, we demonstrate how it can be done using a combination of light illumination and mm-wave irradiation. Using a new Reverse ELection-electron DOuble Resonance (RELDOR) sequence we observe interactions between different components in the NV EPR spectrum. By combining insights from the EPR, RELDOR and DNP spectra, we propose a model to account for this effect.

We also show that optically hyperpolarized NV centers can polarize bulk ^{13}C nuclei in the diamond via the solid effect mechanism with efficiency comparable to that of the cross-effect based P1 center DNP under similar conditions. A surprising observation is that the nuclei polarized via the NV centers are distinct from those that are polarized via the P1 centers, and the total polarization of the sample can be almost doubled when the two effects are combined.



Triplet Excitons in Carbon Nitride Photocatalysts

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Carbon nitride (CN) is a bi-dimensional semiconductor which has raised much interest over the past few years as a versatile photocatalyst. However, due to rapid charge-carrier recombination, its efficiency is usually low. Furthermore, CN is a solid-state material with a poorly defined structure and catalytic properties that strongly depend on its morphology. Therefore, a thorough understanding of the mechanisms underlying light harvesting and conversion into charge carriers and how these are related to the morphology of the material is crucial. In particular, a number of studies have highlighted an empirical correlation between the catalytic efficiency and the number of native and photoinduced paramagnetic species present in the material [1].

Among the common studies on the photophysics of CN, the nature and role of excited triplet excitons has not yet been fully investigated. In this contribution, I will present the characterization of the metastable photo-excited triplet excitons by means of time-resolved EPR [2]. The present study probes the existence of triplet excitons in CN, derives their energy and investigates the dependence of their properties on the morphology of the material. In order to ensure that such results are intrinsic features of polymeric CN, a comparison with the heptazine-based monomer and a series of heptazine oligomers is performed.

This work showcases how time-resolved EPR methods allow i) to investigate the temporal range corresponding to the time scale compatible with chemical reactivity and; ii) to track the evolution of paramagnetic species formed upon photoexcitation in a complex and polycrystalline semiconductor.

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DNP surface-enhanced ^{17}O MQMAS and D-HOMCOR of ^{17}O labeled metal oxides

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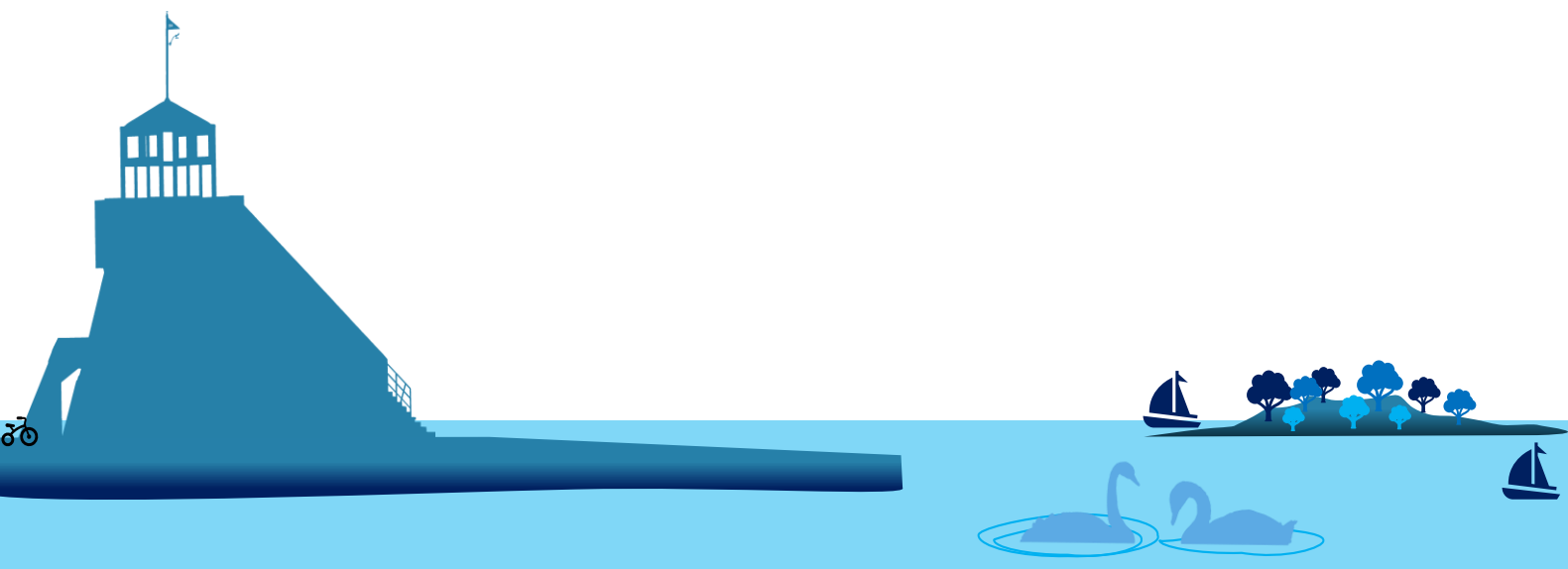
Dynamic nuclear polarization surface-enhanced NMR spectroscopy (DNP-SENS) is a powerful technique for characterizing the surface structure of inorganic nanomaterials, with growing demand for the observation of half-integer quadrupolar nuclei. We have shown that D-RINEPT, employing adiabatic symmetry-based dipolar recoupling, facilitates the polarization transfer from ^1H to quadrupolar nuclei, including low-gamma nuclei such as ^{17}O , $^{47,49}\text{Ti}$, ^{95}Mo , and ^{67}Zn at natural abundance [1].

This presentation highlights recent advancements in the application and methodological development of D-RINEPT-based sequences for DNP-SENS experiments, which incorporate several ^{17}O enrichment strategies for oxide nanoparticles and porous materials. A major methodological achievement is the application of DNP-SENS to obtain high-resolution spectra of half-integer quadrupolar nuclei located on the surface. By combining D-RINEPT and MQMAS sequences, this challenge has been effectively overcome. To further optimize the efficiency, we have developed the D-RINEPT-split-t1-MQMAS-QCPMG sequence, which integrates FAMN or Cos-lp excitation and reconversion pulses within an optimized phase cycling [2]. We present the DNP surface-enhanced ^{17}O MQMAS spectra of both simple and complex metal oxide nanoparticles ($\gamma\text{-Al}_2\text{O}_3$, ZnO , ZrO_2 , CeO_2 , and $(\text{ZnO-ZrO}_2)/\text{SiO}_2$ catalysts), as well as alumino- and titano-silicate zeolites. These samples were prepared using three selected ^{17}O isotope-labeling methods: mechanochemical, slurry, and oxygen-gas techniques.

Furthermore, progress has been made in dipolar-mediated homonuclear correlation (D-HOMCOR) experiments of half-integer quadrupolar nuclei. The development of the D-RINEPT-DQ/SQ-QCPMG pulse sequence with BR2 recoupling enables DNP surface-enhanced 2D DQ-SQ correlation experiments efficiently, which we demonstrate on ^{27}Al and ^{17}O nuclei of $\gamma\text{-Al}_2\text{O}_3$.

[1] H. Nagashima et al, J. Am. Chem. Soc., 2020, 142

[2] H. Nagashima et al, J. Phys. Chem. Lett. 2024, 15



Structures and Dynamics of Self-Assembled and Membrane-Bound Tau from Solid-State NMR

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The microtubule-associated protein tau aggregates into β -sheet amyloid fibrils that spread in a prion-like manner in the Alzheimer's disease (AD) brain. Understanding the structures of tau aggregates, as well as the mechanism of spreading of these aggregates, is crucial for intervention of AD. AD tau is abnormally phosphorylated, truncated, acetylated and modified in other ways. To investigate how these posttranslational modifications promote tau aggregation, we have determined the structures of several full-length tau constructs bearing phospho-mimetic mutations, truncations, and acetylation. These structures are determined by joint use of solid-state NMR and cryoelectron microscopy. Some of these structures resemble the ex vivo tau structures from tauopathy brains. We have also studied the structure of recombinant tau seeded by AD brain paired-helical filament tau. Interestingly, the AD-seeded recombinant tau adopts the precise AD tau structure, but is unable to seed a second generation, indicating that the rigid-core structure alone is insufficient to define true AD tau with the ability for prion-like propagation. Finally, we have investigated how AD-fold tau interacts with lipid membranes. These data shine light on how chemical modifications impact the aggregation propensity and aggregate structure of tau, and imply that the dynamic fuzzy coat impacts the propagation of the rigid core structures.



Exploiting bacterial OMVs as tools for the in-situ characterization of proteins

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The cellular envelope of Gram-negative bacteria is surrounded by a highly asymmetric outer membrane: on the one hand the outer leaflet of which is formed exclusively by lipopolysaccharides and on the other hand the gel-like periplasm, containing proteins at extraordinary high concentrations. Due to its inherent complexity, so far it proved impossible to mimic this environment under laboratory conditions. In addition, its low volume ratio contributing only ~10% to the total bacterial volume poses a formidable challenge for established in-situ approaches such as in-cell NMR spectroscopy.

To overcome these inherent issues and limitations, we recently established a method to modify the protein composition of outer membrane vesicles (OMVs) released from *Escherichia coli* as a unique platform to facilitate the biophysical characterization of envelope proteins within their native cellular environment. OMVs are produced by Gram-negative bacteria in a natural process, having the same asymmetric membrane composition as the parental outer membrane and containing the highly dense periplasmic fluid in their lumen, thus in this way providing an isolated cell-envelope microenvironment.

We utilized methyl TROSY methods in combination with ^{19}F -NMR of aromatic residues, to unravel the dynamics of periplasmic proteins within this crowded cellular environment. Likewise, using solid state NMR spectroscopy allows us to characterize transmembrane domains of outer membrane proteins embedded in the native asymmetric membrane of OMVs. I will update on our endeavours for achieving the first structure determination of the 8-stranded β -barrel protein OmpA in its native membrane environment revealing direct contributions from the lipopolysaccharides on structure and dynamics.



Metabolite partitioning into biomolecular condensates mimics organic solvent behaviour

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Biomolecular condensates form through phase separation and are highly enriched in proteins and nucleic acids. Condensates are increasingly thought to provide distinct solvent environments in cells, challenging the idea of water as biology's universal solvent. There is growing evidence that condensates selectively partition small molecules, which would impact cellular biochemistry and present challenges and opportunities for the druggability of disease-associated condensate phenotypes.

Here we develop and apply an NMR-based method to measure the partitioning of amino acids and nucleotides in condensates. We establish a series of solubility rules that explain why these metabolites are enriched into (or excluded from) different condensates, depending only on the chemical properties of the underlying proteins. Our results show that biomolecular condensates behave like organic solvents, and that the fundamental chemical rule of 'like dissolves like' is a mechanism used by cells to localise biomolecules.

To achieve this, we used a series of pulse sequences to characterise different metabolites; for amino acids, we designed a Seedless optimal control-optimised HACA(CO) pulse sequence to decouple CA and CB and quantitatively resolve overlapped HA-CA peaks. Using linear free energy relations, we established a minimally complex model to explain our partitioning data in terms of chemical properties of both the solutes (metabolites) and solvents (orthogonal condensates and organic solvents). Our analysis suggests these solvents discriminate metabolites via solubility rules based on five molecular properties. Also, the rules for each solvent correlate with solvent properties such as polarity and charge.

This method is widely applicable to characterise the solubility rules of distinct condensates, which will provide insights into their biological functions and aid the development of predictive tools to rationally target therapeutics and chemical probes to condensates.



Switching off a GPCR: different facets of receptor desensitization studied at atomic resolution

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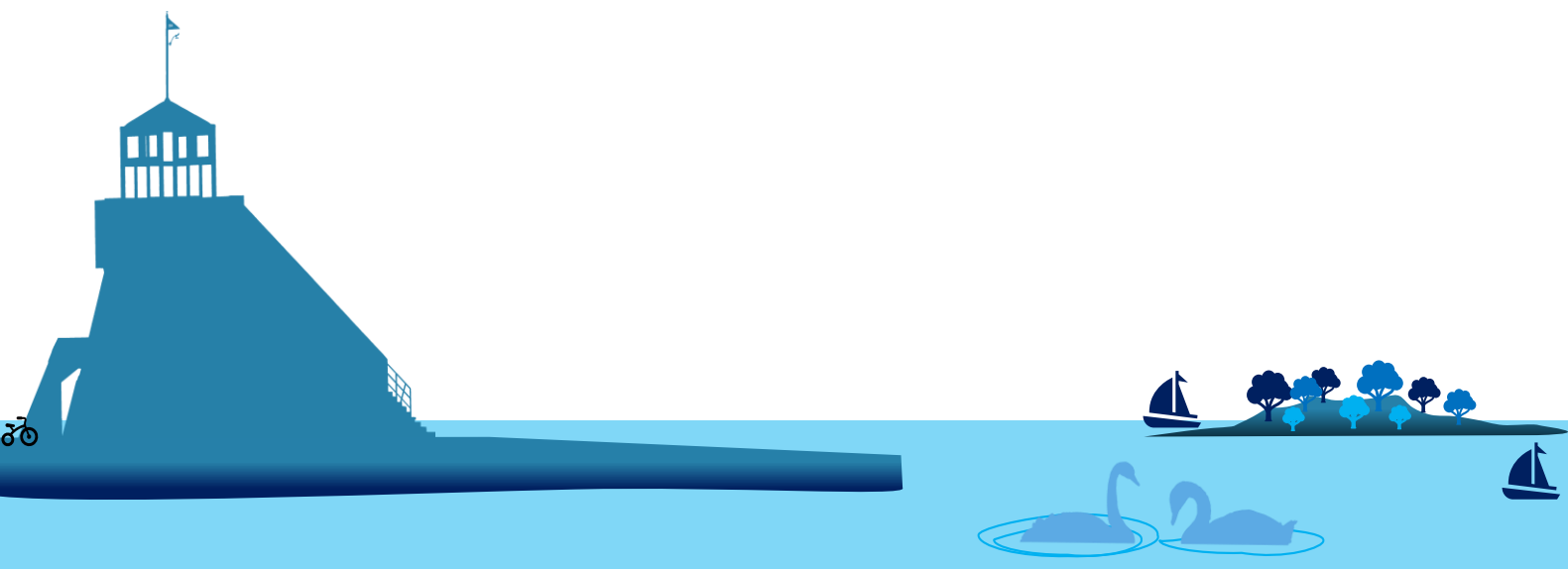
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To control the signaling process of G protein-coupled receptors (GPCRs), cells have developed a tightly regulated system of desensitization where GPCR kinases (GRKs) play a vital role. By selectively phosphorylating the C-terminus and/or intracellular loops of activated receptors, GRKs initiate the recruitment of arrestins to GPCRs, leading to a plethora of cellular responses.

Our aim is to study the allosteric activation of the kinase, its interaction with effector molecules and the resulting phosphorylation pattern in a time-resolved manner using NMR spectroscopy. We performed selective ^{13}C -methionine labeling of GRK1 (60 kDa) and GRK2 (80 kDa) in insect cells to enable the measurement of fingerprint spectra. By recording these characteristic spectra, we can determine distinct conformational changes of the kinase upon binding of physiological effectors (ATP) in contrast to allosteric ($\beta\gamma$ -subunit, anionic phospholipids) or competitive modulators (paroxetine, sangivamycin). Additionally, phosphorylation events catalyzed by GRK1/2 were monitored in real-time on the isolated ^{15}N -labeled C-terminal tails of three GPCRs (hs β 1AR, hs β 2AR, bovine rhodopsin). By recording series of 2D NMR spectra, we were able to determine the individual phosphorylation rates for differing sites. We found pronounced differences in the rates at which certain residues are phosphorylated. Interestingly, we could further identify individual dependencies in the phosphorylation hierarchy. Taken together, the results suggest the prevalence of different phosphorylation patterns over time possibly inducing time-sensitive downstream responses. Moreover, the comparison of two ubiquitously expressed GRK family members, i.e. GRK2 and GRK6, revealed distinct site-specificities of the individual isoforms on peptide substrates.

Unraveling the details of GPCR desensitization in turn may aid in the discovery of new therapeutic agents targeting specific GRK functions.



Towards a nanodiamond-based platform for room temperature hyperpolarization

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As a candidate system for the development of room temperature nuclear hyperpolarization, nanodiamonds (NDs) have attracted attention due to their low toxicity among carbon-based nanomaterials [1] and potentially long nuclear T_1 [2]. NDs can host defects such as the Nitrogen-Vacancy center (NV) [3,4], known for its capability of optical pumping and its favorable coherence time, also at room temperature. We will discuss recent experiments [5], demonstrating the possibility of using NV to hyperpolarize ^{13}C nuclear spins in NDs: applying the PulsePol sequence [6], we obtain a significant, 940-fold, enhancement of the ^{13}C nuclear magnetic resonance (NMR) signal, over the thermal nuclear signal at the polarization field (~ 0.3 T), in 100 nm particles. The approach relies on improvements in material and protocol aspects -in particular, to mitigate the strong inhomogeneous broadening of the NV powder spectrum at the considered magnetic field. As outlook, we will discuss considered developments for achieving polarization transfer to external nuclear spins, in order to enhance the NMR signal of analytes in contact with the diamond surface.

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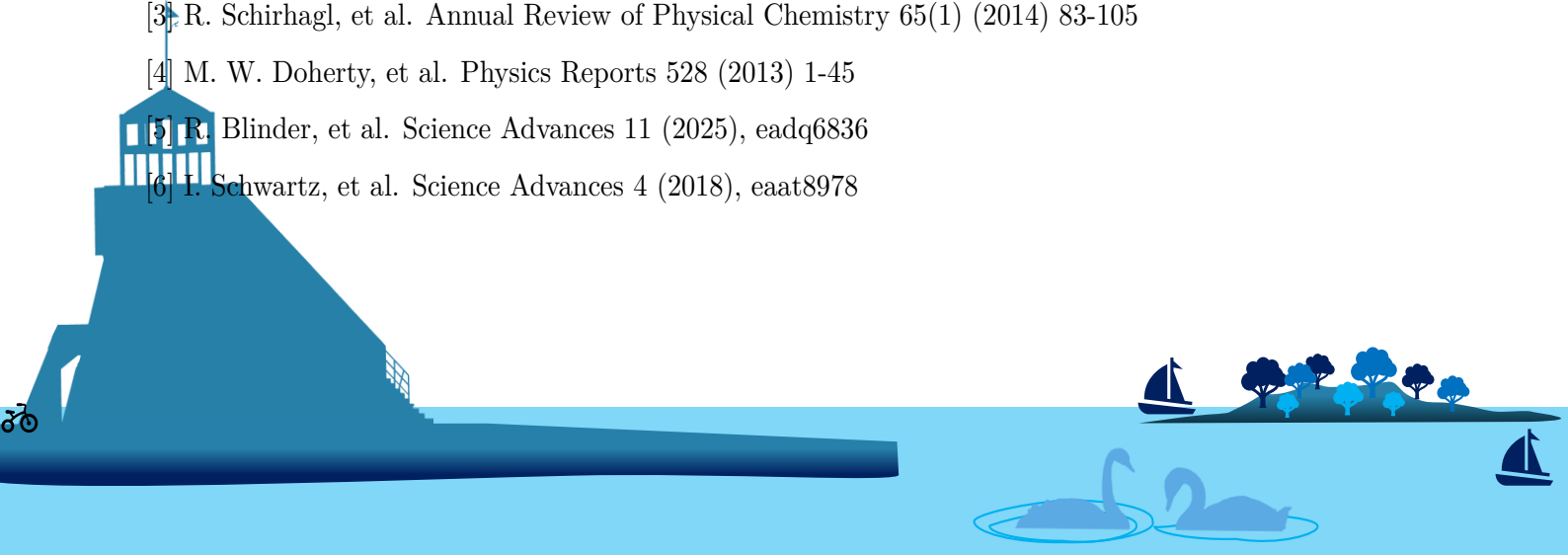
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Equivariant Neural Networks Reveal How Host–Guest Interactions Shape Xenon NMR Chemical Shift in Porous Organic Cages

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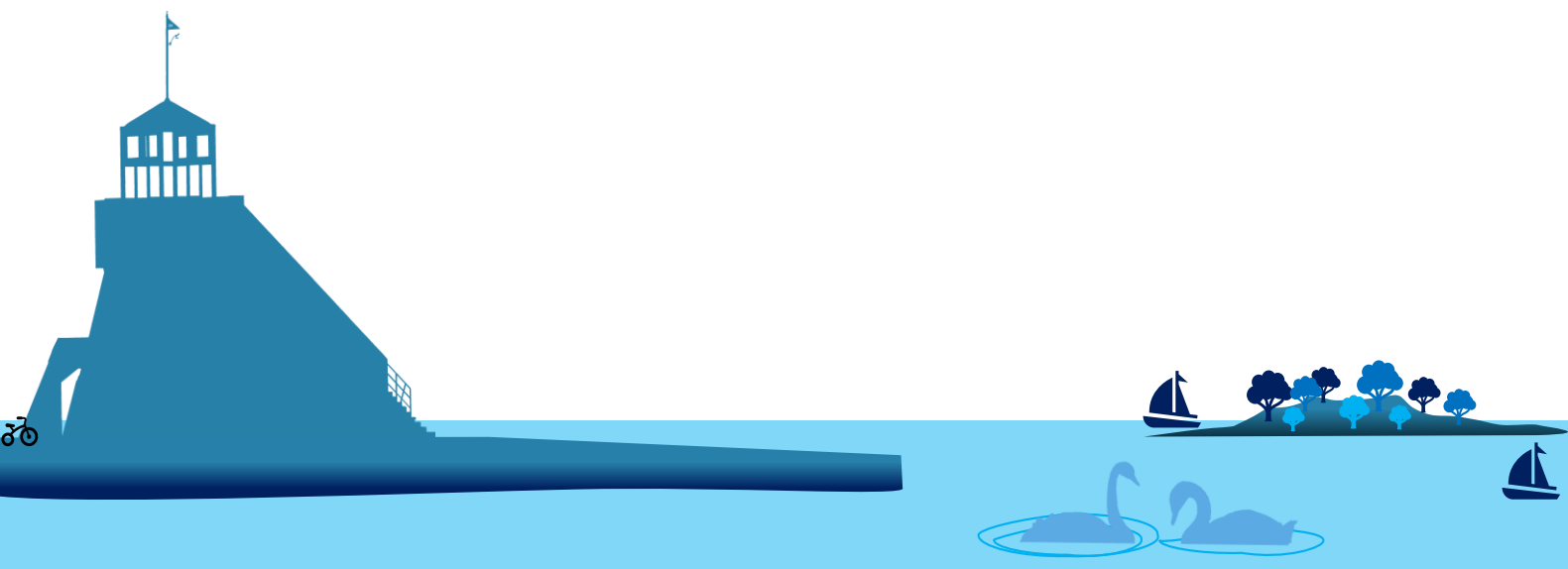
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Porous materials serve diverse applications like molecular separations and catalysis [1], offering energy-efficient methods for capturing greenhouse gases and valuable noble gases including xenon. Despite its importance in medicine and nuclear processes [2], xenon extraction remains challenging due to its low atmospheric concentration (0.087 ppm) and inertness. Porous materials with precise size selectivity [3] and high adsorption capacity are essential for effective xenon isolation. Recent developments in porous liquids (PLs) with cavities formed by porous organic cages (POCs) show promise in addressing these challenges [4]. Understanding the binding, occupancies, dynamics, and equilibrium between the host (POC) and the guest (Xe) is vital for engineering new PLs with targeted properties. While computational simulations are essential for exploring PLs, common approaches face limitations—quantum methods are computationally expensive for large systems, while classical methods are inaccurate.

In this work, we present two complementary machine learning (ML) models: (1) an ML interatomic potential (MLIP) obtained by training E3-equivariant graph neural network (GNN), Allegro [5], on a PBE-D4 theory-level dataset; and (2) an NMR-ML model obtained by training the invariant GNN, SchNet [6], on a PBE-(SVP,TZVP)/BHandHLYP-SVP theory-level dataset. The MLIP model enables accurate, data-efficient, and transferable large-scale molecular dynamics simulations and the NMR-ML model enable predicting Xe NMR chemical shifts. These simulations provide microscopic interpretations of experimental ^{129}Xe NMR observations.

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SLEEPY: Simple simulation of relaxation and dynamics in NMR

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NMR is a key experimental method for dynamics characterization, where measurement of relaxation behavior and residual couplings can be used to perform detailed dynamics analyses. NMR is uniquely flexible, but highly complex, with nearly endless possibilities for pulse sequences to select different pathways for magnetization. Relaxation studies have largely been limited to very simple pulse sequences, because it is not trivial to predict correlation time dependence of more complex sequences, requiring simulation to determine sensitivity of a given experiment to motion. SLEEPY (Spins in Liouville-space for rElaxation and Exchange in PYthon) addresses this challenge by providing a package that simplifies the inclusion of motion and relaxation in NMR simulations. SLEEPY is a complete simulation package, allowing arbitrary pulse sequences for solution and solid-state (static and spinning) NMR, in the rotating and lab frames. Object-oriented implementation in Python makes SLEEPY particularly easy to use for non-experts, and also allows it to be run using online resources such as Google Colab with minimal effort.

We present the basics of SLEEPY usage, demonstrate its range of capabilities (longitudinal and transverse relaxation, paramagnetic effects, DNP, etc.), and also highlight an online tutorial, available at <http://sleepy-nmr.org>, which includes various examples that may be explored interactively via Google Colab.



Hyperpolarizing porous polymer matrices for MAS DNP

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Solid-state NMR (SSNMR) is a powerful tool for studying solid materials' structure, organization, and dynamics. However, its low sensitivity leads to long acquisition times, limiting the observation of transient states in chemical reactions and phase transitions. SSNMR sensitivity can be enhanced by MAS DNP after suitably doping the samples with stable radicals (polarizing agents, PAs). Traditional exogenous doping requires to dissolve nitroxide radicals in a solvent and mixing them with the sample, often in the presence of glassing agents. However, when liquid samples are the object of the MAS DNP investigation, this protocol inevitably leads to sample pollution, which can compromise the analysis.

Porous hyperpolarizing matrices offer a cleaner sample preparation by covalently attaching the PAs to the matrix, preventing solution contamination. While applications of silica matrices have reportedly enabled MAS DNP detection of transient dilute crystallization phases[1], polymer-based matrices have primarily been used in dissolution DNP[2].

Here, we explore the potential of polymer-based hyperpolarizing materials as PAs for MAS DNP, particularly for tracking crystallization from solution of polymorphic solids of pharmaceutical interest[3]. We show how the material porosity aids in stabilizing amorphous and metastable species — critical for pharmaceutical research — via confinement effects, leading to the discovery of new phases. We also introduce novel hydrophilic, high-porosity polymers that enable efficient MAS DNP studies of crystallization in aqueous environments, expanding the technique's applicability in materials and pharmaceutical sciences[4].

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Achieving strong polarization of ^{13}C nuclei at high concentrations with parahydrogen

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Recent advancements in MRI have been driven by nuclear hyperpolarization techniques, which significantly enhance MRI sensitivity. This enables real-time monitoring of tissue metabolism, particularly the metabolic transformation of ^{13}C -pyruvate in cancer cells. Among various approaches, parahydrogen-induced polarization by side-arm hydrogenation (PHIP-SAH) efficiently generates hyperpolarization in ^{13}C -pyruvate by utilizing the singlet spin order of parahydrogen (pH_2).

The efficiency of PHIP-SAH depends on both the design of the SAH precursor and the optimal conversion of singlet order into ^{13}C magnetization. We previously showed that a propargylic ^{13}C -pyruvate ester with a deuterated side-arm enables efficient hydrogenation and extends the proton singlet lifetime, leading to higher polarization levels. However, side-arm deuteration weakens spin coupling between nascent pH_2 protons and the target ^{13}C , requiring an optimized polarization transfer strategy.

In our study, we employ PulsePol, a symmetry-based pulse sequence originally designed for dynamic nuclear polarization but later shown to be effective for PHIP.¹ We demonstrate that PulsePol provides best in class B0 and B1 robustness. Furthermore, we optimize PulsePol theoretically and experimentally, achieving up to 60% ^{13}C polarization at moderate (~ 50 mM) substrate concentrations. At higher concentrations (~ 500 mM), relevant for clinical translation, we demonstrate how excessive ^{13}C magnetization disrupts the polarization transfer via distant dipolar interactions.² To address this, we modify PulsePol to suppress these effects while preserving its inherent robustness.

Our findings represent an important step toward the clinical application of PHIP-polarized ^{13}C -pyruvate, achieving high polarization levels and extending the method's applicability to clinically relevant concentrations of hyperpolarized agents.

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Hyperpolarization and deuterium NMR enable real-time in vitro metabolomics: yeast and intestinal organoids case study

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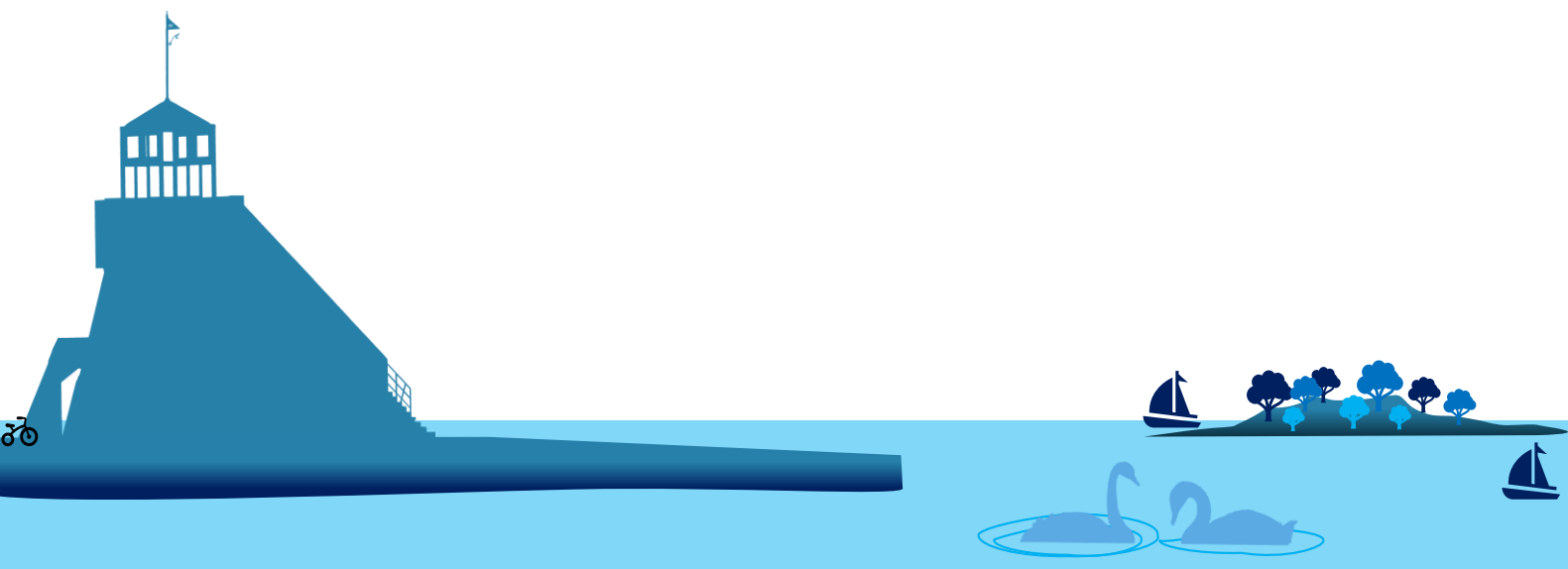
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Low sensitivity of NMR has been a barrier to real-time metabolic probing. Hyperpolarization and isotopic labeling (^2H and ^{13}C) have significantly boosted NMR sensitivity, enabling detailed metabolic studies in vivo.

In this study, we tested over 20 thermally polarized ^2H labeled tracers and hyperpolarized with dissolution dynamic nuclear polarization (dDNP) $[1-^{13}\text{C}]$ pyruvate and $[1,4-^{13}\text{C}_2]$ fumarate to non-invasively monitor metabolism in yeast and organoids. Intestinal organoids from a genetically defined mouse model of spontaneous carcinogenesis (Rnaseh2b/Xbp1 Δ IEC) were examined with or without a targeted therapeutic intervention (mTOR inhibition by rapamycin). dDNP was performed using a cryogen-free dDNP system (SpinAligner, POLARIZE) operating at $\sim 1.4\text{ K}$ and 6.7 T and 25-50 mg samples with trityl radical (AH111501).

Using baker's yeast as a convenient metabolic factory, we demonstrated that yeast cells provide a robust and rapidly metabolizing phantom for hyperpolarized pyruvate and fumarate, including substrates with a natural abundance of ^{13}C and thermally polarized ^2H -tracers[DOI: 10.1021/acs.analchem.4c02419, DOI: 10.21203/rs.3.rs-5312977/v1]. We observed the production of ethanol, carbon dioxide, bicarbonate, lactate, alanine from pyruvate, malate, oxaloacetate from fumarate, nicotinic acid from nicotinamide, and more. High cell densities of 500 million per experiment allowed monitoring pathways with low metabolic activity.

In gut organoids, hyperpolarized NMR revealed a 6.6-fold reduction in lactate production in rapamycin-treated organoids, indicating suppressed metabolic activity[DOI: 10.21203/rs.3.rs-5551528/v1]. Unlike traditional methods that destroy cellular integrity, hyperpolarization enabled repetitive, non-invasive metabolic assessments, bridging the gap between preclinical and clinical applications and affirming the method's potential for targeted metabolic imaging as a novel diagnostic and treatment control approach in cancer medicine.



Ultrafast Correlation Spectroscopy with multi-solvent suppression for the analysis of wine

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NMR is an important tool for analysing complex mixtures such as biological or food matrices. Limitations associated with 1D ¹H NMR can be solved by improved peak distribution and additional structural information from 2D experiments [1]. However, 2D NMR often suffers from long acquisition times, which hinders favourable high-throughput screenings. Fast 2D NMR methods such as ultrafast 2D COSY offer an efficient approach for enhanced profiling of complex samples previously shown on different matrices [2-4]. In the special case of wine, the concentration of water and ethanol highly surpasses the concentration of other metabolites of interest which poses dynamic range issues and hinders the detection of relevant signals.

We present a tailored ultrafast COSY pulse sequence for wine matrix. It provides multi-solvent suppression of water and ethanol signals and enables high-throughput screening. By spatial encoding along the z-axis, ultrafast NMR methods can record the entire 2D spectrum in approx. 20 min for wine samples.

The method showed reliable suppression of both water and ethanol with minimal interleaving artefacts. 20 additional metabolites were identified in comparison to only 8 major metabolites from an ultrafast COSY spectrum without solvent suppression.

The combination of water and ethanol suppression with an ultrafast 2D homonuclear NMR method gives possibilities for a more in-depth analysis of different wine matrices, by providing enhanced structural information in a short time frame. Thus, this is an interesting new method for future metabolomics studies and high throughput screenings with a special focus on the wine industry or extracts with similar solvent ratios.

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Pure Shift NMR: A Robust and General Method for Determining Quantitative Metabolic Profiles in Biofluids

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Recent developments in Pure Shift NMR have allowed to address structural and dynamic questions in increasingly complex samples, providing a deeper insight into molecular and supramolecular systems that are nowadays the focus of our attention in many areas of Chemistry. This is particularly the case in the biological field, where the combination of Pure Shift sequences with methods for suppressing the water signal has opened the way to studying metabolism in aqueous samples such as cell culture media, and even to the precise quantification of key metabolites involved in the metabolism of cancer cells.[1]

This achievement has been followed by a renewed interest in the development of Pure Shift sequences combined with other solvent suppression methods, reflecting a real need in the analytical chemistry community for a generalized approach giving access to a wide variety of aqueous samples. One key question raised by these latter results is the generality of the quantification methods that can be developed based on Pure Shift spectra to address metabolite quantification in a broad range of biological samples.[2]

Here we will show how we have studied the influence of sample preparation on the quality of metabolite quantification using Pure Shift NMR and demonstrated the high reproducibility of the proposed analytical protocol regardless of the physicochemical conditions in the sample. We will also report the implementation of a quantification protocol to determine metabolite levels in real urine and plasma samples, which paves the way to the accurate and quantitative metabolic profiling of a broad range of aqueous samples.[3]

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CO₂ Speciation in Amine-Modified Porous Silicas for Direct Air Capture by DNP Enhanced Solid-State NMR

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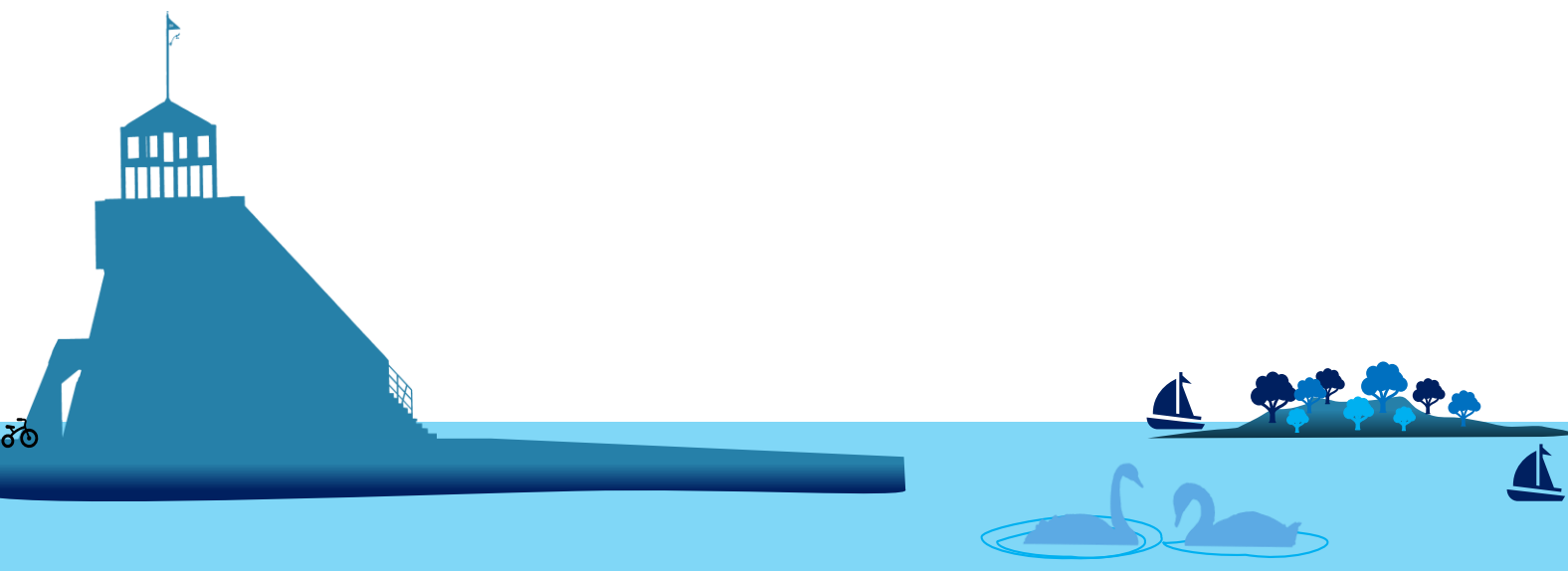
Direct air capture (DAC) is a promising solution to mitigate atmospheric CO₂, especially in low-concentration environments. Amine-functionalized adsorbents are central to DAC efficiency, but optimizing their performance requires understanding molecular-level capture mechanisms. Chemisorption relies on strong chemical interactions with CO₂, yet precise speciation characterization under realistic conditions (~400 ppm CO₂) remains challenging. Traditional methods struggle to resolve adsorbed species at trace levels, limiting insights into material degradation or saturation. Solid-state (ss) NMR can distinguish chemisorbed and physisorbed CO₂ [1,2], but its sensitivity is limited by the low natural abundance of ¹³C (~1%). Dynamic Nuclear Polarization (DNP) enhances sensitivity for studying adsorbed CO₂ in porous materials under DAC conditions. However, sample preparation must preserve the fragile gas-adsorbent interface. Current methods, like incipient wetness impregnation, risk displacing adsorbed CO₂ during radical doping, altering speciation. We present a novel radical impregnation method for MAS DNP NMR that minimizes perturbation of adsorbed CO₂. Our technique maintains speciation integrity while achieving high DNP signal enhancement. We demonstrate its application on amine-modified SBA-15 exposed to ambient air, resolving chemi- and physisorbed CO₂ species. This methodology enables DAC material characterization under operationally relevant conditions, guiding next-generation adsorbent design.

Acknowledgments:

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Pushing the Frontiers of Performance Polymers with Fast-MAS Proton NMR and DFT

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Over the past two decades, the atomic scale characterization of complex synthetic polymers was mostly done through either liquid-state NMR or conventional ^{13}C CPMAS NMR in the solid-state, which continues to serve as a benchmark especially in industrial settings. These methods have significant drawbacks, however. For example, the stability of crosslinks in polyurethanes is questionable when dissolved in polar solvents, and ^{13}C CPMAS NMR at natural abundance (NA) is insensitive, requiring long experimental times. Our research [1] shows that multinuclear ^{13}C and ^{15}N NMR combined with fast-MAS ^1H NMR is a reliable method for in-depth characterisation of polyurethane like polymers in their native state, providing short experimental times and inherently quantitative results. We demonstrate that, this approach facilitates the identification of various chemical moieties, including crosslinks, and enables the identification of subtle structural differences among multiple polyurethane samples.

In second part, I will focus on Polyaramids, commonly known as Kevlar. The chain arrangement within these fibers is believed to be critical to their performance but has remained a subject of debate for over 30 years due to their paracrystalline nature. Utilizing high-resolution ^1H -detected 2D ^1H - ^{13}C and ^1H - ^{15}N correlation experiments (at NA), recorded at high-field (950 MHz and 1.2 GHz) and 60 kHz MAS, in conjunction with DFT calculations, the chain packing is elucidated, revealing the proximity of dissimilar rings and the displacement of amide bonds by 0.5c in adjacent chains. These experiments further highlight structural differences in fibers with distinct moduli, uncovering the possible origins of their strength and providing guidance for optimizing chemical processes to achieve theoretical performance limits.

[1] Akshay Kumar*, Arno Kentgens, “Unravelling Crosslinking and Molecular Structure in Complex Polyurethanes via Advanced Multinuclear Solid-State NMR” Polymer, 2025, 128245



Pulse Sequence Engineering using Single-Spin Vector Effective Hamiltonians

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The recent advent of fast arbitrary waveform generators (AWGs), that permit pulse shaping in the microwave region, has led to increasing attention on the opportunities of realizing pulsed DNP. This approach is particularly enticing since it is believed that it will allow a significant increase in the desired control over spin dynamics, opening new avenues for the applications of DNP. Using single-spin vector effective Hamiltonian theory (SSV-EHT) [1], we illustrate the importance of analysing the effective first-order Hamiltonian for periodic pulsed DNP sequences. Key to this analysis is how the ‘unravelling’ of the pulse sequence complexity can reduce to interplay between bilinear two-spin and linear single-spin terms from the effective Hamiltonian which, in turn, dictates the overall pulse performance [2,3]. This establishes a solid basis for optimal-control-based design strategies to optimize periodic pulsed DNP sequences, leading to pulse sequences with exceptional experimental performance [4]. Lastly, we show how advanced NMR and pulsed DNP experiments—whether on static or rotating samples, single crystals, or powders—can be unified at the experiment design stage [5]. We further demonstrate these principles by showing how a pulsed DNP sequence can be applied to heteronuclear solid-state NMR magic-angle-spinning (MAS) dipolar recoupling experiments, enabling the design of ultra-broadband ^{15}N - ^{13}C and ^2H - ^{13}C cross-polarization experiments [5].

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SIMPSON++: Simulations and time propagation for large spin-systems

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SIMPSON has been developed over decades to become an indispensable simulation tool for NMR. As the years pass the number of developers of this software have remained the select few, whereas the requirements of magnetic resonance calculation software have increased – specifically, there is a very steep learning curve for developers of the software. This brings an additional requirement to SIMPSON; that the software now needs to be easy to modify for research groups' needs.

We present SIMPSON++, an object-oriented software design, retaining all the functionality of previous SIMPSON. Functionality of code has been reorganised to three layers, where the scientific programmer is intended to code in the central layer without need to worry about a mathematical back-end or the operating-system interactive front-end. Conversely, a mathematician need only apply numerical methods to the back-end.

One of the challenges of SIMPSON is optimisation and calculations of time propagation involving large spin systems. SIMPSON++ will showcase novel tools for acceleration of the numerical time-propagation of the spin-system states using an adaptive optimal control method, QOALA (quantum optimal control by adaptive low-cost algorithm).

This method shows that an approximate control manifold can be successfully navigated with gradient following optimal control methods when far from an optimal solution, with accurate manifolds only needed close to the optimal pulse. The potential for QOALA is that its computational demands scale much better than other optimal control methods as the spin system size increases. This gives the potential for it to be applied to proton-rich environments currently out of reach of conventional optimal control methods.



42 Tesla Miniature Magnet

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High-field NMR magnets are always in demand because of the improved spectral resolution and sensitivity. Conventional magnets with fields higher than 40 Tesla requires combination of superconducting and resistive magnets, which are huge and consume megawatts of power. Development cycles of magnets with fields higher than 28 Tesla are usually years, thus access to these magnets is limited. Advances in high temperature superconductors (HTS) enable higher magnetic fields and smaller magnets. We envision a more vibrant and innovative development of NMR magnets to enable more accessible NMR technology.

Here, we present the development of small high temperature superconducting (HTS) magnets from 7 Tesla to 42 Tesla within a 10-month timeframe. The 7 Tesla magnet is a watch-sized magnet which uses only 10-meter HTS tape. The 42 Tesla magnet is a cylindrical double pancake coil only 6 cm in diameter and 3 cm high; small enough to fit in the palm of your hand. We can fabricate a 42 Tesla magnet in only 4 days. First, the two pancake coils are wound using a custom-built automated winding machine in a matter of hours. Next, thin copper plates are soldered onto the coils to provide mechanical stability and a low-resistance electrical path in the case of over-charging and quenches. The 42 Tesla was charged to 1248 Amperes, while consuming less than 1 Watt of power. The inductance of the miniaturized magnets is small, enabling energization to 42 Tesla in less than 5 days of charging. Other advantages of the new handheld sized magnets include low stray fields, easy access to the magnet center (a few cm), and small amount of stored energy in case of a quench. Furthermore, we performed NMR experiments inside the 3 mm magnet bore to calibrate Hall sensors. These miniature magnets offer significant potential for widely accessible benchtop high-field NMR.



A parallel line probe for spatially selective electrochemical NMR spectroscopy

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In situ NMR is a valuable tool for studying electrochemical devices, including redox flow batteries and electrocatalytic reactors, capable of detecting reaction intermediates, metastable states, time evolution of processes or monitoring stability as a function of electrochemical conditions. Here I present a parallel line detector for spatially selective in situ electrochemical NMR spectroscopy. The detector consists of 17 copper wires and is doubly tuned to $^1\text{H}/^{19}\text{F}$ and X nuclei ranging from ^{63}Cu (106.1 MHz) to ^7Li (155.5 MHz). The flat geometry of the parallel line detector allows its insertion into a high electrode surface-to-volume electrochemical flow reactor, enabling a detector-in-a-reactor design. This integrated device is named “eReactor NMR probe”. Combined with B1-selective pulse sequences, selective detection of the nuclei at the electrode-electrolyte interface, that is within a distance of 800 μm from the electrode surface, has been achieved. The selective detection of ^7Li and ^{19}F nuclei is demonstrated using two electrolytes, LiCl and LiBF₄ solutions, respectively. A good B1 homogeneity with an 810° to 90° pulse intensity ratio of 68–72 % was achieved. Using electrochemical plating of lithium metal as a model reaction, we further demonstrated the operando functionality of the probe. The new eReactor NMR probe offers a general method for studying flow electrochemistry, and we envision applications in a wide range of environmentally relevant energy systems, for example, Li metal batteries, electrochemical ammonia synthesis, carbon dioxide capture and reduction, redox flow batteries, fuel cells, water desalination, lignin oxidation etc.

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Structure and dynamics in filamentous phage life cycle

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Ff Filamentous phages are one-micron long viruses containing a single-stranded DNA with ~8000 bases wrapped by ~3500 copies of a major coat protein, g8p.

The solid-state NMR structure of M13 phage shows that the g8p-capsid is strengthened by strong hydrophobic stacking interactions. In order to assess the dynamic properties of g8p in the viral capsid, we used pseudo-3D experiments to measure ^{13}C and ^{15}N CSA, as well as backbone and sidechain C-N dipolar order parameters. The rigid-limit CSA interactions (6-12 kHz) were almost fully recoupled suggesting a rigid capsid on this time scale. On the other hand, the REDOR curves, which were resolved using either single-quantum (DARR, RFDR) or double-quantum J-based spectroscopy, report on capsid motions on a time scale of ~1 kHz and on the highly dynamic N-terminus. C-N dipolar order parameters are also shown to be an excellent tool to characterize the dynamics of electrostatic interactions with the DNA.

The structure of g5p in the pre-mature capsid was determined by SSNMR to a backbone RMSD of 0.37Å for the well-defined regions. Distance and torsion angle restraints were obtained from multi-dimensional experiments (on fully and sparsely labeled samples) and then given as an input to Xplor-NIH. Convergence to a successful structure was achieved by using (i) weak restraints from X-ray DNA-free dimeric form structure; (ii) avoiding structure deformation due to the homodimer interaction; (iii) repeated steps of ambiguity and violation analysis, and (iv) refinement. Free and DNA-bound g5p show significant changes in key locations having an RMSD of 6.4Å, which is accompanied also by loss of several secondary structure elements but with preservation of the main core of the beta barrel. The changes point to a more compact DNA binding pocket and flattening of the protein side facing the dimer-dimer interface explains the cooperative binding which promotes the cellular assembly of this pre-mature virus.



Calcium-Mediated Inactivation of the MthK Potassium Channel: Insights from Solid-State NMR and MD Simulations

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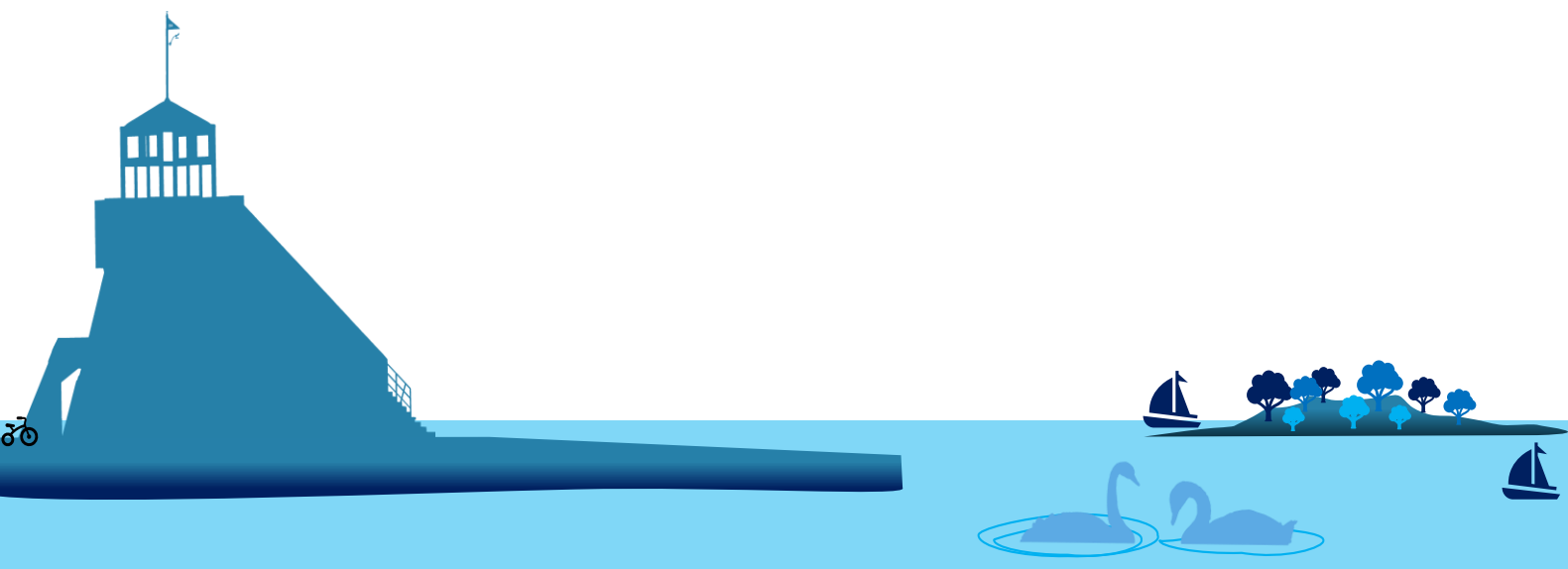
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Ion channels are essential for living cells due to their role in electrical signaling, which is critical for many biological functions. MthK is a calcium-gated potassium-selective archaeal ion channel. It functions as a model system for eukaryotic large-conductance potassium channels (BK channels), which play key roles in muscle contraction, neurotransmission, and blood pressure regulation. The gating mechanism of MthK, which depends on calcium ions binding to a cytosolic regulatory domain, is well understood. However, calcium ions may also play a role in MthK inactivation by binding to the transmembrane pore domain and blocking potassium ion transport out of the cell.

Our approach centers on ^1H -detected solid-state NMR experiments. We investigate the effects of calcium ions on MthK conformation using triple sensitivity-enhanced 4D experiments(1). ^{15}N -labeled ammonium ions serve as potassium ion mimics(2), enabling us to detect bound ions in the MthK selectivity filter and even observe interactions between adjacent ions.

By integrating solid-state NMR data with atomistic molecular dynamics (MD) simulations, we demonstrate that a physical block caused by calcium ion binding to the transmembrane domain is responsible for the inward rectification of the MthK channel. Furthermore, direct ion-ion contacts between ions bound at different sites provide new evidence for water-free ion conduction in potassium-selective ion channels (3, 4). These findings contribute to a deeper understanding of fundamental ion channel mechanisms, with potential implications for eukaryotic ion channel function.

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Precise vicinal J-couplings to determine rotamer populations in aliphatic chains by spin-chain zero-quantum spectroscopy

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The analysis of a novel form of sparse zero-quantum (ZQ) spectra [1] combined with crowded conventional single-quantum (SQ) spectra allows one to determine scalar J-couplings in aliphatic chains with three methylene groups with unprecedented precision. The linewidths in the ZQ spectra are on the order of 0.1 Hz, regardless of the homogeneity of the static field [1]. Such a resolution cannot be achieved with conventional spin-echo J-spectra, where the linewidths are limited by transverse relaxation, as the long-lived ZQ coherences have lifetimes T_{LLC} that are longer than the relaxation times T_2 of transverse magnetization. It is shown that for the molecules under investigation (DSS and homotaurine), the extracted ZQ frequencies depend linearly on the temperature varying in range from 5 to 25 °C and are determined predominantly by the vicinal couplings. Precise values of vicinal J-couplings are then used to determine the energy differences between the rotational conformers in aliphatic chains.

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ShimNet: the data-driven deep learning approach to post-acquisition improvement of NMR spectra distorted by magnetic field inhomogeneity

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Despite commonly applied corrections, known as shimming, the magnetic field in an NMR spectrometer is never perfectly homogeneous. This undesired effect distorts the lineshapes, degrades the resolution, and lowers the signal-to-noise ratio in the collected spectra. As a remedy, numerical techniques have been developed to correct the spectra after acquisition, with reference deconvolution being the most popular example. However, these methods require a precarious parameter guess from the user, making them inconvenient and prone to errors. We propose a self-contained numerical shimming tool named ShimNet based on a convolutional neural network with the attention mechanism. The program learns the spectrometer distortion characteristics from a series of calibration measurements. Once trained, the network can correct any spectrum from that given machine fully automatically. It achieves better reconstruction quality than the existing methods and is considerably faster. This makes ShimNet an excellent tool for laboratories performing routine and massive NMR measurements for chemists. As an exemplary application, we demonstrate that the model properly corrects liquid-state spectra of small molecules, such as azarone, styrene, 2-ethylnaphthalene, or sodium butyrate. The open-source Python code is freely available from GitHub.



Paramagnetic materials: challenges and opportunities for solid-state NMR

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Establishing links between the structure and properties of a material is the key to understanding how the material works, why it fails, and how to improve performance. This requires the careful measurement and evaluation of a variety of structural features, including particle size and morphology, long-range order, local chemical environments, and dynamics. For paramagnetic systems, the local structure of a particular atom/ion is determined by its chemical bonding environment, which is dominated by the nearby open-shell metal ions. In solid-state NMR, the unpaired electrons can be used to probe differences in local structure via the hyperfine interaction to the observed nucleus, and to identify disorder in structure or composition. However, this information is often difficult to obtain since these same unpaired electrons induce large chemical shifts and shift anisotropies of the signals in the spectrum, and accelerate the relaxation of these signals. The resulting signals are difficult to excite, detect, and interpret using standard solid-state NMR methods. We have developed methods for solving these problems, including tailored pulses for broadband excitation, new two-dimensional schemes for separation of overlapping signals from complex disordered materials, and new theoretical formalisms and computational methods for calculating the chemical shifts, and assigning and interpreting the spectra. Here we highlight these methods on different systems, including inorganic battery materials and organometallic catalysts, demonstrating the far-reaching potential of paramagnetic solid-state NMR in different areas of chemistry and materials science. In each case we learn something about the local structure of the NMR-active nuclei, disorder in structure, and metal-ion composition, and the role of dynamics. This information is integrated with data from other structure determination methods to obtain a complete picture which is then used to understand the material properties.



Room-Temperature Overhauser DNP on luminescent poly-TTM radical films with engineered electron delocalization

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Organic radicals hold great promise for optoelectronic applications due to their unique electronic properties. Trityl radicals based on the tris(2,4,6-trichlorophenyl)methyl (TTM) [1] scaffold exhibit exceptional stability and luminescence, making them attractive candidates for organic light-emitting diodes (OLEDs), near-infrared emitters, and biosensing technologies. Despite this potential, direct experimental quantification of electron delocalization remains challenging, and the mechanisms underlying electron-to-nuclear polarization transfer at film state are yet to be explored.

In this study, we investigate the distinct spin properties of mono-, di-, and poly-TTM radicals linked via fluorene (FLr)[2] and cyclopenta[2,1-b:3,4-b']dithiophene (CDT)[3] using variable-temperature electron spin resonance (VT-ESR). To achieve site-specific mapping of electron spin density, we introduce a novel solid-state nuclear magnetic resonance (NMR) approach leveraging paramagnetic Fermi shifts, providing a robust method for quantifying electron delocalization.

Notably, we observe Overhauser dynamic nuclear polarization (DNP) at room temperature in poly-TTM radical films, marking a significant advancement in applying DNP to these materials. By employing DNP-enhanced ^1H - ^1H 2D NMR, we further demonstrate the ability to resolve film packing structures through linker modifications. These findings offer new insights into electron-nuclear polarization transfer mechanisms in conjugated radical systems and enable high-sensitivity structural characterization of amorphous films.

The integration of DNP-enhanced NMR with optoelectronic studies establishes a predictive framework for optimizing radical-based materials, accelerating their development for next-generation optoelectronic technologies [4].

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Hyperpolarized ^{129}Xe Uptake Dynamics in Porous Media and Polymeric Dissolved Phase for Industrial Catalysis and Clinical Applications.

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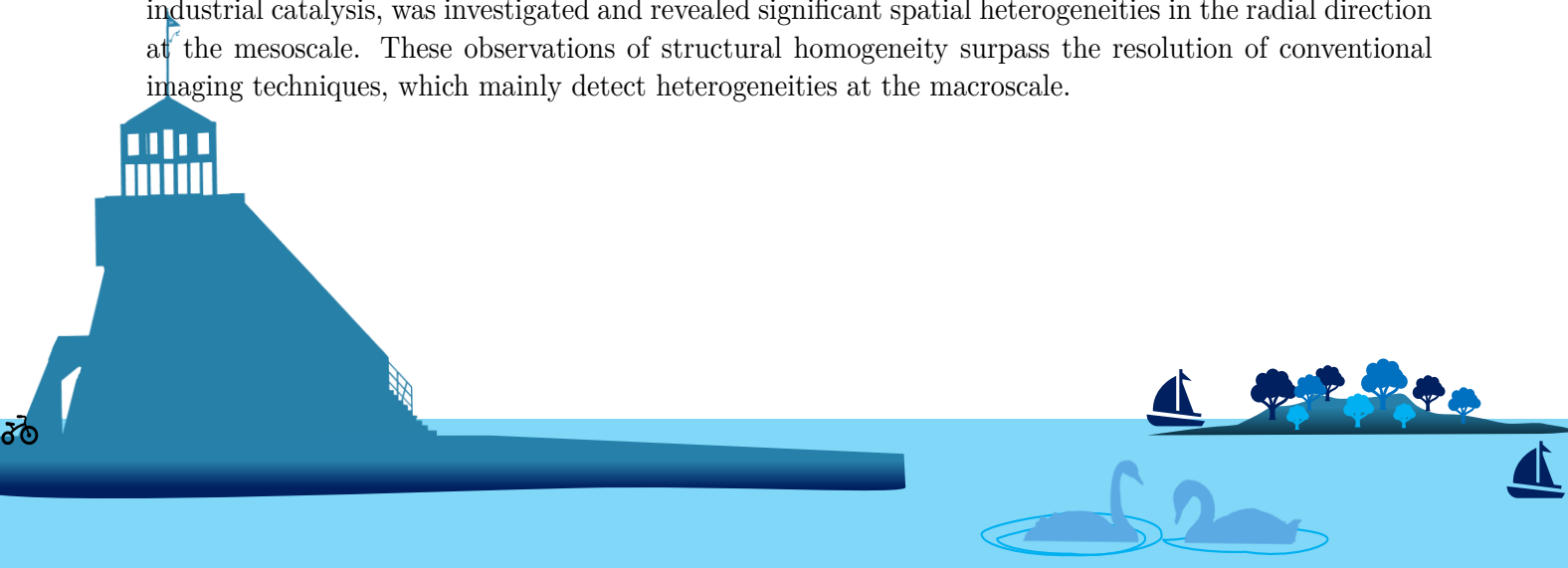
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NMR spectroscopy and MRI with hyperpolarized ^{129}Xe (hpXe) enable unique insights into gas flow and gas uptake dynamics in a variety of applications ranging from materials science to clinical pulmonary care. For example, the ^{129}Xe chemical shift provides distinguishable, characteristic MR signals between xenon dissolved in lung parenchyma and vascular phase. Therefore, monitoring the exchange of hp ^{129}Xe through the parenchyma into the blood stream becomes viable and can be leveraged for functional pulmonary MRI. This methodology is gaining increasing attention for clinical assessment of gas exchange in multiple diseases. However, comparing the hp ^{129}Xe uptake quantitatively across different studies may pose challenges due to varied experimental conditions and different pulse sequence protocols.

A solution to this problem arises from materials science applications of hpXe where xenon dissolved in polymers and liquids can display a wide range chemical shifts with vastly different uptake dynamics. Here, we demonstrate the generation of MR spectral profiles with chemical shifts that closely resemble those observed in human lungs. The uptake kinetics into the non-toxic materials was found to be sufficiently similar to pulmonary signal uptake to enable hpXe MRI with dissolved phase signal ratios that closely resemble clinical data of lungs in health and disease. A phantom assembly was developed to allow for gas handling protocols that match clinical protocols and enables rapid testing of basic experimental protocols without the requirement for regulatory approval and governance.

Applying similar NMR protocols, the hpXe uptake dynamics in millimetre sized pellets, as used for industrial catalysis, was investigated and revealed significant spatial heterogeneities in the radial direction at the mesoscale. These observations of structural homogeneity surpass the resolution of conventional imaging techniques, which mainly detect heterogeneities at the macroscale.



The role and properties of lignin for bio-based carbon fibers

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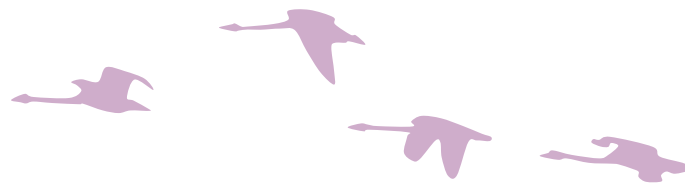
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In light-weight applications, carbon fibers (CFs) show desirable weight-to-strength ratios due to their high specific strength and stiffness, which makes them very efficient reinforcements in composite materials. Cellulose–lignin blends have emerged as a promising, sustainable, and cost-effective alternative to the expensive fossil-based polymers currently used as precursor fibers. Forest-based CFs precursors can be made by wet spinning of lignin-cellulose ionic liquid solutions, where cellulose and lignin are first dissolved (in e.g. ionic liquid) then coagulated (in e.g. water) to form rigid fibers, that later can be thermostabilized and carbonized to CFs. During coagulation, the solvent for dissolution and the anti-solvent for coagulation play an important role impacting the properties of the fibers. Bio-based resources vary depending on their origin and how they have been extracted. The characteristics of the obtained precursors including their crystallinity, the functional groups, and the composition of biopolymers have a significant influence on their carbonization behavior and the properties of the final product.

In this study, NMR and MRI techniques were used to explore various steps of the production process of cellulose and lignin-based carbon fibers, including coagulation and thermostabilization. In-situ MRI measurements on a model system were conducted to analyze mass transport during coagulation, focusing on solvent diffusion and lignin leaching. Additionally, the study investigated the behavior of cellulose–lignin blends after coagulation and thermostabilization using a multi-faceted approach that combined solid state and 2D solution NMR techniques. The findings, derived from a wide range of NMR-based methodologies, provided valuable insights into how raw material variations, coagulation conditions, and thermal treatments influence the properties of the produced fibers, aiding in the optimization of their performance and potential applications.





POSTERS



High Field EPR Investigation of ^{13}C DNP Mechanisms of P1 Centers in Diamond

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DNP can enhance NMR signals by orders of magnitude, vastly expanding the range of NMR applications. P1 centers in diamonds were recently shown to provide efficient ^{13}C DNP at room temperature and high fields. Their DNP lineshape analysis indicates a complex interplay of multiple DNP mechanisms. Correctly identifying the contribution of each mechanism, in order to optimize the DNP enhancement, requires knowledge about electron spin dynamics, available only through Electron Paramagnetic Resonance (EPR) experiments performed at the same fields as DNP, since electron spin properties are field-dependent.

Using the unique capabilities of the high-field dual DNP/EPR spectrometer constructed over the past six years in our group, we investigated the spin dynamics of P1 centers electron spins under DNP conditions. P1-EPR spectra acquired at 7 and 14 T revealed an unexpected broad signal between the sharp lines of isolated P1 centers, which we assign to P1 clusters with strong internal exchange and dipolar coupling. We suggest these clusters to be the cause of efficient P1-DNP at high fields, as they allow for fulfillment of the cross-effect matching condition. This is substantiated by electron-electron double resonance experiments which reveal efficient electron-electron spectral diffusion between the two P1 populations indicating they co-exist in close proximity. This is typically observed in diamonds with high (> 100 ppm) nitrogen concentration. To prove that P1 clusters are essential for efficient DNP, we show that in line with our prediction, in a diamond with low P1 concentration and no P1 clusters, high-field P1-DNP is very inefficient, as only the solid-effect mechanism is active, shown by a combined analysis of EPR and DNP spectra.

This work shows the importance of P1 center clusters for efficient DNP in diamonds, as well as the necessity of EPR experiments conducted under DNP conditions for the identification of the active DNP mechanisms.



J-coupling interactions and Hydrogen/Ligand Exchange in SABRE Systems

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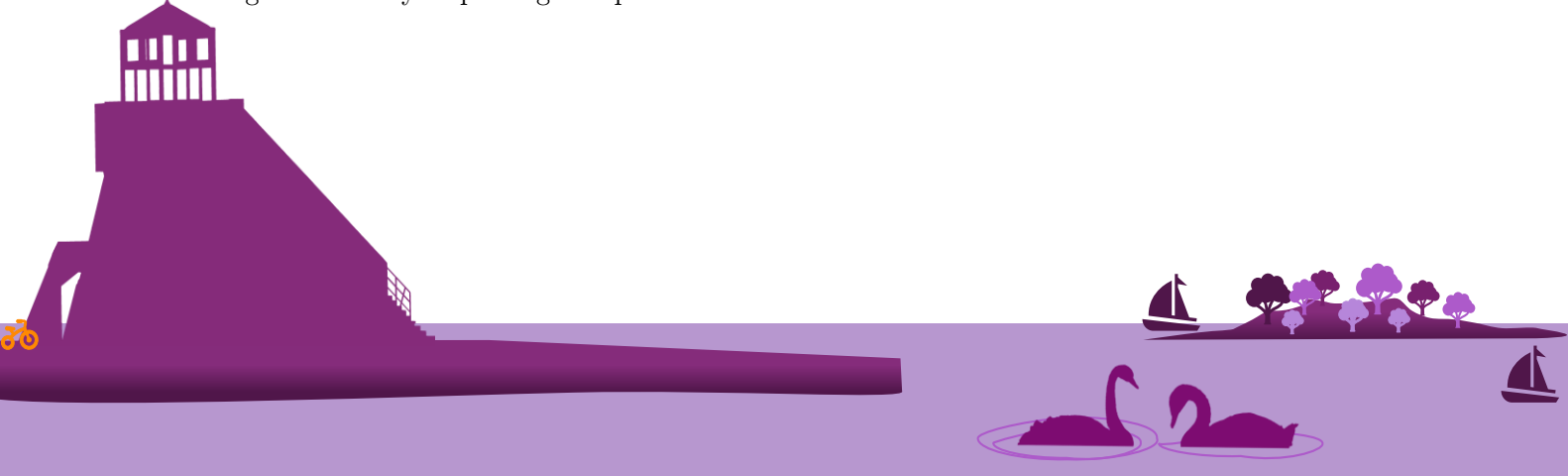
Signal amplification by reversible exchange (SABRE) relies on parahydrogen to hyperpolarize substrates like pyruvate (pyr) a central component in cellular metabolism. The hyperpolarization process is achieved by harnessing J-coupling interactions within the transient polarisation transfer catalyst created during SABRE. Selectively exciting protons in this complex allow us to probe these ^1H - ^{13}C interactions that mediate polarization transfer to pyr ([1] = DOI: 10.1021/acs.jpcclett.3c02980). Understanding and measuring the individual elements of this process, particularly the J-coupling and exchange constants, are essential for increasing the hyperpolarization yield.

Here, we evaluated the accuracy of different kinetic models for SABRE ([2] = DOI: 10.1021/acs.analchem.4c01374) and tested several monodentate polarization acceptors like pyridine, acetonitrile, and metronidazole ([3] = DOI: 10.1038/s42004-024-01376-z) alongside bidentate ligands like pyruvate.

We found that in situ ^{15}N hyperpolarization transfer by SABRE-INEPT and ESOTHERIC sequences allowed a more accurate mapping of substrate dissociation rates than traditional ^1H EXSY NMR techniques.[2,3]

Unlike monodentate ligands, pyruvate requires an additional co-substrate, DMSO, to bind, and more than three interchanging complexes are involved. We developed new pulse sequences SEPP-SABRE and SEPP-SPINEPTplus-SABRE to probe the effects of $[\text{H}_2]$, $[\text{DMSO}]$, $[\text{pyr}]$, and temperature on the chemical exchange pathway, determining the rates of H_2/pyr exchange, and hence decipher the intricate interplay between chemical transformation and SABRE performance for the first time.

These insights will allow us to significantly advance SABRE by enabling accurate simulations and experimentally tuning the chemical exchange and spin-spin interactions. Establishing a reliable tool for achieving this is a key step along this path.



Suitability of Various Cryptophane-A Derivatives for Gadolinium Detection in Solution using ^{129}Xe HyperCEST Spectroscopy

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The increasing use of gadolinium-based contrast agents in MRI has manifested through reports about the higher deposition of free gadolinium (Gd) ions in sewage and urban ecosystems. A destruction-free method to quantify and locate free Gd, especially at low concentrations, is therefore essential for environmental monitoring and safety.

In HyperCEST, the exchange of free hyperpolarized (hp) ^{129}Xe and ^{129}Xe bound to a functionalized host-molecule is detected via chemical exchange saturation transfer (CEST), enabling the detection of molecular targets at low concentrations. Recently, Xe HyperCEST binding and competition-based assays have shown, that free Gd ions exhibit a competitive affinity for the host Cryptophane-A monoacid in solution. The z-spectra revealed that as free Gd concentration increases, a reduction in the HyperCEST signal of up to 50% of Xe bound to CrA-ma is observed.

This study investigates the suitability of Cryptophane-A (CrA) and its acid derivatives (Cryptophane-A monoacid (CrA-ma), Cryptophane-A diacid (CrA-da)) for Gd detection by analyzing hp Xe depolarization rates in HyperCEST NMR spectroscopy. Z-spectra are acquired using CrA, CrA-ma and CrA-da, with the impact of free Gd assessed in each case. Lorentzian fitting of the data allows us to extract the depolarization rate, which is a quantitative measure of the observed CEST efficiency decrease as cage-bound Xe is being displaced by Gd. Initial experiments demonstrate higher sensitivity for Gd in the low μM range with CrA-ma compared to CrA-da. Ongoing experiments with in-house synthesized CrA cages will determine whether sensitivity can be further improved.

Additionally, the potential binding site of Gd to the cage molecule is being investigated by the cooperation partner using Gd EPR spectroscopy.



Glucose as a Versatile Glassing Agent for Hyperpolarizing Key Metabolites in Biological Studies

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Dissolution-dynamic nuclear polarization (D-DNP) significantly enhances nuclear magnetic resonance (NMR) signals¹, paving the way for the exploration of cellular metabolism. Among many targets, glutamine has drawn particular attention for its role in cancer and cardiovascular diseases², due to its high concentration in cancer cells, which produce energy and proliferate more than healthy ones³. In fact, as a key metabolite for energy supply, glutamine's conversion into glutamate and alpha-ketoglutarate can be monitored over a biologically relevant timescale.

However, glassing agents used in standard DNP sample formulations can affect cellular media and kinetic analysis.

Here we will show that glucose can be used as a performant, cost-effective and biologically safe glassing agent, to formulate biological samples with competitive polarization levels. We demonstrate how this formulation can be optimized to achieve significant ¹H polarization levels in the range of 69% and ¹³C polarization higher than 30%. We will report the excellent biocompatibility of glucose, enabling the monitoring of the enzymatic conversion of glutamine to glutamate via glutaminase.

Using this formulation, we have then been able to monitor in real-time glutamine and glucose metabolism with hyperpolarization in *E. coli* bacteria and cancer cells. These promising results suggest the potential for simultaneous metabolic studies of glutamine and glucose, opening the way for applications in cancer cell research. This approach could enable the assessment of glutamine inhibition, metabolic pathways favored by different cell lines, and potentially the response of cells to cancer treatment, in particular regarding anti-metabolic drugs.

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Field-dependent ^{15}N Relaxation and Chemically Induced Deceleration of Nuclear Spin Relaxation (CIDER)

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Longitudinal magnetization dynamics is a key feature of magnetic resonance. T_1 -relaxation enables signal averaging, suppression, and anatomical contrast, therefore T_1 -shortening Gd-based agents are widely used. T_1 -prolonging agents, however, remain unexplored, with previous methods focusing on reducing relaxation sources (deuteration of solvent or molecule, chelating complexes). Long T_1 maybe less useful for routine MRI than short T_1 , but it is essential e.g. for hyperpolarization experiments. Here, complete loss of polarization may occur in particular at low fields (or certain pH).

We discovered an hitherto unknown effect that prolongs T_1 . Instead of reducing intra or inter molecular relaxation, agents suppress exchange-driven relaxation, in particular at low fields, by several orders of magnitude. This chemically induced deceleration of nuclear spin relaxation (CIDER) is very effective for molecules with pH-sensitive R_1 relaxation (DOI: 10.21203/rs.3.rs-4668036/v1). The effect enabled us to polarize molecules with otherwise prohibitively short T_1 , and increasing polarization e.g. from 5 % to 29% $t=18\text{s}$ after polarization for $1\text{-}^{15}\text{N}$ nicotinamide.

^{13}C and ^{15}N signals were acquired after dissolution using a 1T benchtop and a 9.4T NMR. dDNP was performed using a cryogen-free dDNP operating at $\sim 1.4\text{K}$ and 6.7T and $\sim 50\text{mg}$ samples with different compositions of the tracer, trityl radical (AH111501), water and trehalose.

This is the first report of relaxation agents that prolong T_1 , which holds great potential e.g. for hyperpolarization. Additionally, using field cycling, we observed the CIDER effect on NAM and other molecules at fields from a few μT to 9.4T. As an indicator for exchange driven relaxation, T_2 relaxation was used to estimate the rate of rapid hydrogen exchange.

Our data provides new insights into CIDER and relaxation mechanisms of ^{13}C and ^{15}N labeled molecules. This boosted observed polarization enabled the use of previously prohibited molecules for imaging.



SCREAM-DNP for Site-Specific Polarization Transfer to Heteronuclei and Ring Puckering Dynamics

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Typically, dynamic nuclear polarization (DNP) is used to enhance magic-angle spinning (MAS) NMR signals uniformly, but in recent years there has been an interest in using DNP to also achieve site specificity.¹ One such approach is Specific Cross Relaxation Enhancement by Active Motions under DNP (SCREAM-DNP), which exploits the fast reorientation dynamics of methyl groups, even at low temperatures.^{2,3} The scope of this application has recently been expanded by combining it with rotational resonance, which allows a high degree of sensitivity and spectral specificity.⁴

To further expand the application of SCREAM-DNP in a biomolecular context, we shift the focus away from enhancing the methyl carbon atom and instead explore other types of mobile groups as well as polarization transfer to different heteroatoms.

One example for such system is proline where the dynamics are expected to be caused by the change between ring pucker conformers.⁵ Here, we present a systematic approach on how the SCREAM-DNP efficiency or better ring puckering dynamics is influenced by the incorporation into slightly larger molecules. The distinct SCREAM-DNP activity of each carbon atom in proline suggests that the nitrogen may also be influenced, as our initial findings indicate. This leads to a broader investigation into the efficiency of SCREAM-DNP in transferring polarization from methyl protons to heteronuclei, both in proteinogenic and non-proteinogenic amino acids.

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Low-field dynamic nuclear polarization for the investigation of batteries

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The performance and lifetime of rechargeable lithium batteries are governed by complex interfacial processes at the electrode-electrolyte interface. One critical component is the solid electrolyte interphase (SEI), a passivation layer that forms on the electrode surface during cycling. However, its structure and composition remain challenging to characterize. In pursuit of higher energy densities, battery concepts are shifting toward anode-free configurations, in which lithium is plated directly onto a current collector such as copper, instead of using a traditional lithium-based anode. Although promising, this approach introduces new challenges, particularly the formation of lithium dendrites—needle-like structures that can pierce the separator and cause short circuits or battery failure. Despite extensive research on lithium plating and dendrite formation, the molecular formation processes are not yet fully understood. Dynamic nuclear polarization (DNP) offers the potential to shed more light on this phenomenon. By transferring electron spin polarization to nuclei, DNP can overcome the low sensitivity of NMR under low-field conditions. Here, we demonstrate correlative EPR and DNP-enhanced ^7Li NMR measurements of lithium on copper using a custom setup operating at 0.34 T. The resulting enhanced ^7Li NMR signal allows for the observation of electrochemically deposited lithium on copper, harvested from a Cu vs. Li cell, with an enhancement greater than 400. By changing the magnetic field strength by a few Gauss, the saturation of the conduction EPR transition was varied, leading to an alteration in the Knight shift for metallic ^7Li . Moreover, an approximately twofold enhancement of the ^1H signal from the surrounding electrolyte near the electrochemically deposited lithium was observed, indicating the potential to investigate the SEI. The setup employed a battery cell housing developed for EPR, demonstrating its suitability for future in operando experiments.



NMR masers: from single mode DNP hyperpolarization at 1.2K to multimode masers in solution through electronic control of radiation feedback.

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We have recently observed sustained NMR masers in DNP-hyperpolarized nuclei at low temperature.[1,2] These originate from the natural radiation feedback from the coil due to the huge sample magnetization, when the continuous microwave irradiation that builds up a negative magnetization. Signals lasting for minutes (hours) can thus be obtained and broad maser pulses lead to line widths of the order of 10 Hz in a non rotating solid. Particular features involving the large dipolar field in the sample will be discussed.

Alternatively, NMR masers can also be generated at ambient temperature on thermally polarized proton spins via the electronic control of the radiation feedback (radiation damping - RD) from the probe by manipulating the phase and gain of the RD feedback field.[3] Recently, we have shown [4] that this technique can also be used to generate multimode masers from thermally polarized protons with different chemical shifts in ethanol. Our observations illustrate the breakdown of the usual three-dimensional Maxwell-Bloch equations for radiation feedback and can be explained by a simple toy model of few coupled classical moments.

Finally, we introduce a new multi-purpose NMR simulator, PyOR (Python On Resonance) [5], which incorporates radiation damping (feedback) in the Liouville equation, thereby allowing for the simulation of more complex spin systems that possibly include J coupling or dipolar relaxation.

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Nitroxide-Doped Solid Matrices: a New DNP MAS NMR Approach to Polarize Sensitive Surfaces

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Dynamic Nuclear Polarization (DNP) has emerged as a key method to enhance the sensitivity of solid-state Nuclear Magnetic Resonance (NMR) under Magic Angle Spinning (MAS). In a MAS DNP NMR experiment, the polarization of unpaired electrons is transferred to nuclei of interest, leading to substantial signal amplifications, theoretically up to ~ 660 for protons. Implementing MAS DNP NMR requires optimizing several aspects, with sample formulation being one of the most critical. Typically, exogenous DNP involves coating or impregnating the substrate with a glassy solvent containing a soluble organic free radical. However, such formulations remain impractical for characterizing reactive surfaces that readily reduce polarizing agents.

To address this, we prepared polarizing solid matrices designed to prevent direct contact between the polarizing agent and the target surface. This was achieved by incorporating a biradical into a silica-based matrix, yielding porous solid particles with controlled texture and biradical concentration. Several polarizing solids with variable biradical concentrations were synthesized. Their DNP efficiency was assessed at 9.4 T and 100 K by measuring solvent and surface enhancement factors as well as polarization build-up times after incipient wetness impregnation. The best solid matrix exhibits performances similar to AMUPol in water/glycerol mixtures.

We then show that these polarizing beads can polarize external objects, i.e., objects outside the porous structure. An enhancement of ~ 30 was obtained for a large analyte molecule that does not penetrate the pores. Significant surface enhancement factors on target solids were achieved by homogeneously mixing the polarizing beads with other solid particles. An overall sensitivity gain of ~ 50 was notably obtained for ligands grafted on alumina surfaces. Preliminary data on reactive surfaces are finally presented.



Systematic study of hyperpolarization with hybrid polarizing solids – moving towards pure hyperpolarized solutions after dissolution-dynamic nuclear polarization

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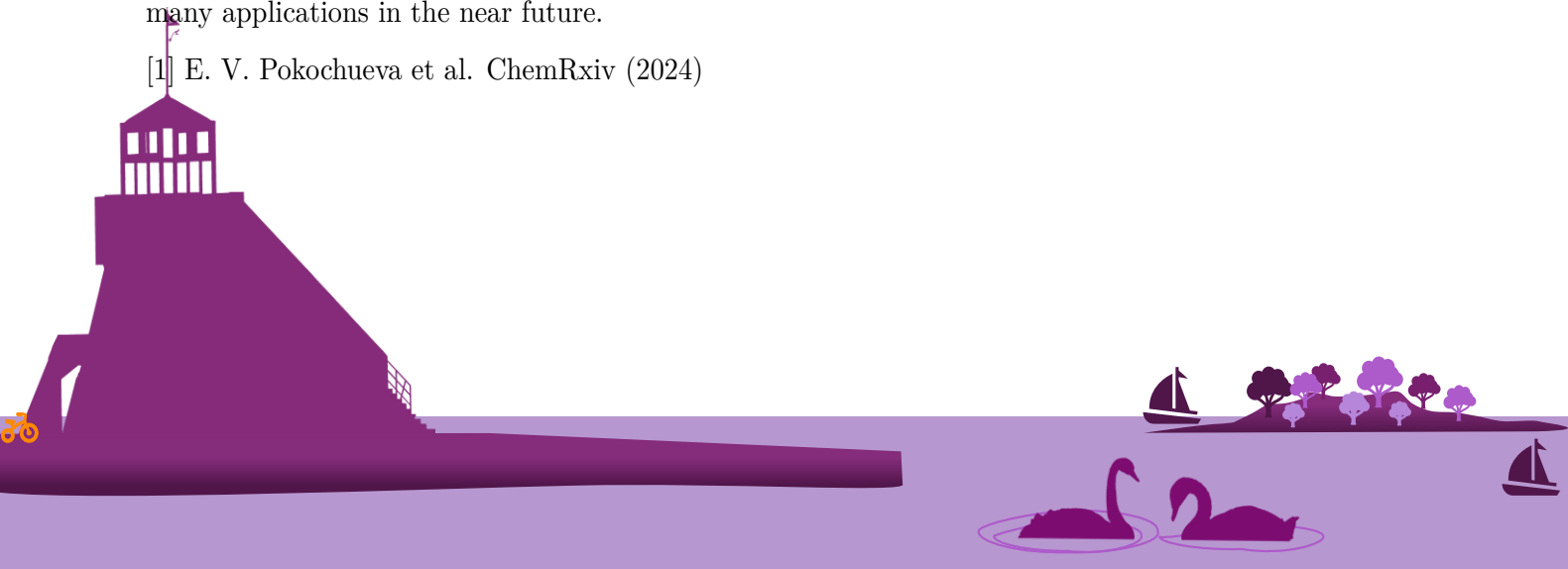
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Dissolution dynamic nuclear polarization (dDNP) is one of the most powerful and versatile hyperpolarization techniques, able to provide NMR signal enhancements of the order of 10'000 in high fields and even more at low fields. To perform dDNP, radicals are added to the substrate to be polarized, and while the transfer of polarization from electrons to ^1H is very effective, the presence of the radicals in the final solution causes several problems. It contaminates the sample, leads to paramagnetic relaxation and causes significant paramagnetic lines broadening at low magnetic fields. As a solution, mesoporous silica-based matrices with radicals covalently immobilized on the surface (HYPSO, hybrid polarizing solids) were proposed.

Here we report a first comparative ^1H and ^{13}C hyperpolarization study with HYPSOs of different pore sizes (4 – 28 nm) and concentrations of radicals (9 – 67 $\mu\text{mol.cm}^{-3}$). We show that the concentration of polarizing agents can be tuned to achieve optimum dDNP even with the large pores. With such optimization, liquid-state ^{13}C polarizations of 15% can be achieved on small molecules (here ^{13}C -labeled sodium acetate) resulting in a signal enhancement of ca. 12'000-fold compared to thermal equilibrium at 14 Tesla. We implemented an in-line filtration of the HYPSO materials right after the dissolution, and we observed that nuclear spin-lattice relaxation times were extended (compared to the standard sample formulation), due to the absence of paramagnetic free radicals in the hyperpolarized solutions (confirmed by EPR measurements).[1]

HYPSOs is an alternative sample formulation that may become a versatile polarization agent for the dDNP community. For now, HYPSOs already offer the possibility to effortlessly remove hyperpolarizing agents without additional delays while retaining significant hyperpolarization, which may be used for many applications in the near future.

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Monitoring pyruvate to lactate conversion in biological solutions at low magnetic fields

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In this project, spectral acquisition of metabolic pyruvate to lactate conversion using a built-in-house, low-field multinuclear scanner (66 mT) is aimed. To enhance signal detection, pyruvate is hyperpolarized via para-hydrogen-induced polarization (PHIP) [1], allowing cost-effective monitoring of metabolisms with compact devices. In high magnetic fields mostly the chemical shift difference is used to distinguish between pyruvate and lactate. Since chemical shift differences are proportional to the magnetic field, this is not possible in lower magnetic fields. Instead, the JCH coupling of [2-13C]-lactate ($JCH = 145$ Hz) stemming from [2-13C]-pyruvate is employed [2].

A new polarization procedure was developed to obtain [2-13C]-pyruvate in a bio-compatible aqueous (D₂O) solution with a polarization of 5%. To observe the metabolic conversion, the pyruvate solution was added to a lactate dehydrogenase (LDH) solution and pancreatic cancer cells (Panc02), catalyzing the pyruvate to lactate conversion. ¹³C-NMR spectra of hyperpolarized free pyruvate and after adding hyperpolarized pyruvate to LDH solution and Panc02 cell suspension were recorded in the low-field scanner. When adding the hyperpolarized pyruvate to the LDH solution and Panc02 suspension, both pyruvate and lactate could be detected in the low-field scanner, indicating that polarization procedure and hardware of the low-field scanner are capable of detecting the conversion in both enzymatic solution and Panc02 cell suspension.

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Hyperpolarized ^{19}F NMR for multi-channel detection of biomarkers

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Multi-channel ^{19}F NMR is characterized by a wide range of chemical shifts in its spectra, absence of background signal interference, and sensitivity to chemical environments, making it widely applicable in drug metabolism, protein structure and function studies, as well as in the research of fluorinated drugs and ^{19}F -labeled biomolecules. However, the low detection sensitivity of ^{19}F NMR requires high sample concentrations and longer detection times, especially for benchtop NMR spectrometers with low magnetic fields (<80 MHz). The detection time for high-concentration (>20 mM) multi-channel samples using ^{19}F NMR typically requires 3 minutes or more, whereas benchtop NMR is generally inadequate for detecting common biological sample concentrations (<2 mM). Here, we report a synergistic co-ligand strategy based on SABRE hyperpolarization to enhance the detection sensitivity of multi-channel ^{19}F NMR on a benchtop spectrometer (60 MHz). When fluorinated pyridine is mixed with a ^{19}F -labeled substrate, they act as co-ligands to synergistically enhance the signal-to-noise ratio, achieving up to a 5600-fold enhancement factor in ^{19}F NMR signal (about 2.5% polarization) and a detection limit as low as $25\text{ }\mu\text{M}$ (single scan). Furthermore, by combining this method with substrate concentration adjustment, we achieved rapid and highly sensitive quantitative detection of three enzyme-responsive ^{19}F molecular probes related to liver and kidney damage in vitro. Our work utilizes a co-ligand strategy to achieve simultaneous, rapid, and highly sensitive ^{19}F NMR detection, and this method holds potential application value in medicine, biology, and drug metabolism.



Parahydrogen-induced polarization with solid catalysts – from theory and practice to advanced applications

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NMR signal enhancement in gas-solid and liquid-solid hydrogenations of unsaturated substrates with parahydrogen can be useful to readily produce continuous streams of catalyst-free hyperpolarized substances as well as for advanced operando studies of industrially important heterogeneous catalytic processes, but some important issues need to be resolved. First, heterogeneous catalysts normally do not favor pairwise H₂ addition to substrates. Pairwise selectivity of H₂ addition is thus a highly important characteristics of the catalyst/process which determines the achievable level of NMR signal enhancement. Yet, it turns out that for products with magnetically equivalent nuclei it is systematically underestimated in previous studies (e.g., by ca. 2.2-fold for propane). Through a thorough search we identified more efficient heterogeneous catalysts among intermetallic compounds, immobilized metal complexes, supported liquid phase, etc. A more reliable progress requires the studies of the reaction mechanism - valuable information is provided by accessing S-T₀ mixing in the intermediates, indirectly detecting their NMR spectra, and DFT modeling of physics and chemistry on a catalyst surface.

All these advances bring practical applications closer, with the use of hyperpolarized hydrocarbon gases for lung ventilation imaging being one notable example. Yet, operando NMR/MRI studies of heterogeneous catalysis are obstructed by severe distortions of magnetic field homogeneity. One solution is based on double-quantum 2D NMR of PASADENA-type antiphase signals. Another uses an appropriate structuring of the catalyst particles. As a result, the buildup and decay of PHIP within an operating catalyst layer was visualized for the first time, providing the means for chemical engineering level optimization of a heterogeneous PHIP reactor to efficiently produce hyperpolarized liquids and gases for a variety of applications.

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Revealing aging processes of nanodroplet emulsions with hyperpolarized Xe in different exchange regimes

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Hyperpolarized xenon-129 (hp Xe) in combination with chemical exchange saturation transfer (HyperCEST) reveals even minor spin pools that typically escape conventional NMR detection due to pool size and/or relatively fast exchange. This study of variable Xe exchange in emulsions of nanodroplets with decreasing size shows that HyperCEST can monitor the aging process through different stages that involve exchange rates even outside the typical NMR timescale for compartmentalized systems.

We performed Xe NMR on aqueous emulsions containing antifoaming nanodroplets (AFNDs) by bubbling hp Xe gas into the sample. Dynamic light scattering (DLS) measurements confirmed an aging process in which the mean diameter of the nanodroplets shrinks continuously with the number of applied bubbling cycles. This results from the emulsification of the AFNDs during foam suppression [Bergeron et al., 1997; 10.1016/S0927-7757(96)03774-0]. We initially observed a conventional NMR signal originating from Xe@AFND. While this directly detected signal disappeared over the course of a few bubbling cycles, a second signal associated with the AFNDs and indirectly measured by HyperCEST persisted in the z-spectrum even for several bubbling cycles.

We explain the vanishing of the AFND signal and delayed loss of the HyperCEST signal by a continuously decreasing residence time of Xe in continuously shrinking droplets. The HyperCEST signal, however, is more immune to the associated accelerated Xe exchange.

To further extend the detection to the smallest droplets, we used the Xe host cryptophane-A (CrA), which enters the AFNDs and slows down the Xe exchange by transiently binding Xe. The third signal originating from Xe inside CrA@AFND was detected by HyperCEST even after a large number of bubbling cycles.

Overall, the three detected signals provide evidence of nanodroplets throughout their aging stages and cover a wide range of exchange regimes that would otherwise escape conventional NMR detection.



Targeted DNP for Solid-State NMR Studies of microRNA-mRNA Duplexes by Covalent Spin-Labeling with AsymPol

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Low sensitivity poses a challenge for studying complex biomolecular systems with ssNMR spectroscopy, especially using state-of-the-art approaches with limited sample amounts in native, cellular environments. DNP provides a considerable sensitivity boost and enables otherwise unfeasible detailed investigations. In conventional DNP, a polarizing agent such as AsymPol is added to the sample in a glass-forming matrix and subjected to microwave irradiation under MAS. Built up polarization is transferred to protons in the matrix via cross effect and dispersed uniformly via spin diffusion to enhance NMR signals arising from the entire sample.

We have recently applied this method to a microRNA-mRNA-hAgo2 protein complex to study non-canonical conformations of model RNAs that had eluded other structure elucidation methods, creating new opportunities for understanding microRNA-based gene regulation with atomic precision. With the future aim of moving to larger RNA systems over a background of interfering species in cells, it is desirable to restrict polarization enhancement only to the system of interest for more specific measurements.

We present here a targeted DNP approach using site-specifically AsymPol-tagged Sirt1 mRNA fragments produced via amine-isothiocyanate click reaction paired with site-specifically isotope-labeled microRNA-34a to yield up to 28x ¹³C signal enhancement. Notably, in conventional "DNP juice" the polarization build-up still propagates via spin diffusion and is therefore not localized. Reducing the proton concentration in the environment by 85% mitigates this and results in localized DNP enhancement only in tagged RNA.

This modular approach can be exploited to synthesize targeted DNP enhancement carriers bearing multiple biradical moieties which base-pair to specific target RNAs and is easily extended to other polarizing agents besides AsymPol. It will therefore remain useful for future in-cell experiments even as the field continues to develop.



Recognizing DNA structures using Dissolution Dynamic Nuclear Polarization

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Aims: Dissolution Dynamic Nuclear Polarization (dDNP) application to larger biomolecular systems has thus far been mostly limited to proteins. Employing dDNP to DNA studies has the potential to alleviate the sensitivity drawback of standard NMR. Conveniently, DNA spectrum contains weak imino proton signals that can receive hyperpolarization via water. We aimed at amplifying the weak, structure specific, signals of both canonical and non-canonical DNAs by dDNP.

Methods: We optimized dDNP experiment with hyperpolarized water (hyperW) [1] that was originally designed for proteins to study DNA. The most efficient transfer of hyperpolarization to the molecule of interest is via direct proton exchange between water and labile moieties i.e. amino and imino protons of DNA. Interestingly, imino protons are major indicators of DNA structure [2].

Results: We applied hyperW dDNP approach to several different DNAs showing that hyperW can enhance signal of various DNAs. Imino protons are generally weak signals that only appear in NMR spectra when base-pairs form hydrogen bonds. Our results on DNAs that vary in both the structure and the degree of stability show that hyperW can be applied generally on DNA samples without altering the DNA structure. In addition to the standard buffered solution we achieved signal enhancement in the blood plasma.

Conclusions: We have demonstrated the suitability of hyperW approach on DNA samples that contain different types of structures without the need for specific isotope enrichment. In addition, our approach enabled us to perform the first direct observation of DNA secondary structures in blood plasma. Overall, we present hyperW as a promising technique that can be generally used to boost the signal on various nucleic acid structures.

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Precision chemically modified fullerenes enhance optical nuclear hyperpolarization in random orientations

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Magnetic resonance imaging (MRI) is indispensable for medical diagnostics due to its non-destructive and non-invasive nature. However, its low sensitivity remains a significant challenge. Triplet-DNP using photo-excited triplet electrons enable MRI sensitivity enhancement through nuclear hyperpolarization at moderate temperatures because the high electron spin polarization between triplet levels is independent of temperature. [1]. Pentacene and their derivatives has been used for polarizing agent since first triplet-DNP reported. However, since pentacene has broad ESR spectra, there were a strict requirement to prepare a large single crystal and control its orientation relative to the magnetic field to obtain high nuclear polarization [2]. Furthermore, there is the essential problem that MRI probes such as pyruvate cannot be introduced into single-crystal samples. These factors have made practical applications of triplet-DNP toward the clinical MRI difficult.

We proposed the use of fullerene as a new polarizing agent [3]. Fullerene is promising for polarizing agent because of its delocalized electronic structure shows very sharp ESR spectra even in random orientations. However, fullerene have never used before because of its high symmetric structure induce pseudo-rotation result in short triplet electron spin polarization lifetime (T_{1e}) about few μ s. We revealed that two substituents modification at exact position on fullerene effectively suppress pseudo-rotation and elongate the T_{1e} (87 μ s) while retains sharp ESR spectra. Triplet-DNP with di-substituted fullerenes achieved a first practical level ^1H polarization ratio of 14.2%, which is about 10 times higher than the conventional standard polarizing agent pentacene in random orientations.

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Investigation of exchange processes in SABRE

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SABRE is a hyperpolarization technique that enhances NMR signals by transferring spin order from para-hydrogen to a substrate nuclei without chemical modification. This study investigates the substrate and hydride exchange rates in the SABRE hyperpolarization process at different temperatures ranging from 270K to 320K. We compare the experimental results with the simulations to understand the SABRE exchange dynamics. We also employ chemical exchange saturation transfer (CEST) to reveal short-lived intermediate complexes. Four molecules: pyridine, pyrazine, 3-fluoropyridine, and 3,5-difluoropyridine were analyzed. Substrate exchange rates were measured using Selective Inversion Recovery (SIR) and variable delay CEST (vd-CEST) experiments. Hydride exchange rates were determined through vd-CEST experiments, while Inversion Recovery method (IR) was used to determine T1 relaxation time constants for free and complex-bound substrates. Spin dynamics simulations of a 14-spin system incorporating the hydride protons and two equatorial substrates were performed using an in-house developed Python package. Both the substrate and hydride exchange rates increased with temperature, with fluorinated pyridine derivatives exhibiting higher rates due to electronic and steric effects. However, simulation results indicate that higher substrate exchange rates lead to decrease in the polarization levels, while longer T1 relaxation times in free and complex-bound substrates favor higher polarization levels. SABRE efficiency depends on a balance between substrate exchange rates, polarization transfer dynamics, and relaxation properties. Better understanding of the interplay between these parameters can provide insight into designing improved SABRE systems for enhanced NMR hyperpolarized signals.



Automated PHIP polarizer with large sample volume

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Hyperpolarization helps to boost the NMR sensitivity, enabling real-time in vitro and in vivo molecular spectroscopy and imaging.

Parahydrogen (pH₂) induced polarization (PHIP) is a cost-efficient and fast method for polarizing nuclear spin either by reversible exchange or direct hydrogenation with pH₂. In the absence of an unsaturated precursor, hydrogenating and cleaving a sidearm was shown to be very effective for obtaining the desired, polarized molecule.

Major steps include: (i) hydrogenating the side arm, (ii) transferring polarization to the target nucleus, (iii) cleaving the side arm and purifying the hyperpolarized precursor. This process is called PHIP with side arm hydrogenation (PHIP-SAH).

Most of the research in literature, however, was performed in 5 mm NMR tubes, which constricts the pH₂ flow mechanically, and with very low volumes (0.2 ml). Here, we demonstrate an automated PHIP-SAH polarizer for producing up to 2 mL solutions, sufficient for small animal imaging, based on our previous work.

We designed and constructed custom high-pressure multiport tube caps for standard 5- and 10-mm NMR tubes and 16-mm microwave tubes. The larger tube enabled over 2 mL sample polarization with a sample height of 20 mm, requiring a homogeneous sphere of 2 cm for the polarizer. The system was also automated for fast processing, to counteract the relaxation mechanisms.

We used a 20 mm bore permanent benchtop MRI system as a polarizer with a magnetic field of 0.445 T. This magnet provides a 1H FWHM of 2.492 ppm = 47 Hz for the 2 mL sample and is better for smaller tubes.

The hyperpolarization process was automated and reproducible (30 repetitions): injected volume of the hyperpolarized precursor was (2.215 ± 0.014) mL; the standard deviation of hyperpolarization between shuttlings is 7.7%. Current studies include optimization of SOT, cleavage, and purification of precursor. These studies will pave the way for in-vivo imaging research with a fast and repeatable process.



Exploring a Broad Molecular Pool for PHIPNOE-Driven NMR Signal Enhancement

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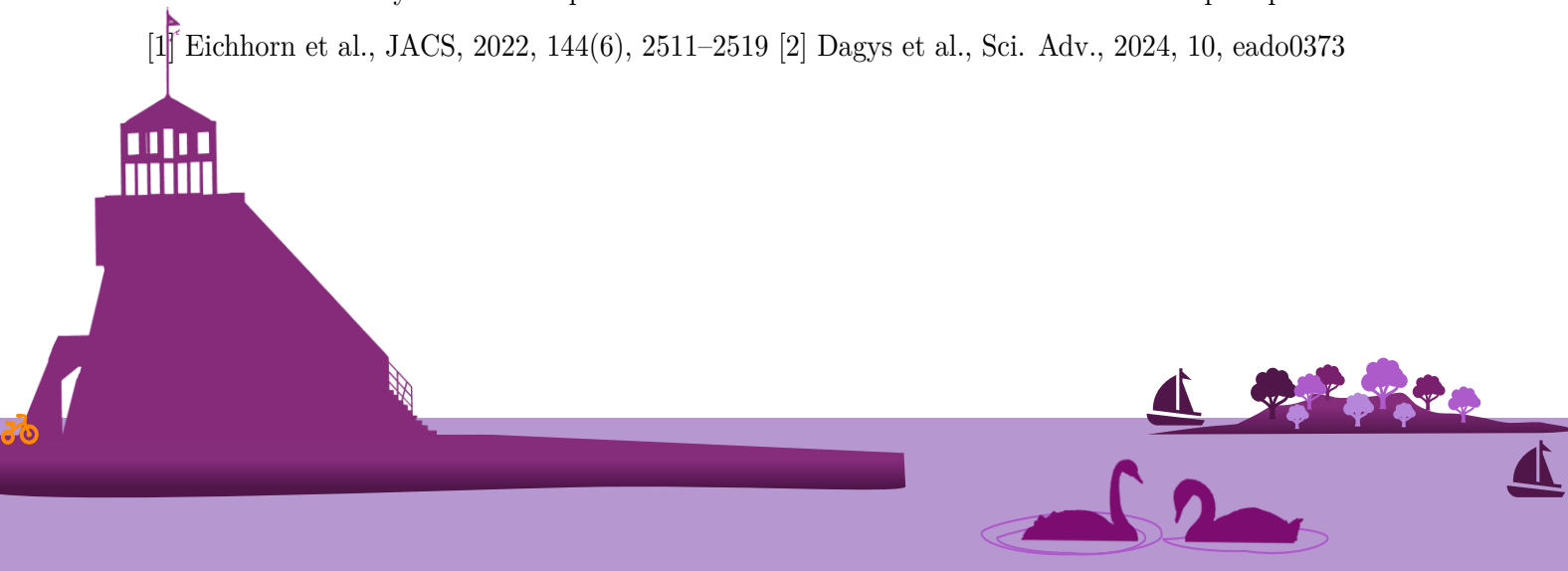
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Despite its widespread applications, NMR suffers from inherently low sensitivity due to the weak interaction between nuclear magnetic dipoles and an external magnetic field. This results in low macroscopic magnetization, as thermal energy dominates over the nuclear spin alignment. Various hyperpolarization techniques have been developed to overcome this limitation. Recently, we demonstrated that highly polarized ^1H nuclear spins on a source molecule—initially, naphthalene from optically polarized pentacene-doped naphthalene crystals—can transfer polarization to a wide range of target molecules in solution [1]. To address scalability challenges, we adopted the Parahydrogen-Induced Polarization (PHIP) method, where substantial polarization is achieved through the transfer of hydrogen singlet order. We refer to this approach as PHIPNOE.

In this study, we systematically investigate the achievable enhancements across a diverse set of molecules, attaining signal enhancements of up to 30 over 400 MHz. We analyze the dependence of polarization efficiency on key parameters, including source molecule polarization, concentration, and relaxation properties. The enhanced polarization can be readily transferred to X-nuclei using conventional cross-polarization techniques. To model the observed enhancements, we propose an extended Solomon equation incorporating diffusion effects, which successfully correlates enhancement levels with molecular mass and T_1 relaxation time.

The PHIPNOE approach required a specialized polarization transfer protocol to mitigate the effects of distant dipolar fields, enabling high molar polarization solutions. While initial implementations relied on continuous wave irradiation [2], we now employ a modified MREV pulse sequence to achieve the same effect more efficiently. We discuss potential refinements to further enhance the technique's performance.

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Optimising SABRE for the detection of weakly binding pharmaceuticals.

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Since the first report of the catalytic process Signal Amplification by Reversible Exchange (SABRE),[1] it has become a powerful tool for enhancing magnetic resonance sensitivity. This technique efficiently transfers polarisation from nuclear spin-aligned parahydrogen to target molecules through a ligand exchange mechanism that is dovetailed to match the spin-spin couplings involved in polarisation transfer. Traditionally, the key SABRE catalyst contains a complex-to-synthesise N-heterocycle carbene ligand whose properties are varied in order to achieve optimal NMR sensitivity.[2] More recently, co-ligands like simple dimethylsulfoxide have been introduced to allow SABRE to polarise molecules like pyruvate.[3] This step also serves to reduce the number of sites available within the metal complex for target substrate binding which means there are fewer magnetisation receptors leading to more efficient polarisation transfer.[4]

This study explores the role of electronic effects in the hyperpolarization of weakly binding 2,5-lutidine and 2,5-dimethylpyrazine using a series of eight diphenylsulfoxide derivatives as co-ligands. By introducing different para-substituents with electron-donating or electron-withdrawing properties, the stability of the catalyst is systematically modulated in a process that can be rationalised through Hammett substituent constants. The Hammett constants prove to directly link to the rate of substrate dissociation, and propagating JHH couplings, leading to a predictable enhancement in hyperpolarization efficiency. The new catalysts for SABRE deliver at least 10% 1H and up to 20% 15N hyperpolarization within seconds, suggesting a new route to successfully detect pharmaceuticals has been created.

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Systematic Evaluation of Polarizing Agents for Dynamic Nuclear Polarization Enhanced NMR at 9.4 T, 14.1 T, and 21.1 T

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Dynamic nuclear polarization (DNP) can significantly enhance the sensitivity of NMR spectroscopy, via the transfer of polarization from unpaired electrons to nuclei. This sensitivity enhancement has successfully opened up applications of magic angle spinning (MAS) DNP solid-state NMR to areas such as nanoparticles, catalysts, batteries, proteins, and pharmaceutical formulations.

Today the development of polarizing agents (PAs) for efficient DNP remains an active research field. With many design principles identified, further optimization of PAs is a complex process that requires fresh perspectives and fine tuning of multiple-factors.

In this work, we evaluate a series of 32 PAs, including 26 dinitroxide and 6 hetero biradicals, of which 11 are introduced for the first time. Their DNP performance are compared at magnetic field strengths of 9.4 T, 14.1 T, and 21.1 T. We find that the narrow-line-broad-line hetero PAs not only yield the best performance at high field (21.1 T and 14.1 T), but remain among the best at intermediate fields (9.4 T). Specifically, SNAPol-1 and HyTEK2 are consistently among the best performing PAs at any field. Furthermore, hetero PAs require only 20% of the microwave power as compared to dinitroxides at 9.4 T, which could be of particular interest when only low power sources are available. Among the dinitroxides, notably at 9.4 T, the AsymPol-POK and M-TinyPol families stand out in proton-dense aqueous matrix, and a newly-developed dinitroxide, NaphPolCbo, provides the best performance in organic solvent.

Overall, the work here clearly highlights the outstanding performance and potential of hetero PAs at all field strengths studied. We anticipate considerable efforts will now be diverted towards the development of new efficient hetero PAs for optimal DNP efficiency.



D-DNP and LLS in the study of amine-CO₂ reaction

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CO₂ is a major greenhouse gas emitted in industry. Post-combustion capture, the most promising industrial technique, involves absorbing CO₂ in an amine solution to form carbamates and then heating the solution to release CO₂ for storage¹. We propose to explore the potential use of developing NMR methodologies, such as dissolution-Dynamic Nuclear Polarization (dDNP)² and the use of long-lived spin states (LLS)³ to help characterize the reaction pathway of CO₂ release from carbamate.

We have performed series of natural abundance ¹³C and ¹H dDNP experiments on a hyperpolarized solution of the reaction medium containing ethanolamine and carbamate obtained from ethanolamine by capture of CO₂. Based on these experiments, we used the ¹³CO₂ signal to potentially monitor the kinetics of the CO₂ release upon dissolution. During the “dissolution” stage of a dDNP experiment, the overheated solvent (water at 180° and ~ 10 bars) is flushed onto the frozen (hyperpolarized) solution in the polarizer, which provokes a quick melting of the polarized sample before it is transferred to the NMR spectrometer for detection. As it turns out, our observations suggest that this process stimulates the desorption of CO₂ and makes it possible to access the kinetics of the reaction.

Alternatively, in dDNP experiments, LLSs involving coupled protons are produced directly by the polarization process, which may be beneficial through the potential sensitivity gain provided by ¹H detection. The use of such LLSs to probe the reaction kinetics of the CO₂ release will be discussed.

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Enhancing Non-Labile Protein NMR Residues through Dissolution Dynamic Nuclear Polarisation of Hyperpolarised Water

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Dissolution dynamic nuclear polarisation (dDNP) has the potential to transform liquid state NMR by boosting sensitivities by orders of magnitude. In dDNP, samples mixed with radicals such as TEMPO are polarised at cryogenic temperatures, then rapidly melted into a diluted solution which is rapidly transferred to an NMR spectrometer for observation.

Enhancements of over three orders of magnitude are readily achievable on small molecules but application to large molecules such as proteins is currently limited by their short T_1 relaxation times. Amide groups in proteins and nucleic acids can be polarised via the exchange of labile protons with hyperpolarised water [1]. Good quality 2D NMR spectra can be acquired in tens of seconds using SOFAST-HMQC experiments [1,2].

The present study explores the possibility of using a hyperpolarised water strategy to polarise non-labile protein protons. Methyl groups are attractive targets to study large proteins. While there is no direct exchange between the non-labile methyl groups and water, surprisingly large enhancements in these signals can still be imparted by the HyperW route. The increases in signal intensity are site-specific, with almost ten-fold improvements in particular signals observed in 2D ^1H - ^{13}C HMQC experiments. The importance of individual methyl sites, and their accessibility, and of protein size, and its folding state, are currently under study.

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Dynamic Nuclear Polarization and Nuclear Spin Diffusion in Nanoscale Si Particles

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We investigate dynamic nuclear polarization (DNP) of ^{29}Si nuclei in silicon (Si) nanoparticles (NPs), comparing spherical Si NPs and irregular porous Si NPs with crystalline cores of 25–50 nm. Spherical Si NPs were synthesized via laser gas-phase synthesis, while PSi NPs were fabricated using low-load metal-assisted catalytic etching of Si wafers with varied doping (boron/phosphorus, from $4 \times 10^{18} \text{ cm}^{-3}$ to nominally undoped). Paramagnetic Pb centers formed at the Si/SiO₂ interface provided unpaired electron spins for DNP.

Hyperpolarization between 3.4 T and 7 T as well as 1.4 K and 3.4 K achieved around 6% bulk ^{29}Si polarization with buildup times of 6–7 h. Room temperature relaxation time of 75 ± 3 min was the longest in lightly boron-doped PSi NPs. The results suggest that microwave-induced triple spin flips (electron-electron-nuclear) initiate a rapid polarization transfer from the Pb electron spin to the central ^{29}Si nucleus due to the large hyperfine coupling of 210–417 MHz. As a competing process, microwave radiation itself induces additional relaxation during DNP[1]. Nuclear spin diffusion within the 25–50 nm crystalline cores is fast and equalizes the polarization within seconds[3]. The rate limiting step in low temperature DNP and room temperature relaxation is then the nuclear polarization transfer across the spin diffusion barrier - a barrier between the strongly hyperfine coupled central ^{29}Si nuclei and ^{29}Si in the bulk[2]. Doping type/density has minimal impact except in highly donor-doped Si beyond the metal-to-insulator transition. Finally, the polarization between the hyperfine coupled ^{29}Si and ^{29}Si in the bulk is discussed using the two-electron two-nuclei spin model[4].

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Understanding signal amplification by reversible exchange through large-scale spin dynamics simulations

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Signal amplification by reversible exchange (SABRE) is a popular hyperpolarization technique where spin order originating from parahydrogen is transferred to a substrate in a temporary organometallic complex. The generated polarization levels are determined by an interplay between spin physics and chemical exchange, which makes the optimization of SABRE challenging.

To advance the understanding of SABRE, we have implemented a Python package tailored for performing versatile spin dynamics simulations in restricted Liouville space. The package offers a detailed picture of the SABRE mechanism by simultaneously accounting for both the coherent and incoherent dynamics as well as the chemical exchange.

We performed spin dynamics simulations on a 14-spin system composed of hydride protons and two equatorial pyridine ligands using the Python package. Comparison of the simulated and experimental polarization levels shows that the SABRE process can be modelled with great precision. Qualitative behaviour of polarization transfer field dependence is reproduced accurately with the simulated polarization levels being comparable to the experimental results.

This work highlights the potential of using computational methods in improving the theoretical understanding and practical applicability of SABRE, enabling quicker advancements in its development.



In vivo Overhauser MRI at ultralow-fields

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Introduction:

Most hyperpolarization (HP) techniques involve the external polarization of a small bolus and its rapid transfer into the MRI scanner. Examples include dissolution dynamic nuclear polarization (dDNP) and parahydrogen-based methods, which have been successfully applied in vivo to study molecular dynamics.

An alternative method is Overhauser MRI (OMRI), which directly hyperpolarizes the sample using Overhauser DNP. By transferring spin order from unpaired electrons of radicals to nuclear spins in its surroundings via RF irradiation at the electron Larmor frequency, OMRI can achieve signal enhancements greater than 100. Although this is lower than dDNP or parahydrogen-based techniques, OMRI allows continuous HP of the sample.

Aims: To develop efficient in vivo imaging techniques optimized for hyperpolarization by OMRI in the ultralow field (ULF) MRI regime.

Methods: OMRI experiments were performed using a SQUID-based ULF MRI system operating between 1 – 50 kHz. Carboxy-PROXYL (CP) free radicals were injected subcutaneously (SC), intraperitoneally (IP) and intravenously (IV) into rats. The sample was continuously rehyperpolarized during phase encoding. A modified balanced steady-state free precession (bSSFP) sequence was used to suppress banding artifacts.

Results: While SC injections enhanced the MR signal near the injection site, no detectable signal was observed for IP injections. Following IV administration, the bladder consistently showed signal enhancement after 10-20 minutes. Continuous IV infusion allowed imaging of the kidneys in addition to the bladder, but the required injection rate varied significantly between experiments.

Conclusions: This study demonstrates the feasibility of in vivo OMRI at ULF. We successfully obtained the first in vivo ULF MRI images of rat kidneys using CP free radicals. However, reproducibility remains a challenge due to the variability between subjects and thus further optimization is needed.



Exploring Complex Enzymatic Reactions and metabolic Pathways of Glutamine and Glucose With Dissolution Dynamic Nuclear Polarisation

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The high signal enhancements brought by dissolution dynamic nuclear polarisation (D-DNP) have made it a promising method to analyse fast enzymatic reactions, making of it a tool of choice to study complex networks of reactions such as the ones appearing in cellular metabolic pathways. Among the metabolites of interest, we focused on glucose and glutamine which play a key role in energetic supply to cells, and can be very desirable targets to detect a deregulated metabolism, such as an increased consumption in cancerous cells [1].

In vitro studies on the pentose phosphate pathway (PPP) of glucose as well as the one leading glutamine to enter the TCA cycle were performed after polarising ^{13}C at 1.2 K and 6.7 T. First, we studied the oxidation of glucose-6-phosphate (G6P) performed in the PPP by the G6PDH enzyme. This enabled a thorough analysis of the robustness of the experimental protocol of our D-DNP experiments and of our fitting method used to monitor and characterise kinetics in real time. In this complex kinetic pathway, the anomerisation of G6P was taken into account and we could provide quantitative evidence of the selectivity of the enzyme for the beta anomer [2].

Then, after sample optimisation to efficiently polarise glutamine using nitroxide radicals, we studied and compared in vitro its conversion to glutamate with glutaminase and L-asparaginase, as well as its consumption by *E. coli* lysates and lymphoma cell extracts [3]. Finally, we developed a sample formulation combining both metabolites, using glucose both as a glassing agent and a potential metabolic probe. It has enabled to obtain competitive polarisation levels ($> 70\%$ for ^1H and $>30\%$ for ^{13}C), and has been successfully tested on the conversion of glutamine by glutaminase, and then also on live *E. coli* bacteria and lymphoma cells, paving the way to co-polarisation of glutamine and glucose and simultaneous observation of their metabolism.



Unperturbed Direct Detection of [1-¹³C]Pyruvate and [1-¹⁵N]Acetonitrile Hyperpolarised by SABRE-SHEATH

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Attaining sufficient SNR has been a consistent challenge that has defined the methodologies in common practice for nuclear magnetism-based techniques. In NMR, nuclei align along the B_0 holding field, generating their own magnetic field B_1 along it. However, due to poor SNR, B_1 cannot normally be directly detected. It is only after a system perturbation that B_1 can be detected. Hyperpolarisation techniques offer an opportunity to enable the unperturbed direct detection of B_1 by increasing the strength of B_1 by orders of magnitude. Here, we present how the use of hyperpolarisation by SABRE-SHEATH enables the unperturbed direct detection of both [1-¹³C]pyruvate and [1-¹⁵N]acetonitrile. Unperturbed direct detection through both hyperpolarisation and spin-lattice relaxation was performed using an ultrasensitive SQUID setup within a moderately shielded magnetically shielded room. Buildup of polarisation was characterised by acquisition during bubbling of pH_2 gas into the sample within a holding field on the order of 100s~nT, while spin-lattice relaxation was characterised similarly, while no pH_2 gas was bubbling into the sample. For [1-¹³C]pyruvate, the characterisation of the spin-lattice constant was shown to be consistent with prior NMR work. Additionally, we show how unperturbed direct detection presents itself as an attractive technique for probing hyperpolarisation complexes via observing variation in polarisation buildup as a function of the hyperpolarisation field B_{hyp} . These results are in agreement with prior work probing hyperpolarisation complexes by varying temperature. With the recent expansion of SABRE techniques to in vivo studies, unperturbed direct detection presents an attractive methodology to characterise both hyperpolarisation and spin-lattice relaxation to enable the future use of SABRE for clinical studies.



Hyperpolarized Kinetics Enabled By Microfluidic NMR

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Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for monitoring chemical reaction kinetics. However, its effectiveness is often restricted by the inherently low sensitivity of NMR. To address this limitation, hyperpolarization techniques such as parahydrogen-induced polarization (PHIP) have been developed which can boost NMR signals by several orders of magnitude, enabling the observation of rare species. However, chemical kinetic studies using hyperpolarized agents are complicated by the non-equilibrium nature of the spin states involved, and quantification of the enhanced NMR signals remains challenging. Unlike in traditional NMR, there is not a direct correlation between integral area and analyte concentration; many further factors, such as chemical reactivity and relaxation properties of intermediates need to be considered. In many cases not all these factors can be known a priori.

Herein we report a microfluidic system for parahydrogen-induced polarization (PHIP) that operates under continuous flow, and allows switching of reactant concentrations without altering the conditions and timing of the hyperpolarization process. We demonstrate a system where flow rates for both the reactant and hyperpolarized solution are controlled, in addition to variables such as parahydrogen pressure and employing in situ valving to initiate or halt reactions as needed. This device enables the separation of nuclear spin relaxation from chemical kinetics and that microfluidic implementation of PHIP turns hyperpolarized NMR into a reliable tool for chemical kinetics.

We report the implementation of microfluidic side arm hydrogenated PHIP (PHIP-SAH) and cleavage of a hyperpolarized species in situ within the microfluidic device.



Indirect Zero Field NMR Spectroscopy

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Two-dimensional NMR spectroscopy is a hallmark of modern NMR enabling peak assignment in otherwise crowded 1D NMR spectra. 2D NMR also provides more detailed information on molecular connectivity and dynamics. Low-field and zero-field NMR can also benefit tremendously from 2D approaches. Previous work on 2D zero-field NMR was performed at two different magnetic field strengths for signal evolution and detection to resolve complex spectra. However, this approach requires the sample to be physically moved between magnetic fields.

In contrast this study demonstrates in situ two-field correlation spectroscopy (COSY) of hyperpolarized molecules in the zero-to-ultralow-field (ZULF) regime without sample shuttling. The experiments were performed with a superconducting quantum interference device (SQUID)-based system inside a three-layer shielded chamber. Continuous signal amplification by reversible exchange (SABRE) hyperpolarization was used to increase the sensitivity at ZULF. A reaction chamber housed the sample and allowed the supply of parahydrogen for SABRE at pressures up to 10 bar.

ZULF spectra and two-field COSY spectra of $[1-^{13}\text{C}]$ pyruvate, $[3-^{19}\text{F}]$ pyridine, and $[^{15}\text{N}]$ acetonitrile were acquired at three different magnetic field regimes: the J-coupling dominant regime (< 1 nT), an intermediate coupling regime (~ 25 nT) and the Zeeman regime (> 0.5 μT).

Two-field COSY proved to be an excellent alternative to direct zero-field NMR with significant advantages. First, the 2D approach directly reports on the J-connectivity. Second, more sensitive Faraday coils can be used in contrast to the typically used magnetometers. Third, the near-DC frequency noise can be avoided by detecting at slightly elevated magnetic field, which is particularly useful for pyruvate with $J = 1.25$ Hz only. In conclusion, hyperpolarization and two-field COSY provide broader access to strongly coupled zero-field regimes as an alternative to total correlation spectroscopy (TOCSY).



Steady-state photo-CIDNP experiments - theoretical studies and applications

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Hyperpolarization techniques, such as photochemically induced dynamic nuclear polarization (photo-CIDNP), can overcome the inherently low sensitivity of Nuclear Magnetic Resonance (NMR) spectroscopy. While steady-state photo-CIDNP experiments with low laser power and prolonged irradiation times offer practical advantages, they are more complex to quantify than time-resolved experiments, which can be directly correlated to hyperfine interactions. The prolonged irradiation in steady-state experiments affects the gained hyperpolarization due to relaxation processes, F-pair contributions, and polarization transfer. Additionally, the suitability of individual molecules for photo-CIDNP experiments must be experimentally verified, as their performance strongly depends on the interaction between the molecule and the dye, and this search typically requires laborious screening efforts.

Here, we report progress towards a semi-quantitative and quantitative prediction of the photo-CIDNP enhancement in steady-state experiments. We calculate molecular properties relevant for the photo-CIDNP mechanism and employ machine learning to predict from them the signal enhancement in steady-state experiments. Such predictions can accelerate the identification of highly photo-CIDNP-active molecules by in-silico screening. Secondly, we report progress in methods development of photo-CIDNP for diagnostics. Our data show that quantification in biological samples remains challenging due to strong matrix effects caused by triplet quenchers, competing photo-CIDNP-active species, and other factors. We are developing strategies to mitigate these effects towards potential diagnostic applications of photo-CIDNP in high and low magnetic fields.



Molecular dynamics simulation study of spin exchange optical pumping in Xe/Rb complexes

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Hyperpolarization techniques enable us to enhance signal intensities in NMR measurements by increasing the spin polarization of target nuclei beyond their thermal equilibrium values [1]. Hyperpolarized xenon (HP-Xe) is widely used in applications such as pulmonary MRI [2] and materials science [3]. Spin-exchange optical pumping (SEOP) [4] is the primary method to produce large volumes of HP-Xe. In this process, the spin polarization of the rubidium electron is transferred to the nuclear spin of xenon through collision-mediated interactions. The dominant contribution to this polarization transfer arises from long-lived Rb–Xe van der Waals (VDW) complexes [5]. Recently, Rantaharju et al. provided the first computational insight into these complexes [6]. The lifetime and stability of these complexes may depend on factors such as external pressure, temperature, gas composition, and number density. Furthermore, the presence of other species such as N₂ and He may also influence the formation and behavior of the complex. In this work, we employ high-accuracy molecular dynamics simulations to investigate the formation and stability of Rb–Xe VDW complexes. We carry out high-throughput calculations to identify the experimental conditions that maximize the spin transfer efficiency to xenon nuclei.

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Halogen bond mediated ^{13}C Overhauser DNP at 9.4 Tesla

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Liquid-state high-resolution ^{13}C NMR is a powerful analytical technique but often encounters sensitivity issue due to low gyromagnetic ratio and natural abundance of ^{13}C . Overhauser-effect dynamic nuclear polarization (OE-DNP) can theoretically boost ^{13}C NMR signal intensity by 103-fold while allowing repeat sample hyperpolarization. [1] We recently reported a 9.4 T liquid-state OE-DNP setup, which allows 3 to 10^2 -fold ^{13}C enhancements for small molecules in 1D and 2D experiments without significant loss of NMR resolution. [2]

^{13}C OE-DNP enhancement depends on chemical functionality. To date, rational design of systems with large ^{13}C enhancements still remains a challenge. Previous observations indicated polarizing agent-analyte halogen bond facilitating ^{13}C OE-DNP for halogenated carbons. [2-4] However, how halogen bond properties, such as bond strength and geometries, influence OE-DNP were not well understood.

Enabled by the aforementioned setup, we investigated ^{13}C OE-DNP of a series of halogenated model compounds with 4-oxo-TEMPO radical as the polarizing agent. [5] We showed up to 10-fold boost in the enhancement of halogenated ^{13}C by adjusting the chemical functionalization of the analytes. Computational analyses supported halogen bond-mediated polarizing agent-to-analyte spin polarization transfer, which induces isotropic hyperfine interaction crucial for scalar-driven ^{13}C OE-DNP. We further explored possible influence of halogen bond on OE-DNP dynamics. Our results provide a potential approach for broadening the application of OE-DNP, particularly in designing hyperpolarized molecular probes.

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Indirect Measurement and Hole burning of Proton Spectrum via Electron Detection

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In a Dynamic Nuclear Polarization (DNP) experiment the much larger polarization of the electron spin is transferred to the nuclei of interest to increase the sensitivity of a nuclear magnetic resonance (NMR) measurement [1]. As shown recently [2], the reverse polarization- transfer step is also possible and polarization can be transferred from the nuclei to the electrons. Using reverse DNP, one can selectively observe the nuclear spins close to the electron spin. Those nuclei play an important role for the initial polarization transfer in DNP experiments. However, the spectral detuning of those spins due to the large hyperfine coupling and the fast relaxation of nuclei near an electron spin makes direct detection via regular NMR experiments difficult.

We have characterized the nuclear spins (protons) involved in the DNP process with an extension of the reverse DNP scheme using a band-selective inversion pulse on the nuclear spins. We were able to record an electron-detected proton NMR spectrum with a full width at half maximum (FWHM) of almost 600 kHz. With the inclusion of a second band-selective pulse on the nuclear spin we managed to burn a hole into the NMR spectrum. Increasing the time shift between the two band-selective pulses revealed a spin diffusion process. We carried out our experiments using OX063 Trityl radical at X-band (0.35 T) and temperature of 80 K using three different matrices, glycerol-d8:D2O:H2O, (v/v 6:3:1), glycerol-d8:D2O, (v/v 6:4) and glycerol- d8:H2O, (v/v 6:4).

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SABRE-enhanced metabolites in urine extracts

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Metabolomics is a powerful technique to investigate biological processes, based on metabolic marker concentrations in tissue, biofluids and derivatives. Nuclear Magnetic Resonance (NMR) spectroscopy is one of the primary analytical methods for metabolomics studies, but is hindered by its low sensitivity. This shortcoming can be overcome by employing hyperpolarization techniques.

Hyperpolarization allows for significant enhancements in signal amplitude. A source of spin alignment is used to generate a non-equilibrium population distribution of nuclear spin states. This boosts sensitivity, which in turn reduces the number of acquisitions necessary to generate quantifiable spectra. We propose hyperpolarization employing Signal Amplification By Reversible Exchange (SABRE). This method is based on parahydrogen, a spin isomer of molecular hydrogen.

SABRE does not involve chemical modification of the target molecules. Instead, polarization is generated by both parahydrogen and a target molecule reversibly binding to a homogeneous metal complex, which facilitates the transfer of spin alignment. Polarization buildup does not require a high magnetic field, which enables the use of benchtop NMR spectrometers for acquisition. This transfer step also introduces a layer of chemoselectivity, which can further aid analysis.

Chemoselective processes are able to differentiate between chemical compounds based on their structural motifs. For SABRE, this differentiation is based on exchange dynamics at the coordination site. Thus, markers of interest can be hyperpolarized, while background signals remain close to the noise level.

Our preliminary results demonstrate SABRE in a solid-phase extract of urine. SABRE experiments were performed on a 60 MHz benchtop spectrometer and compared to spectra acquired using a 500 MHz spectrometer. This paves the way for further analyses using SABRE in complex matrices, with applications in metabolomics and other disciplines.



Towards High Molar Polarization for Nuclear Magnetic Resonance Applications

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Hyperpolarization techniques like SABRE (Signal Amplification By Reversible Exchange) have dramatically enhanced NMR sensitivity, yet the quest for high polarization of molecules present ideally at high concentrations — such as solvent molecules — remains a major challenge. Our work demonstrates the critical influence of solvent polarity and substrate identity on the activation of SABRE polarization transfer catalysts. Building on these insights, we develop a reliable protocol for long-term SABRE hyperpolarization of acetonitrile solvent, supported by benzylamine coligand. The resulting molar polarization can be maintained for several hours under continuous parahydrogen bubbling. Our results provide new perspectives on spin-transfer catalysis and mark an important step towards scalable hyperpolarization.



DNP-enhanced MAS NMR of paramagnetic frozen solutions

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In solid-state NMR spectroscopy, dynamic nuclear polarization (DNP) is one of the most prominent methods for generating hyperpolarization in order to enhance sensitivity. DNP is based on the transfer of polarization from the electron spin of a paramagnetic polarization agent (PA) to nuclear spin via hyperfine interaction (HFI). However, paramagnetic relaxation enhancement (PRE) induced by the PA often leads to comprised spectral resolution due to signal quenching of the nuclei close to the PA, giving rise to the occurrence of a ‘blind sphere’ around the PA. Yet, no quantitative model of paramagnetic relaxation under cryogenic conditions relevant for MAS DNP exists.

In order to develop strategies for mitigation of this effect, we want to gain a deeper understanding of paramagnetic relaxation under the conditions typically encountered during MAS DNP NMR. To this end, we investigated the influence that the addition of paramagnetic salts (additives) has on DNP parameters. We performed MAS DNP measurements of standard DNP formulations (1 M ^{13}C -urea in 60:30:10 d_8 -glycerol/ H_2O / D_2O with 10 mM AMUPol as PA), to which we added various paramagnetic salts in varying concentrations. Here, we find that the paramagnetic additives lead to a lower steady-state signal enhancement, while the polarization build-up rate was increased. The strength of this effect depends on the spin quantum number S of the additive as well as on its concentration, with the tendency of lower spin quantum numbers and higher concentrations leading to a stronger effect. For some additives, also the effect of peak broadening has been evaluated.

Our results will aid the development of a quantitative model of paramagnetic relaxation under MAS DNP conditions which is crucial for further developments for site-specific DNP. Furthermore, it provides important information for MAS DNP of paramagnetic (biological) materials, such as heme-containing tissue or blood.



Quantum J-oscillators improve spectral resolution for zero-field NMR

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Nuclear magnetic resonance (NMR) lasers generate coherent radiofrequency signals via hyperpolarization-driven population inversion. They have applications in quantum sensing, chemical analysis, and magnetic resonance imaging. However, their reliance on Zeeman-split energy levels makes them susceptible to magnetic field fluctuations, limiting long-term frequency stability and spectral resolution. Furthermore, achieving the necessary population inversion for operation can be experimentally demanding. To overcome these limitations, we introduce quantum magnetic J-oscillators that operate at zero magnetic field by exploiting intrinsic nuclear spin-spin J-coupling transitions, achieved by coupling in situ SABRE-hyperpolarized samples to a programmable digital feedback system that digitizes, delays, and amplifies the sample-generated signal before feeding it back. Due to the J-coupling's insensitivity to magnetic field drifts, we achieved coherent J-oscillations lasting over 3000 s, with a linewidth of 337 μHz limited primarily by acquisition time, achieving the Cramér-Rao lower bound in frequency measurement errors. The ability to control the feedback delay and gain enabled us to improve the spectral resolution, making possible on-demand spectral editing. Crucially, the active applied feedback gain relaxes the need for population inversion and lowers the polarization requirements, enabling operation even in samples present with natural isotopic abundance. Application of quantum oscillators was demonstrated on a diverse range of molecules (nitriles, heterocycles, organic acids). The J-oscillators produce highly resolved, sharp spectra, reveal near-DC transitions, and may allow distinction of complex mixtures that are difficult to resolve in conventional zero-field NMR. As a result, this approach would potentially expand the scope of zero-field NMR for analytical chemistry, biomolecular characterization, and fundamental physics.



Optically Generated Hyperpolarisation in Solution-State Magnetic Resonance

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A newly developed approach to solution-state dynamic nuclear polarisation (DNP) uses optical illumination instead of microwave pumping. Upon illumination of a suitable dye, a long-lived triplet state is formed, which can undergo spin-selective quenching with radicals in solution to generate dynamic electron polarisation on the radical via the radical triplet pair mechanism (RTPM): i) the triplet dye and radical can diffuse together to form a doublet-quartet pair in an encounter complex, ii) this encounter complex lives long enough to generate substantial polarisation of the radical's electronic transitions due to spin-allowed relaxation from the doublet manifold. Subsequent cross-relaxation of the radical with nearby protons produces in-situ nuclear polarisation. Persistent radicals, such as TEMPO, can be used in the RTPM, avoiding any competitive kinetic decay processes that affect other mechanisms of generating chemically induced dynamic electron polarisation (CIDEP).

The work presented applies a method of rapid mechanical shuttling of NMR samples on a benchtop spectrometer from low to high magnetic field strengths, to generate sensitivity enhancements in solution state NMR using the RTPM. Illumination of the sample occurs at low-field where the RTPM and subsequent cross-relaxation is most efficient, with sample transfer to higher field for improved chemical shift dispersion. Sample transfer occurs on the sub-second timescale, minimising any nuclear relaxation. We present our findings on DNP substrate enhancements, the effect of different chemical systems on the enhancements, and the optimal magnetic field for polarisation in the shuttle system. Finally, we present time-resolved EPR data which gives an insight into a salt effect on the RTPM, first explored in DNP studies.



SABRE Hyperpolarisation of pyruvate in non-alcoholic solution.

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SABRE[1] can hyperpolarise pyruvate in a fast, low cost, and reversible fashion that does not involve technologically demanding equipment compared to comparable techniques[2]. Most SABRE hyperpolarization studies have been done using methanol-d₄ as a solvent, which is not suitable for in vivo application[3,4]. The main goal of this work was to obtain hyperpolarized pyruvate in a solvent other than methanol and study the opportunity to obtain hyperpolarized singlet order in [1,2 - ¹³C₂]pyruvate, as the use of long-lived states in NMR spectroscopy and imaging presents many opportunities, such as hyperpolarised molecular imaging.

This work has shown hyperpolarization of [1-¹³C]-, [2-¹³C]- and [1,2 - ¹³C₂]-pyruvate by SABRE in non-alcoholic solutions as an alternative to methanol at room temperature with detection of NMR signals using a 1.1T benchtop NMR spectrometer. Upon transfer into a benchtop magnet, hyperpolarised ¹³C resonance corresponding to a free pyruvate was observed.

In this work we have investigated the effect of different catalyst concentration and of DMSO presence as a co-solvent on the signal enhancement. We studied SE dependence for singlet order of [1,2 - ¹³C₂]-pyruvate from the magnetic field where bubbling of p-H₂ took place.

For developing future purification methods a study of the relaxation times for ¹³C enriched pyruvates has been done for various type of solutions used for hyperpolarized experiments. The field dependence of LLS time constant was measured. The influence of the presence of paramagnetic oxygen in non-degassed sample to a ¹³C singlet lifetime of [1,2-¹³C₂]-pyruvate was investigated as well.

Our work opens new possibilities for obtaining an aqueous solution of pyruvate for potential in vivo applications.

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Probing Hydrogen Activation in a Nitrogenase Model Complex by Symmetric Exchange with Parahydrogen

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This work investigates symmetric hydrogen exchange between a dinickel(II) dihydride complex (1), mimicking nitrogenase enzymes, and parahydrogen (pH₂) to generate hyperpolarization in NMR. Despite mirror symmetry, the dihydride complex shows enhanced longitudinal magnetization, explained by a novel nuclear relaxation interference mechanism. Notably, initial symmetry breaking is also attributed to nuclear relaxation dynamics, eliminating the need for an asymmetric intermediate (2). Advanced parahydrogen-NMR techniques and simulations support these findings. Polarization persists upon dihydrogen elimination, resulting in an enhanced hydrogen resonance. This study helps to understand the mechanism of hydrogen activation in this complex and provides unique insights into hyperpolarization in symmetric systems, broadening PHIP applicability. By examining a bioinspired system, we aim to extend our methodology to native enzymes (3). This work may facilitate comprehensive investigations of hydrogenase and nitrogenase enzymes, aiding in the development of more efficient hydrogen-activating catalysts.

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On the water tolerance of ansa-aminoboranes and PHIP

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Hyperpolarization is an effective tool for signal enhancement in NMR. One such technique is parahydrogen-induced polarization (PHIP). This method is based on chemical reactions of parahydrogen that can lead to hyperpolarized substances. Understanding correlations between the chemical processes and NMR signal enhancement allows for improving PHIP experiments. In this study we examine water tolerance of metal-free ansa-aminoboranes (AABs) — molecules that can react with parahydrogen (para-H₂). AABs are considered as nitrogen-boron frustrated Lewis pairs (FLPs). This moiety plays a crucial role in the reaction of AABs with para-H₂, which results in NMR signal enhancement of the corresponding AAB-H₂ adducts [1]. At the same time, this moiety is typically water-intolerant. To investigate water tolerance of AABs and the mechanism behind para-H₂ activation, a series of NMR experiments were performed. Namely, ¹H NMR measurements were conducted for AABs with two different chemical groups at the boron site: mesityl and phenyl. The experiments were performed at various temperatures with a significant excess of water in toluene-d₈ and acetonitrile-d₃. The experiments were conducted with para-H₂ and normal H₂. Our results show a pronounced effect of moisture on the hyperpolarization effects. For the AAB with mesityl groups, strong signal enhancements were observed. In the case of AAB with phenyl groups, hyperpolarized signals were not observed due to the irreversible formation of the AAB adduct with water. Through understanding these effects, we obtained valuable information on the para-H₂ activation using AABs and the potential design of water-tolerant FLPs [2]

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Reactor designs for SABRE SHEATH and their influence on the polarization level

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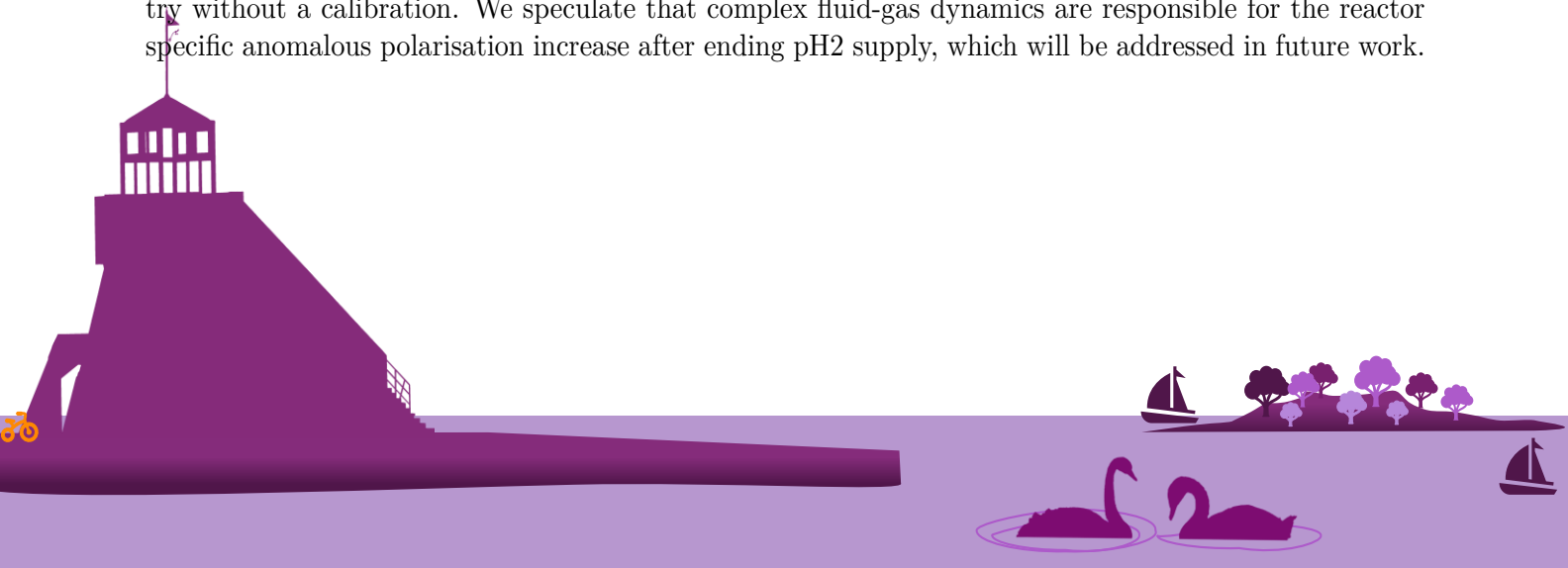
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Aim: Parahydrogen-induced polarization can be used to boost the signal-to-noise ratio of NMR. The variant SABRE-SHEATH enables continuous generation of polarization on the selected substrate that transiently interacts with constantly replenished parahydrogen (pH₂). The SABRE process typically involves bubbling pH₂ for a fixed time through a reactor containing the liquid sample. This consists of the target substrate and a catalyst, typically [IrMesCOD]Cl, dissolved in methanol. Creating a defined and reproducible hyperpolarization reactor is a critical step toward the reliable and quantitative use of SABRE.

Methods: We present a comparison of two reactor designs used for SABRE-SHEATH experiments. In situ observation at ultra-low magnetic field was achieved using a SQUID-based detection scheme with a non-adiabatic field switch. Reactor I has a sample volume of ~3.1 ml and is 3D printed using a stereo lithograph apparatus. It features a Kinecs mixer to generate turbulence between pH₂ and the sample and was operated at 5 and 20°C. Reactor II, with a sample volume of ~2.0 ml, is built from PEEK and allows temperature control from -10 to 40°C. Polarization levels were quantified by simulating the measurement setup.

Results: Polarization levels of ~0.5 % were achieved in [1-¹³C]pyruvate for both reactors but anomalous behaviour was also found. Reactor I exhibited a significant polarization increase after pH₂ delivery was stopped and a delay prior to detection was introduced. This effect was most pronounced for a delay of 10 s, where an increase in polarization by factor 2 was observed. Reactor II showed a maximum after ~2.5 s with a much less pronounced increase of ~30 %.

Conclusion: The accurate geometry of the reactors and the SQUID-based detection setup allow polarimetry without a calibration. We speculate that complex fluid-gas dynamics are responsible for the reactor specific anomalous polarisation increase after ending pH₂ supply, which will be addressed in future work.



Enabling Dynamic Nuclear Polarization Enhanced Fluorine Solid-State NMR by a Spin-Diffusion Booster

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Solid-state NMR under MAS is an essential method in structural biology. Given the inherently low sensitivity of NMR, hyperpolarization techniques are often sought to enable otherwise unfeasible experiments. The most promising method for solid-state MAS NMR is dynamic nuclear polarization (DNP).[1]

A notable trend in the investigation of biological systems is the use of fluorine spin-tags.[2] Fluorine has the inherent advantage that it does not occur naturally in biological systems, and its high gyromagnetic ratio and natural abundance make it an easily accessible nucleus. Moreover, fluorine is commonly used in active pharmaceutical ingredients. Despite these benefits, the sensitivity issue persists, making the extension to fluorine DNP a promising endeavor. DNP on fluorine was first shown in 2019 by Emsley et al.[3] and Polenova et al.,[4] and although it has great potential, it is currently constrained by the limited availability of DNP probes capable of ^{19}F irradiation.

In this investigation, a methodology for measuring direct and indirect fluorine DNP is proposed without the need for a specialized ^{19}F DNP probe. The standard glycerol- d_8 and H_2O DNP matrix is modified by adding 1,3-Difluoro-2-propanol as a spin-diffusion booster. To this end, the fluorine atom concentration is increased, resulting in a dense fluorine network that facilitates hyperpolarization propagation. We demonstrate the efficacy of this approach on a simple model compound, sodium 2,2,2-trifluoroacetate, by showing its potential to enhance the sensitivity of direct fluorine DNP and indirect carbon DNP through fluorine. This may serve as a foundation for its application in biochemical systems, thereby increasing the relevance of fluorine DNP.

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TEMPOL-induced solid-state ^1H relaxation in DNP samples

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The radicals used to hyperpolarize nuclear spins in dissolution dynamic nuclear polarization (dDNP) also cause nuclear relaxation, such that the sample composition needs to be carefully balanced to achieve fast hyperpolarization build-up and only limited subsequent relaxation before measurement [1]. Optimized recipes for dDNP have so far been reported for various combinations of target molecule and radical, but do not apply for bullet-DNP [2], where the transfer conditions differ dramatically.

We report fast-field-cycling measurements of proton relaxation at low temperature (5-40 K) and field (0.01-2 T) for a standard DNP-sample (0.5 M acetate in $\text{H}_2\text{O}/\text{D}_2\text{O}$ /glycerol- d_8 (1:3:6)) that corroborate the difference in optimal TEMPOL-concentration for bullet-DNP compared to standard dDNP.

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High H₂ Solubility of Perfluorocarbon Solvents and Their Use in Reversible Polarization Transfer from para-Hydrogen

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First pioneered by Duckett et. al. in 2009,(1) Signal Amplification by Reversible Exchange (SABRE) is a hyperpolarization technique which uses the polarization potential of parahydrogen (pH₂) to realise a major sensitivity increase for NMR detection. A critical limitation of SABRE is the low pH₂ solubility under standard SABRE conditions of ca. 4 bar pH₂ when using methanol-d₄ as a solvent.(2) In this work we show how the emerging use of perfluorinated solvents for SABRE,(3) can improve the SABRE efficiency while remaining at the typical 4 bar pressure. By measuring the Henry's constants of the solvents used, we show that the H₂ solubility can be an order of magnitude higher in these perfluorinated solvents (59.3 ± 5.0 mmol L⁻¹bar⁻¹ for perfluorooctane) than typical organic solvents used (5.6 ± 5.0 mmol L⁻¹bar⁻¹ for methanol-d₄). We demonstrate how this high H₂ solubility can deliver substantial improvement in polarization efficiency, however, it is limited by catalyst solubility in the perfluorinated solvents. Up to 67,000-fold signal gains (22% polarization) were achieved for the pyridyl ¹⁵N in nicotine when using the solvent methylperfluorobutylether, a comparative sample in methanol achieved only 3.8% polarization.(4)

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Applying parahydrogen hyperpolarization to horticulture research: nhPHIP on affordable instrumentation

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There are limited opportunities to use high-cost equipment in horticulture, making detailed biochemical analyses difficult. This can sometimes limit access to advanced analytical techniques like very high field NMR. Therefore, this study was done to explore whether metabolic analysis of plant material can be carried out on more affordable NMR spectrometers, while still achieving sufficient sensitivity and resolution to measure proline in its biological matrix and to identify its relationship with plant stress. Achieving this goal required developing and adopting hyperpolarized NMR methodology to make such analyses feasible. As part of this work, we conducted a pilot study using raspberries, a crop widely grown in colder climates, and developed an parahydrogen hyperpolarized NMR method to measure proline in raspberry bud tissue. Using nh-PHIP hyperpolarization and optimizing experimental conditions, we successfully quantified proline concentrations, employing sarcosine as a standard reference for calibration.

This work demonstrates that non-hydrogenative Parahydrogen-Induced Polarization (nh-PHIP) can significantly enhance NMR signal strength in plant metabolomics studies, enabling the detection and resolution of small molecules such as amino acids in a biological background matrix even on lower-field spectrometers (300 MHz). Achieving this involved developing adequate raspberry bud extraction and sample preparation methods, assuring compatibility of these extracts with nh-PHIP, achieving reproducibility of nh-PHIP detection and establishing a calibration for proline quantification. This work is the first larger scale metabolomics study by hyperpolarized NMR, involving a study cohort of 40 distinct biological specimens. Overall, our work shows that PHIP removes previous limitations of NMR in horti- and agricultural applications caused by low sensitivity and high cost, making it possible to use even affordable machines for a wide range of applications.



Detection of D- and L-amino acids in pancreatic tissue

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The detection of D-amino acids in biological tissue is of increasing interest due to their emerging role as potential biomarkers in neurodegenerative diseases and cancer [1]. However, standard nuclear magnetic resonance (NMR) spectroscopy lacks the sensitivity and enantiomer discrimination required to detect these low concentration chiral metabolites. Therefore, there is a clear need for a sensitive and reliable method to detect and differentiate between D- and L-amino acids in biological samples.

To address this challenge, we adopted the nhPHIP (non-hydrogenative parahydrogen-induced polarization) method introduced by Tessari et al, a hyperpolarization technique that can increase NMR sensitivity by up to 1000 fold and allows enantiometric discrimination of amino acids [2]. nhPHIP enables the detection of even submicromolar concentrations of amino acids and distinguishes between both D- and L-forms.

We optimized the extraction and measurement protocol using pancreatic tissue from a one-year-old sheep. Out of several extraction mixtures, methanol-water extraction provided the highest yield and reproducibility. After optimization of the conditions, human postoperative pancreatic cancer tissue samples were analyzed. The D- and L- amino acid levels in cancerous and proximal healthy pancreatic tissues were assessed.

These results demonstrate that nhPHIP-enhanced NMR is a powerful tool for studying amino acid chirality in biological tissues and provides a foundation for future diagnostic applications.

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^{13}C Hyperpolarization of acetoacetate using PHIP-SAH

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Nuclear hyperpolarization (HP) techniques dramatically increase signal-to-noise ratio in magnetic resonance. The combination of heteronuclear MRSI with administration of HP contrast agents opens the doors to 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 (pH_2) as a source of non-equilibrium polarization. Recently, PHIP by side arm hydrogenation (PHIP-SAH) approach was proposed, which expands the capabilities of PHIP technique for obtaining HP carboxylates in a biocompatible form. One of the prospective biomolecules is acetoacetate (AcAc) which converts to 3-hydroxybutyrate in vivo, and kinetics of this reaction is associated with mitochondria redox status. Here, we propose the PHIP-SAH based procedure for hyperpolarization of AcAc.

[1,3- $^{13}\text{C}_2$]Allylacetate ($^{13}\text{C}_2\text{-alAcAc}$) was produced in hydrogenation of the corresponding propargylic precursor using pH_2 . Under optimized experimental conditions, $^{13}\text{C}_2\text{-alAcAc}$ (32 mM) was produced with 100% efficiency and $P(^1\text{H}) \approx 24\%$ was achieved. The ^1H -to- ^{13}C polarization transfer was performed using the magnetic field cycling approach, and the highest observed ^{13}C polarization levels (maximizing at 150 nT polarization transfer field) were estimated as 3.3% for 1- ^{13}C and 1.7% for 3- ^{13}C atoms. It was found that the mild conditions (150 mM NaHCO_3 , r.t.) typically used for hydrolysis of pyruvate esters are not sufficient to hydrolyze [1,3- $^{13}\text{C}_2$]alAcAc since a stable negatively charged enolate is formed. We demonstrate that ^{13}C hyperpolarization is retained during harsh hydrolysis conditions (1 M NaOH , $T \approx 100^\circ\text{C}$, 30-60 s) and HP [1,3- $^{13}\text{C}_2$]AcAc is produced with $P(^{13}\text{C})$ of $\sim 0.5\%$, although some amount of enolate remains unreacted and, moreover, partial decomposition of acetoacetate into acetone and carbonate is observed.

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Hyperpolarization of Long-Lived States of Protons in Aliphatic Chains by Bullet Dynamic Nuclear Polarization

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Long-lived States (LLS) of protons in short aliphatic chains have lifetimes up to seven times longer than longitudinal relaxation times T_1 . By virtue of their remarkable sensitivity to their surrounding environment, new perspectives unfold in the interest of high-throughput parallel drug screening 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.

Here, Bullet Dynamic Nuclear Polarization (B-DNP), which amounts to the rapid transfer of frozen solids from a polarizer designed for DNP to an NMR spectrometer, is a method suggested to achieve this target.

In the solid, DNP leads to an over-population of the lowest-lying spin state. After rapid dissolution, this is partly converted into a long-lived state, i.e., into a population imbalance between combinations of singlet and triplet states of geminal protons. This LLS is allowed to relax slowly prior to its reconversion into observable transverse magnetization by polychromatic Spin-Lock Induced Crossing (poly-SLIC). This reconversion can be achieved simultaneously in several molecules. The reconversion can be carried out in several steps to determine the lifetimes TLLS on-the-fly. The enhancement factors of hyperpolarized LLS-derived signals typically exceed two orders of magnitude compared to experiments carried out at room temperature.

These methods extend applications of B-DNP to protons in molecules containing short aliphatic chains and may be useful for drug screening. Future perspectives now include lowering the concentrations of ligands by orders of magnitudes, extending the LLS lifetimes by degassing the solvent of reception as well as quenching the TEMPOL radical present in the final solution. At last, contrasts of various drug screening experiments will be compared to maximize information content per B-DNP shots.



Quantitative In Situ and Ex Situ ¹²⁹Xenon Polarimetry

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Spin-exchange optical pumping (SEOP) is a common hyperpolarization (HP) method in human and material research. Reliable HP xenon-129 (Xe) signal quantification is critical for quality assurance. Often, it is done after SEOP (ex situ) by comparing high-field NMR signals of HP Xe to a thermally polarized Xe/O₂ mixture. In situ quantification during SEOP at mT fields remains challenging, as thermal Xe signals are undetectable at low field. Here, we present methods for in situ and ex situ Xe polarization quantification.

HP Xe was generated in a SEOP polarizer (Polaris) at 125°C and 2.85 mT. The SEOP cell had been in use for four years, resulting in Rb oxidation and reduced polarization. In situ polarization was analyzed by measuring the ¹H and HP Xe signals with a surface ¹H/¹²⁹Xe loop coil placed on the SEOP cell. The ¹H reference signal was acquired from a 6.3 ml sample of 1-decanol (boiling point 229°C) in a PEEK container. Ex-situ polarization from 1 bar HP Xe was quantified using a 60 MHz benchtop multi-nuclei spectrometer (SpinSolve, Magritek) and four reference samples: 1 bar post-SEOP HP Xe diluted with 6 bar O₂; a premixed 3.5 bar Xe/8 bar O₂ sample; 0.5 mL water; 8 M ¹³C-urea. Xe polarization was calculated considering different flip angles, enrichments, pressures, nuclei count, NMR tube geometries, gyromagnetic ratios, and coil quality factors.

In situ reference ¹H signal of 1-decanol gave an SNR of 4.64 after 500 repetitions (TR = 10s); the HP Xe signal reached an SNR of 11.83 after 25 averages (TR = 1s). Coil calibration is ongoing to enable in situ polarization quantification. Ex situ Xe polarization was (0.13 ± 0.04)% and (0.16 ± 0.03)% in two experiments with uncertainties from four reference samples. The method will next be applied to a fresh SEOP cell. We demonstrate a robust cross-nucleus quantification approach that accelerates analysis via denser reference media, offering higher SNR than Xe gas.

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Experimental approach for pyruvate hyperpolarization using SLIC-SABRE

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Magnetic resonance imaging (MRI) with hyperpolarized (HP) contrast agents has enabled real time metabolic imaging in vivo, a paradigm shift for modern diagnostics. Most methods to create HP biomolecules, however, are either slow, expensive or require a synthesis seconds before application.

A novel technique, signal amplification by reversible exchange (SABRE) combined with spin-lock induced level anti crossing (SLIC), mitigates most of these matters, and allows high HP of $1\text{-}^{13}\text{C}$ pyruvate e.g., at low cost, in seconds and in large quantities. However, no commercial equipment is available, and only few setups were reported in literature.

Here, we analyse the process, describe optimization and quality assurance methods. Critical parameters for high and reproducible polarization include B_0 homogeneity, B_1 homogeneity, B_0 power supply stability, shielding, and high-frequency (HF) amplifier noise. Based on our findings, we developed a dedicated SLIC-SABRE polarizer for $[1\text{-}^{13}\text{C}]$ pyruvate. The major components include: (i) NMR unit consisting of a 4-layer μ metal shield (Twinleaf, MS-2), a two-layer solenoid B_0 coil (360 mm length, 120 mm diameter), a power supply (Twinleaf, CSBA-30), a one-layer saddle shape B_1 coil (100 mm diameter), and an RF amplifier (Onkyo, TX8555); (ii) homemade gas control unit supplying up to 10 bars of para-hydrogen (iii) a process control unit consisting of a custom software and a digital-analog converter (Matlab, National Instruments). For example, we achieved an average hyperpolarization of $(15.15 \pm 0.02) \%$ of $[1\text{-}^{13}\text{C}]$ pyruvate (7.97 mM, $N=10$), demonstrating the robustness and reliability of the experimental design.



Detection of adenosine phosphates in blood by nh-PHIP

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The practical NMR limit of detection in the low- to mid-micromolar range¹ and signal crowding hinder its metabolomics applications, allowing only the higher concentration analytes to be targeted. Yet, diagnostically significant metabolites in the human metabolome are often found at lower concentrations ($<5\ \mu\text{M}$), beyond the reach of standard NMR. Several nuclear hyperpolarization methods are being developed to overcome this sensitivity and resolution limitation in analysis of biological samples.

nh-PHIP (non-hydrogenative parahydrogen-induced polarization)² is a parahydrogen-based hyperpolarization method where an iridium N-heterocyclic carbene complex complexes with various analytes and facilitates NMR signal enhancement in the hydride region of $-20\text{...}-30\text{ ppm}$. nh-PHIP analysis requires alcohol environment, with up to 20% water tolerance. The method has been shown to be compatible with various matrices e.g. urine.³ However, blood metabolites have not been studied by nh-PHIP previously. Blood is a much more complex matrix compared to urine and achieving such analysis would have to overcome challenges related to blood cellular fraction, high protein and lipoprotein content, and large alcohol insoluble fraction.

We focused on the nh-PHIP detection of ATP, ADP, and AMP⁴ in red blood cell (RBC) fraction of the blood, since these molecules play a vital role in how the body produces and uses energy. This work presents the first nh-PHIP spectra of both RBC and plasma fraction of blood, a significant step toward sensitivity-enhanced blood-based NMR metabolomics.

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Fluorescent Ir complex for SABRE-based nuclear spin hyperpolarisation

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Aims: Signal amplification by reversible exchange (SABRE) enhances the nuclear spin polarization of the target molecules which transiently interact with Ir-complex in the presence of parahydrogen. The development of new Ir complexes plays an important role in the rapid development of SABRE. To date, the maximum hyperpolarization was demonstrated in methanolic solutions. To extend the list of potential solvents, we have synthesised a new Ir complex that is also active in diethyl ether. This enables two-phase transfer catalysis and separation of the toxic catalyst from hyperpolarized target for future studies on living objects. Furthermore, the new complex can also be detected by UV-light – necessary for a rapid quality control of purification.

Methods: In this study, a new imidazolium salts containing a 1,4-benzoquinone group was first synthesised. Afterwards, this then reacted as a carbenium ion in the presence of KOtBu and $[\text{Ir}(\text{COD})\text{Cl}]_2$ to form a precursor for SABRE active Ir complex. The fluorescent properties of the formed SABRE precursor were examined in CHCl_3 . After column chromatographic purification, the complex obtained was spectroscopically examined for its suitability for SABRE.

Results: The resulting complex is insoluble in water, but readily soluble in alcohols such as methanol or ethanol and diethyl ether (standard IMes precatalyst is not soluble in diethyl ether). After activation of the precatalyst with parahydrogen (pH_2), negative signal amplification of the ^1H NMR signals of 3-fluoropyridine were observed in deuterated methanol. The enhancement factor from initial measurements was observed to be greater than 6 (50% pH_2 , 7 T, 293 K).

Conclusion: For the first time, a fluorescent Ir catalyst suitable for SABRE studies was synthesised and its suitability for hyperpolarization was demonstrated. In addition, its good solubility in diethyl ether enables future two-phase transfer nucleus spin hyperpolarisation.



Enhancing NMR sensitivity and contrast by ^{19}F Overhauser DNP and hyperpolarization transfer to ^{13}C -carbons

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Dynamic nuclear polarization (DNP) in liquids is enabled predominantly by the Overhauser effect (OE) through electron-nuclear cross-relaxation. Polarization is transferred onto nuclei of interest by resonant microwave irradiation of unpaired electron spins introduced to the sample as small stable organic radicals. The DNP efficiency crucially depends on the time modulation of the electron-nuclear hyperfine coupling by molecular dynamics of the radical-target complex.

Previously, we reported ^{19}F NMR signal enhancements up to ~ 25 at high magnetic fields using our new liquid state OE-DNP setup operating at 9.4 T [1]. Because ^{19}F NMR has important applications in biomolecular NMR and drug analysis, we systematically explored the ^{19}F OE-DNP performance on a variety of ^{19}F chemical environments using galvinoxyl radical finding NMR signal enhancements up to 25 [2].

Moreover, we show that hyperpolarized ^{19}F can be exploited as a source for hyperpolarization when transferred to scalar coupled carbon-13 within a molecule to drastically enhance the ^{13}C sensitivity and contrast. With that, hyperpolarization can be transferred to nearby quaternary ^{13}C nuclei, which have so far been challenging to hyperpolarize.

Finally, a quantitative analysis of the DNP coupling factor at various fields revealed fast molecular dynamics that are important for efficient high field OE-DNP. With the help of DFT calculations we identified key features of the radical-target interaction leading to large isotropic hyperfine couplings facilitating the spin polarization transfer. Our findings further establish ^{19}F as one of the most promising target nuclei for effective OE-DNP at high magnetic fields.

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Dissolution DNP at PHIP speed: using a compact and transportable device to generate hyperpolarized ^{13}C and ^1H solutions

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Aims:

Since the seminal work in 2003 and for the following 15 years, the magnetic field of dissolution Dynamic Nuclear Polarization (dDNP) machines has increased with a rate of 0.5T/year from 3.35T to 10.05T. Herein, we present a small foot-print and transportable dissolution DNP polarizer that works at “relaxed” experimental conditions allowing second to minute long polarization times.

Methods: Polarizer design

A previous work inspired the design of this polarizer. The cryogenic liquid reservoir (1.5L) was equipped with a waveguide ending at the bottom of the 0.85T permanent magnet. The $25\pm 1\text{GHz}/2\text{Watt}$ microwave source and solid-state proton NMR cryogenic probe were built in house. Solid-state DNP:

Measurements were performed at 77 K on samples containing $\text{H}_2\text{O}:\text{D}_2\text{O}:\text{d8-glycerol}$ and TEMPOL concentration ranging from 10 to 50 mM TEMPOL, and 5M $[1-^{13}\text{C}]\text{urea}$ dissolved in $\text{H}_2\text{O}:\text{glycerol}$ and doped with 20 mM trityl radical

Dissolution and liquid state acquisition

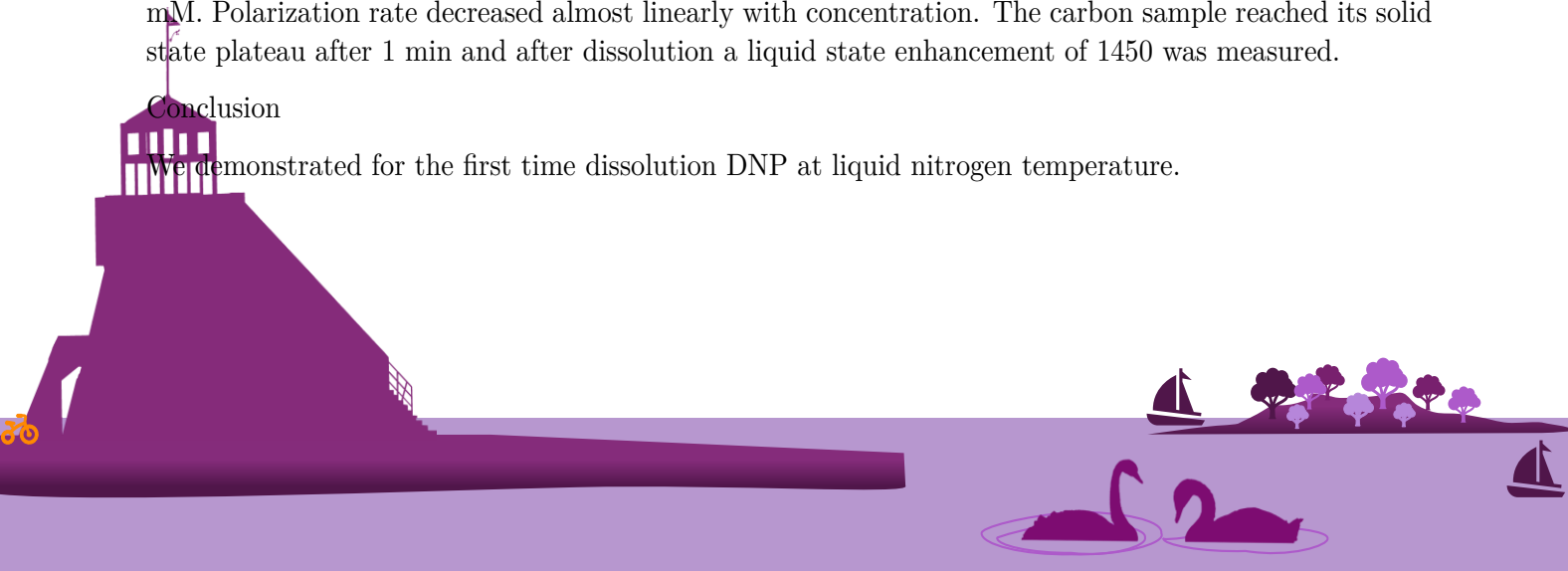
Carbon samples (100 μL) were dissolved in 5 mL superheated phosphate buffer using the fluid path technology. Liquid-state acquisition were performed in a 1 T benchtop NMR spectrometer.

Results:

Nitrogen lasts for 48 h. Proton solid-state polarization using TEMPOL radical, was a good way to test the system. The microwave frequency sweep of the 10 mM and 50 mM TEMPOL samples show that the permanent magnet homogeneity (200 ppm) is good enough for ESR. Indeed, the broadening due to dipolar coupling can be appreciated. Moreover, the source and waveguide system were efficient enough to saturate the enhancement. The latter showed its maximum (210 ± 10) for a radical concentration of 40 mM. Polarization rate decreased almost linearly with concentration. The carbon sample reached its solid state plateau after 1 min and after dissolution a liquid state enhancement of 1450 was measured.

Conclusion

We demonstrated for the first time dissolution DNP at liquid nitrogen temperature.



Investigation of the hyperpolarization of lactic acid via para-hydrogen induced polarization using exchangeable protons

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One of the remaining challenges in NMR is to overcome its inherently low sensitivity. Hyperpolarization of nuclear spins allows for increasing the NMR signal by multiple magnitudes, making it a highly promising approach. Although several methods were proposed to polarize specific compounds, a general technique to polarize small molecules in solution is still challenging. For example, hydrogenative para-hydrogen induced polarization (PHIP) requires the synthesis of suitable precursors with an unsaturated C-C bond.

In this work, we report on optimizing the polarization of lactic acid, an important metabolite, e.g., for hyperpolarized metabolic imaging, with PHIP relayed via proton exchange (PHIP-X). PHIP-X is based on first polarizing a transfer molecule and then transferring the polarization to a target molecule, here [2-¹³C]lactic acid, via proton exchange. Different parameters influence the efficiency of the process, namely the polarization distribution within the transfer molecule, the exchange of the labile protons between the transfer and target molecule, and the spin order transfer (SOT) within the target molecule.

To better understand these processes, experimental and computational methods have been used to disentangle the influence of exchange rate and lifetime of the labile protons and efficacy of SOT in both transfer and target molecules.

We found that within the target molecule, the ¹³C polarization was higher when the SOT pulse sequence at high field was adapted to the J-couplings between the ¹³C and a covalently bound proton than to a labile proton. Additionally, the exchange rate of the labile protons of lactic acid and the transfer molecule strongly affected the polarization, leading us to investigate the solvent system optimization further to maximize the integral SOT efficacy.



Signal enhancement of > 1000 in high field ^1H liquid-state NMR

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Sensitivity is central to nuclear magnetic resonance, and hyperpolarization methods that increase the otherwise weak nuclear spin polarization are the most promising means for substantial sensitivity boosts. The most broadly applicable of these methods for liquid-state NMR, dissolution-DNP (D-DNP) [1] and bullet-DNP (B-DNP) [2, 3], have indeed achieved ^{13}C polarization levels of tens of percent, enabling the detection of ^{13}C -labelled substances at low micromolar concentrations [4]. For protons, however, the observed gains have thus far been limited. In D-DNP, fast relaxation during the liquid-state transfer has limited ^1H enhancements to values often well below 100 [5]. With bullet-DNP we recently estimated ^1H enhancements of up to 400 when using deuterated methanol as a solvent [6].

Here we show that the sample composition has a dramatic effect on the attainable signal enhancement, and that stringent optimization boosts the enhancements by more than an order of magnitude. We report signal enhancements > 1000 at 9.4 Tesla in H_2O on fast relaxing ^1H resonances of a range of small molecules. The enhancements allow us to observe ligand-binding in water using unlabelled ligands at low micromolar concentrations.

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The Dual Role of Electron–Electron Coupling in Polarization Transfer and Nuclear Spin Diffusion

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Dynamic Nuclear Polarization (DNP) and its microwave-free counterpart, photochemically induced DNP (photo-CIDNP), both rely on control of coupled electron spins to enhance NMR sensitivity. In this poster, we explore the pivotal role of electron–electron (e–e) coupling in two distinct but fundamentally linked phenomena: polarization transfer and nuclear spin diffusion. First, we introduce the Electron-Assisted Spin Diffusion (EASD) mechanism, in which e–e flip-flop processes facilitate polarization leakage from core to bulk nuclei, effectively overcoming the spin diffusion barrier—a bottleneck in polarization transfer rate. Next, we explore role of e–e coupling in the Three-Spin Mixing (TSM) mechanism in solid-state photo-CIDNP, where coherent evolution among two electrons and one nucleus leads to efficient nuclear polarization without microwaves. Both mechanisms hinge on the interplay between hyperfine and dipolar couplings, with e–e coupling strength acting as a tunable bridge that links localized polarization to the broader nuclear spin network.



Metal-free Parahydrogen-Induced Hyperpolarization for Polarizing Alcohols

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Parahydrogen-induced polarization (PHIP) is a powerful hyperpolarization technique capable of enhancing NMR sensitivity by several orders of magnitude. Traditional PHIP relies on metal-based catalysts, which can pose challenges for practical applications. This work focuses on studying ansa-aminoboranes (AAB), metal-free parahydrogen activators, for polarizing simple aliphatic alcohols. An important requirement for such experiments is the ability of AABs to tolerate the hydroxyl function of alcohols and reversibly activate parahydrogen.

In these initial experiments, we study the interaction of methyl alcohol with various AABs using NMR spectroscopic methods. We have utilized three different orthophenylene-bridged AABs with mesityl, isopropyl phenyl, and phenyl substituents at the boryl site. These compounds were treated with methanol, keeping the similar experimental conditions. The difference in the stability of the AABs is being investigated for identifying better candidates for reversible parahydrogen activation and polarization transfer to alcohols.

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Accessing High ^{13}C Polarisation Whilst Observing the RASER Effect When Detecting ^1H Signals

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The hyperpolarisation of ^{13}C is desirable for many reasons which include the study of metabolic pathways and reaction monitoring. [1] SABRE (Signal Amplification By Reversible Exchange) has proven to be a convenient method to boost the signal intensity of many signals that is compatible for use with standard NMR spectrometers. [2] One limitation of studies using this method though is the need to use labelled material, either directly or indirectly, to assist the hyperpolarisation effect. [3] This reduces the potential substrate scope to materials that are accessible to labelling whilst imposing the cost penalty of using labelled substrates when compared to unlabelled material at natural abundances. Thus it would be beneficial if high ^{13}C polarisations could be obtained which would abrogate the need to use labelled substrates.

Herein, we create high ^1H polarisations such that the RASER (Radio Amplification By Simulated Emission of Radiation) effect is evident. The RASER effect occurs when molecules spontaneously emit radio waves and this has been shown to occur with very high ^1H polarisation. [4, 5] The RASER generated signal can interfere constructively or destructively with the ^1H NMR signal when a rf pulse is applied, and can be detected by running an NMR experiment with no pulse. Under conditions where proton RASER is seen it is possible to obtain improved indirect ^{13}C polarisation. A range of substrates are explored which with optimisation of the SABRE transfer effect show substantial ^{13}C hyperpolarisation. The enhancements obtained for unlabelled substrates via this method compare favourably with those previously reported for labelled targets, highlighting an advancement in this methods methodology.

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Investigation of the DNP Performances of High Field TinyPol Polarizing Agents

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Dynamic Nuclear Polarization (DNP) applied to MAS solid-state NMR has proved to be a valuable technique to enhance the sensitivity of more than two orders of magnitude. Cross-effect polarizing agent (PA) are among the most efficient radicals and di-nitroxides AMUPol and TEKPol lead to enhancement factors of up to about 250 at 100 K and 9.4 T.

Nevertheless, the performances at 9.4 T are strongly reduced at high field like 18.8 T (800 MHz of ^1H), where the design of optimal PAs is still an open problem. In 2018 we introduced hybrid hetero-biradical, like HyTEK2, which shows enhancements up to 180-200 at 18.8 and 21.3 T, respectively, soluble only in organic solvents. (1,2) In 2020 we introduced novel, water soluble, dinitroxides biradicals, named TinyPols, (3) an last year further major improvements in the radical design were achieved: optimizing a molecular geometry promoting stronger electron-electron magnetic interaction and “antenna” side arms to promote an efficient radical-solvent interaction. In particular, M-TinyPol(OH)₄ showed enhancements up to about 200 even at 65 kHz of MAS frequency and 18.8 T. (4)

Here we present a detailed analysis of the DNP efficiency of these improved TinyPol biradicals, comparing the experimental enhancements with DNP computational simulations that support the experimental data. DNP simulations, performed with an hybrid QM DNP model (5) and Molecular Dynamics, makes it possible to follow the propagation of the polarization within the solvent matrix unravelling the roles of the antenna sidechains in promoting the hyperpolarization spin-diffusion. The detailed comprehension of these processes opens the way to further progress in polarizing agent design.

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Efficient NMR methods to characterize therapeutic antibodies at atomic resolution.

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Monoclonal antibodies (mAbs) are promising therapeutics, and the biopharmaceutical industry's efforts to develop them are increasingly successful. NMR is a powerful technique to obtain spectral fingerprints of mAbs and antibody fragments at natural abundance using 2D methyl NMR [1]. To get structural information at atomic resolution, NMR signals need to be assigned to their corresponding nuclei, which is challenging for a 150 kDa disulfide-rich protein.

An efficient cell-free method was developed to produce isotopically labelled mAb fragments with ^2H , ^{13}C , ^{15}N and $^{13}\text{CH}_3$, optimizing disulfide bridge formation, yield, and cost [2]. This strategy enabled the first backbone and methyl signals assignment of Fc and Fab fragments of a therapeutic antibody.

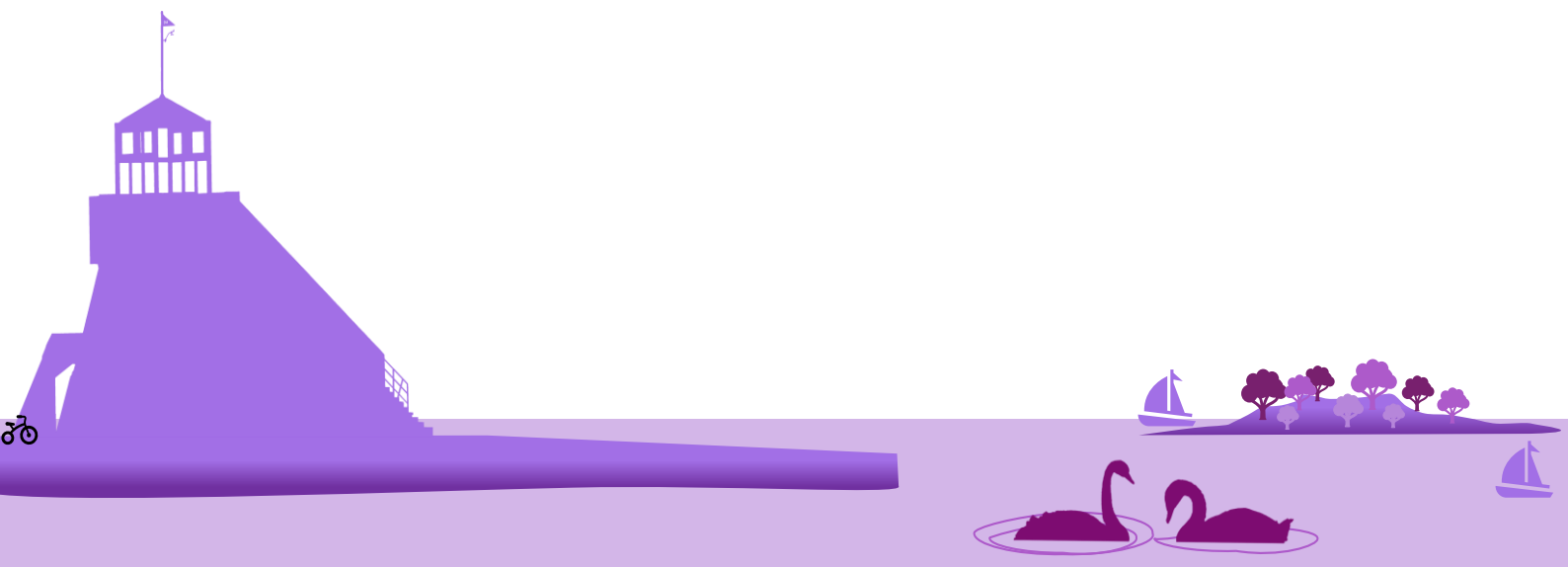
Although advanced labelling was a key innovation for this challenging assignment, NMR data analysis remains tedious. To speed up the assignment of therapeutic mAbs, we designed a method using this first assignment and sequence identities between targeted mAbs. While the Fc fragment is identical in all IgG1, the Fab fragment consists of a light and a heavy chain, each composed of a highly conserved constant part and a variable part. We used an efficient divide and conquer strategy to transfer assignments between the conserved domains and to assign the variable domain with a minimal

amount of small uniformly ^{15}N , ^{13}C -labelled constructs. We will show how this method can be used to assign different therapeutic mAbs.

As an example, these assignments have been used to easily map the amino acids of the antibody interacting with its antigen by NMR. On the Fc side, assignment expedites the assessment of the impact of glycosylation on its structure. These applications underline the strength of solution NMR with optimal labelling to characterize this growing class of therapeutics.

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Isoleucine Side Chains as Reporters of Conformational Freedom in Protein Folding Studied by DNP-Enhanced NMR

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Understanding the dynamic conformations of protein side-chains is essential for deciphering protein function. We employed DNP-enhanced solid-state NMR (ssNMR) at cryogenic temperatures to dissect isoleucine (Ile) side-chain dynamics across diverse protein states. We examined three Ile labeled proteins, representing key biological states: intrinsically disordered (α -synuclein), well-folded (GABARAP) and the whole folding/unfolding/misfolding transition (PI3K SH3).

By analyzing ^{13}C chemical shifts, which reflect Ile side-chain angles, and performing line-shape analysis of 2D spectra, we obtained comprehensive information about secondary structure and protein mobility. Furthermore, through peak volume integrations, we could quantify the prevalence of specific conformations.

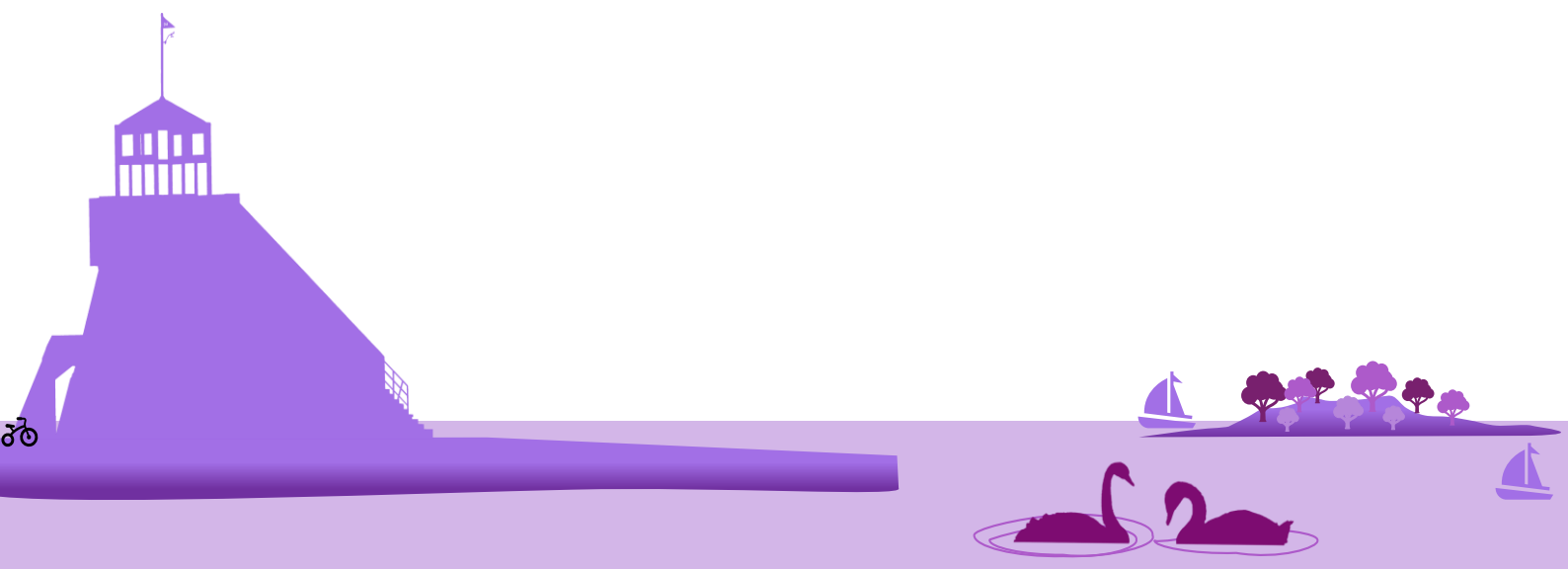
Our results revealed that in well-folded proteins, Ile residues exhibit well-resolved chemical shifts, reflecting structural rigidity. Here, residue-specific conformations could be identified and quantified. In contrast, unfolded and intrinsically disordered proteins displayed significant line broadening, as consequence of higher conformational freedom. We demonstrated that solvent conditions profoundly influence conformational ensembles, distinguishing different unfolded states of PI3K SH3. In amyloid fibrils, β -sheet-like chemical shifts dominated, indicating restricted conformations. Concurrently, broad signals were observed, reflecting the residual disorder of Ile within the fuzzy coat surrounding the fibril core.

Our research demonstrates the capability of DNP-enhanced ssNMR to overcome limitations in studying (partially) disordered biomolecules. Freeze-trapping physiological exchange provides a precise ensemble snapshot, representing each conformation's probability, offering a more accurate representation than time-averaged data. This approach is a significant advancement in structural biology.

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Levorin et al, 2025, JACS (revision)



The peptides within R5-templated silica particles resemble a condensed phase with liquid-like properties

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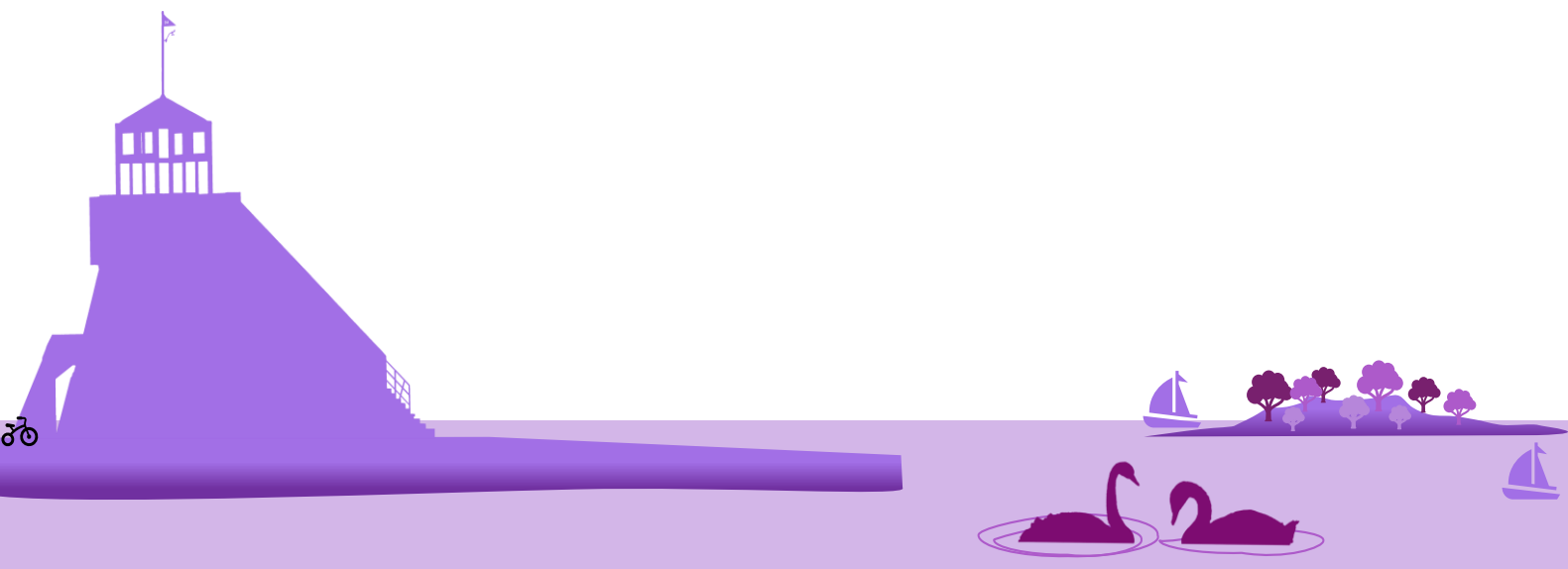
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Biomimetic silica nanoparticles templated by diatom-derived peptides, notably the R5 peptide¹, show significant promise for drug delivery and enzyme encapsulation applications due to their inherent biocompatibility, tunable surface properties, and capacity for molecular encapsulation. Optimizing these nanoparticles for biomedical use requires detailed insights into the structure and molecular dynamics of the peptide matrix that directs their formation. However, the primary technique for probing peptide and protein dynamics in aqueous environments, nuclear magnetic resonance (NMR) spectroscopy, typically struggles with high molecular weight systems when relying on conventional proton detection. To overcome this limitation and study larger peptide assemblies involved in biomineralization, we present an integrated approach that combines carbon-detected NMR² with electron paramagnetic resonance (EPR) spectroscopy with site-directed spin labeling (SDSL) and low-temperature magic angle spinning dynamic nuclear polarization (LT MAS DNP) to characterize the structural dynamics of R5 peptide assemblies both before and after encapsulation within a silica matrix.

Our findings reveal that R5 assemblies exist in a condensed, phase with liquid-like properties prior to mineralization. Upon incorporation into the silica matrix, a portion of the R5 peptides adopts a more restricted dynamic state, while another subset retains liquid-like mobility. This study not only deepens our understanding of the principles underlying biomimetic material design but also underscores the critical role of liquid-liquid phase separation in peptide-guided biomineralization.

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NMR Reveals a Cryptic SH2 Binding Motif in the Disordered Region of Src

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Src tyrosine kinase, the prototypical member of the Src family of non-receptor tyrosine kinases (SFKs), plays a crucial role in cell signaling pathways. Its domain structure includes an N-terminal disordered region, followed by the SH3 and SH2 folded domains, which regulate the kinase activity of its C-terminal kinase domain.

In its inactive state, Src is stabilized by an intramolecular interaction between a phosphorylated C-terminal tyrosine (Y530) and the SH2 domain, locking it in a closed, autoinhibited form.

Phosphorylation at T37 and S75 in Src's Unique domain during mitosis increases its activity without significant changes in Y530 phosphorylation. The underlying mechanism of this activation remains unclear. Using a multinuclear NMR approach and selective methionine methyl labeling, we investigated the effects of the Unique domain phosphorylation in the context of the 60 kDa full-length Src.

The observation of NMR signals from the C-terminal tail and SH1-SH2 linker regions, shifts in methionine signals from the SH1 domain, and diagnostic signals from the SH2 domain demonstrate the release of the autoinhibited Src conformation. This release results from competition between the phosphorylated Thr37 in the disordered domain and phosphorylated Y530 for the same binding site in the SH2 domain within full-length Src.

The discovery that a phosphothreonine-containing region can compete with and displace a canonical phosphotyrosine interaction with an SH2 domain highlights the power of NMR in uncovering the regulatory role of disordered regions.



Concentration-Dependent Mechanisms of Melittin-Induced Membrane Destabilization: An Interdisciplinary Approach

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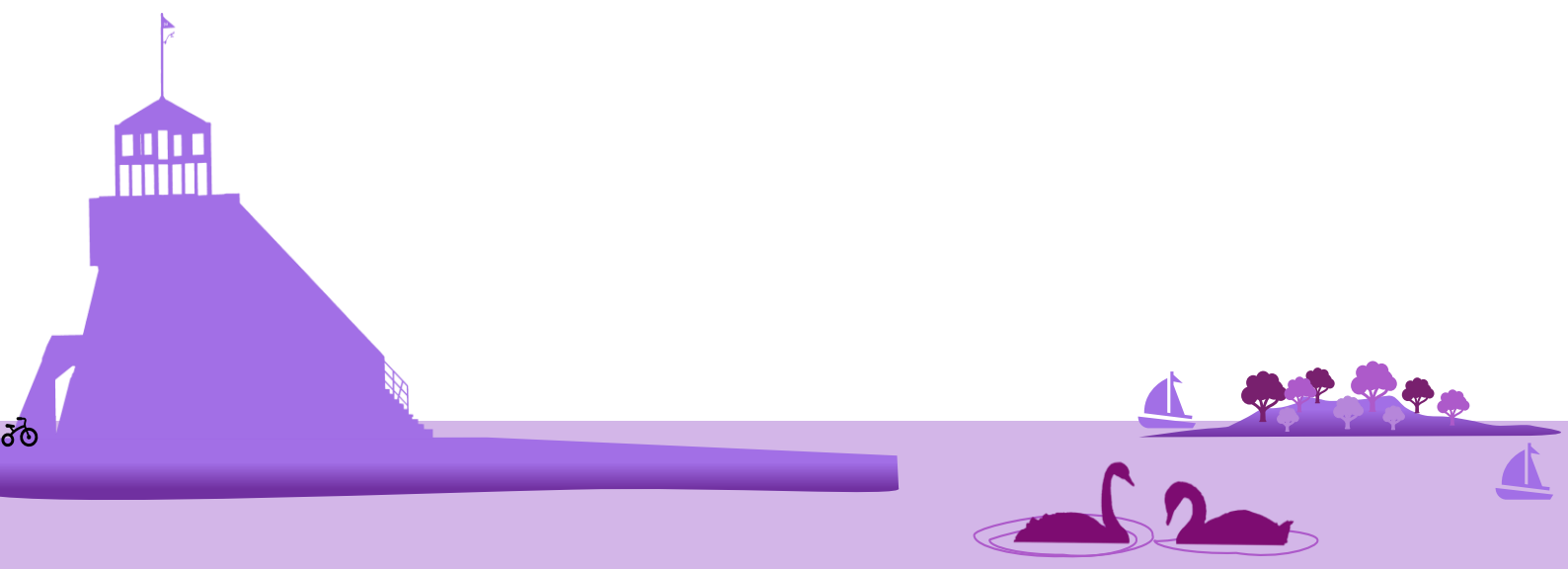
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Understanding how antimicrobial peptides disrupt bacterial membranes is essential for developing new antimicrobial strategies. In this work, we present an interdisciplinary methodology to characterize peptide-membrane interactions at multiple levels, combining solid-state NMR (ssNMR), vibrational spectroscopy, microscopy, Langmuir monolayers, and molecular dynamics simulations. Despite being widely studied, melittin precise mechanism of action remains under debate. Using a model membrane that mimics the *E. coli* inner membrane (DPPG/DOPE), we demonstrate that melittin fully incorporates into Langmuir monolayers, expanding and fluidizing them at high concentrations, while at lower concentrations, it increases local acyl chain order, as revealed by PM-IRRAS and ^1H ssNMR. Vibrational spectroscopy highlights changes in phosphate headgroup hydration, and ^{31}P ssNMR confirms melittin's preferential binding to DPPG over DOPE. Microscopy uncovers concentration-dependent morphological changes, including domain fusion, homogenization, and aggregation. Molecular dynamics simulations support these findings, showing that melittin enhances membrane fluidity, induces bilayer thinning and bending, and has a stronger effect on DPPG than on DOPE. These results reveal a concentration-dependent transition from localized interactions to widespread membrane destabilization. By integrating multiple techniques, this study provides a comprehensive understanding of melittin action, demonstrating the power of an interdisciplinary approach to investigating antimicrobial peptides.



Expression And NMR Characterization Of Labelled P-domains Of Emerging Norovirus

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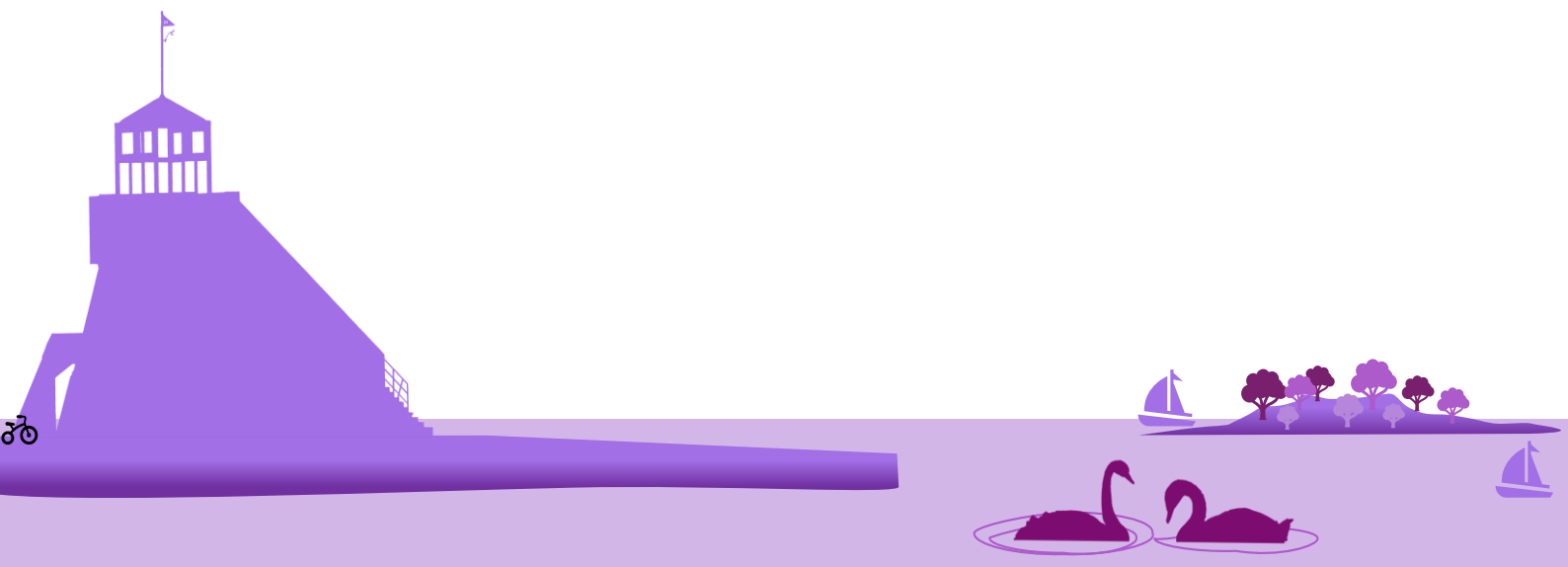
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Noroviruses are members of the Caliciviridae family and are non-enveloped, single-stranded, positive-sense RNA viruses. Norovirus is the major cause of acute gastroenteritis, particularly affecting vulnerable populations such as children, the elderly, and immunocompromised individuals. With millions of illnesses and thousands of deaths yearly, norovirus poses a significant global health burden. The economic impact is equally staggering, with billions of dollars in costs annually. Additionally, new virus strains emerge every 2-5 years, making outbreaks difficult to contain. Among the various norovirus strains, the GII.4 variant is the most prevalent globally.

The ability of norovirus to infect host cells depends on the interaction between the Protruding domain (P domain) of its capsid protein VP1 and Histo-Blood Group Antigens (HBGAs) on the surface of host cells. Furthermore, previous cell-based studies found, that Human Milk Oligosaccharides (HMOs) prevent norovirus infection to cells, by acting as a decoy receptor and blocking the interaction between HBGAs and human noroviruses. Therefore, understanding the structure of the P domain is essential for the development of effective antiviral drugs and vaccines.

In this study, we successfully expressed and purified the P domain from the GII.4. P16 norovirus strain in *E. coli*. To enhance NMR signal resolution, isotopically labeled samples (^{15}N , ^2H , and ^{13}C) were prepared, and multidimensional NMR experiments, along with chemical shift perturbation (CSP)-based NMR experiments, were performed. These experiments will help identify key residues involved in binding to HBGAs and other antiviral molecules, such as analogs of HMOs. By elucidating these interactions, our studies provide crucial insights into the viral infection mechanism and offer valuable information for the development of new therapeutic strategies.



Combined solution- and MAS NMR reveal a locally unfolded state of the bacteriophage tail tube that prevents premature assembly

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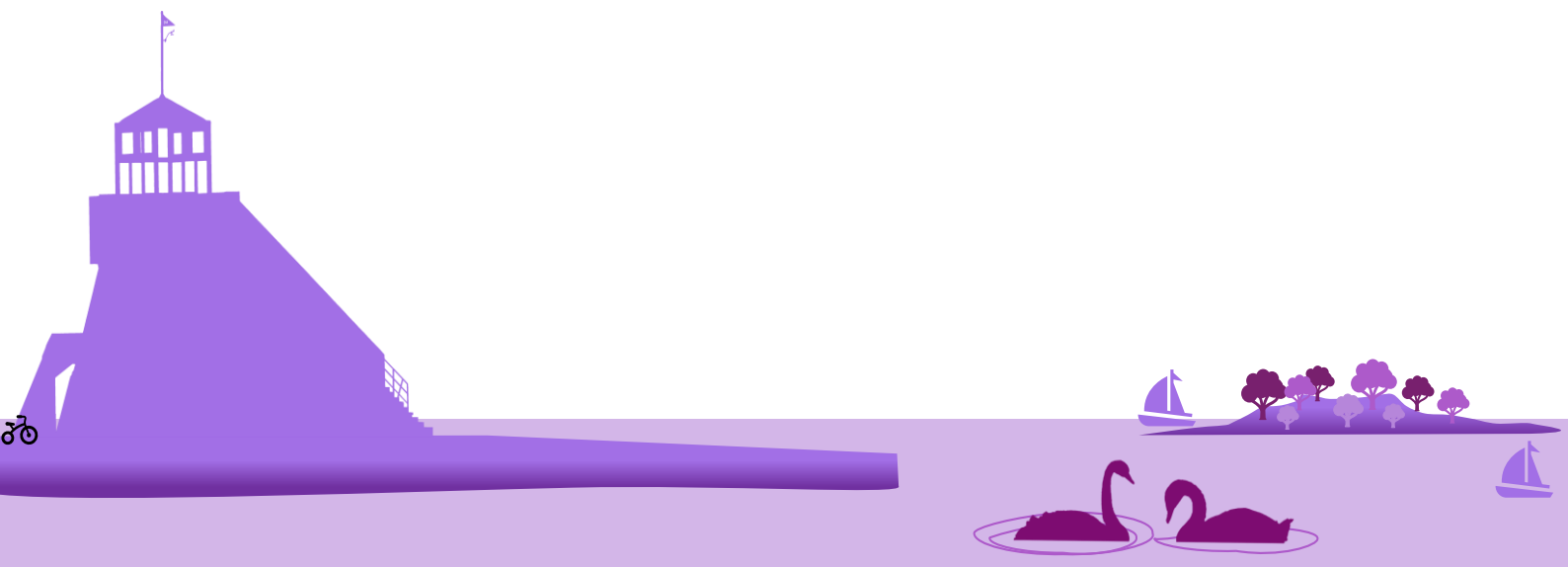
The assembly of large functional protein assemblies, such as virus capsids or phage tail tubes must be a well-orchestrated process, ending at a well-defined and highly complex supramolecular structure. NMR spectroscopy is an excellent tool for studying proteins in solution – but only as long as they are small. MAS NMR, on the other hand, is a versatile tool to probe how the major protein of the bacteriophage tail assembles from monomers to large tube-like architectures. In particular, we interrogated why in the cell the tube forms only when the rest of the bacteriophage is “ready” to be assembled. What keeps the protein from assembling?

With the 50 kDa large tail-tube protein from bacteriophage T5 – which represents a challenge for any NMR study – we thus addressed the dynamics and structure both in the monomeric state (pre-assembly) and the assembled tube state, using both solution and MAS NMR.

Interestingly, an extensive set of dynamics experiments revealed that the monomeric protein exists in a dynamic equilibrium of a partially unfolded state (the major form) and the folded state. CPMG relaxation dispersion experiments quantified the thermodynamics and kinetics of the process. MAS NMR, including dynamics measurements, revealed that upon assembly the protein adopts a rigid structure.

Using MD simulations we gained more detailed insights into the partially folded state. Importantly, guided by these data, we were able to identify mutations which shift the equilibrium from the predominantly partially unfolded state to a fully folded state. This mutant protein has dramatically altered self-assembly, which allowed us to identify the mechanism protecting the protein from premature assembly: the folding-unfolding equilibrium keeps the protein primarily in a state that cannot assemble, until the rest of the phage is in place.

Our study highlights the power of combining solution and MAS NMR techniques to address complex biological questions of self-assembly.



Structural transition from closed to open for the Influenza A M2 proton channel as observed by proton-detected solid-state NMR

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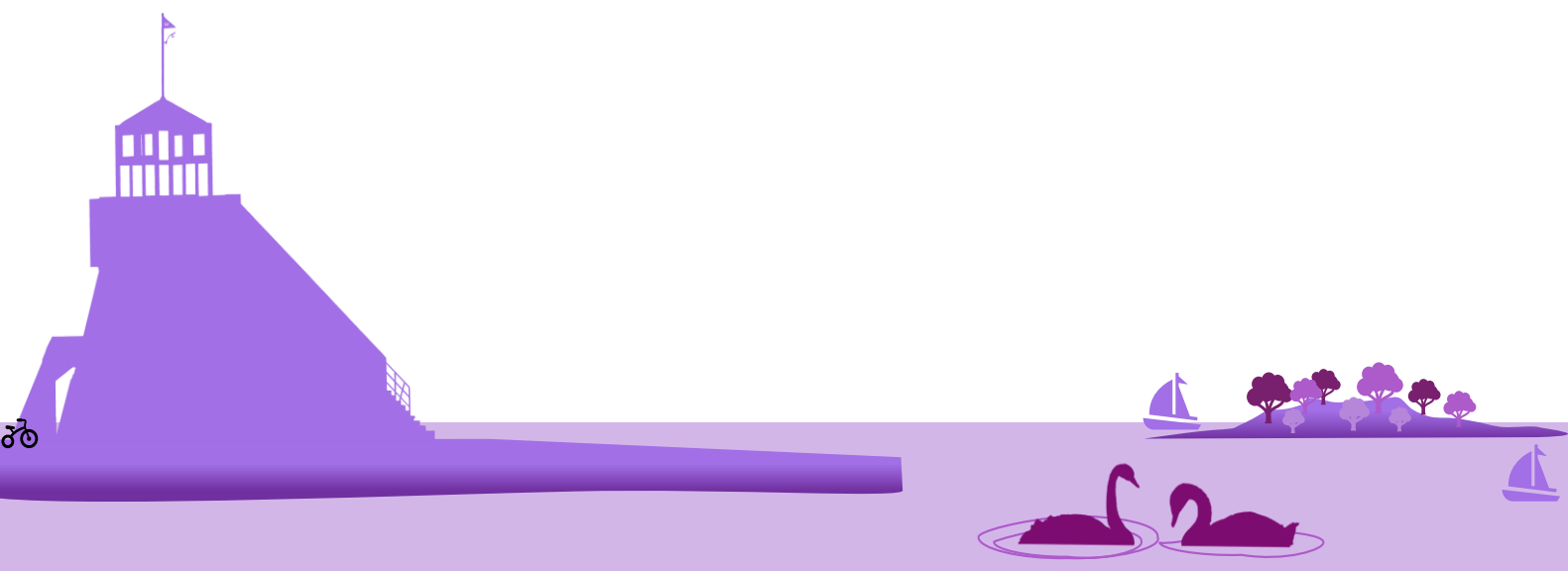
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Viruses and their protein components have been studied extensively in recent years. Nevertheless, some systems appearing in many different virus species need to be analyzed in more detail, regarding their essential role in virus function [1]. The influenza A M2 protein is an acid-activated proton channel and a key target for antiviral drugs. In our study, we employed proton-detected solid-state NMR together with molecular-dynamics simulations and functional studies to investigate the tetrameric M2 channel in proteoliposomes under native-like lipid bilayer conditions. Our analysis covered different functionally relevant pH states, leading to a closed, an opening and a fully open and conducting form. Analyzing side chain chemical shifts, we could discover variable hydrogen-bond networks at residues involved in the conduction mechanism of the channel. Together with QM/MM simulations and functional studies, this led to an improved understanding of the activation mechanism. Backbone signal duplications further suggest conformational heterogeneity only in the closed conformation, where a rigid structural arrangement prevents conduction. In the open state at low pH, the conformational heterogeneity observed at higher pH values disappears completely and a unique set of highly resolved resonances becomes visible. This suggests a well-defined acid-activated state of M2. Our findings support a model of M2 proton conduction based on dynamic versus rigid H37 side chains and provide a foundation for resolving the atomic structure of the acid-activated state. Together with data from surface-enhanced infrared absorption (SEIRA) spectroscopy we employed in a previous study [2], we can add valuable findings to the ongoing discussion on the structure of and mechanics in M2.

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Distinct mechanisms of allosteric modulation at the β 1-adrenergic receptor

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Allosteric modulation of energy landscapes is a fundamental aspect in the control of signalling in the G protein-coupled receptor (GPCR) family of membrane proteins, providing a means to regulate receptor coupling to downstream effectors such as G proteins through distinct alternative regions from the endogenous orthosteric site. However, the direct effects of such modulation on the receptor conformational equilibria of active, pre-active, and inactive states, set by the orthosteric ligand, are currently unclear.

We found two distinct mechanisms of receptor activity modulation, including mutagenesis of the key conformational hub at E130 or binding of an established β 2AR negative allosteric modulator (NAM), induced similar decreases in G protein coupling to the β 1-adrenergic receptor (β 1AR). We investigated the structural mechanisms of this allosteric modulation at β 1AR, using single site labelling coupled with 19F NMR spectroscopy and selective isotope labelling of methyl groups to provide insight into how these factors affected receptor conformational ensembles and dynamics.

We found mutations at E130 imparted a partial efficacy effect on the full agonist, effectively reducing the efficacy of full agonists to activate G proteins as a side-chain dependent rheostat. Receptor equilibria were shifted away from the agonist-bound active state towards existing conformational ensembles with reduced G protein coupling efficiencies, including pre-active and inactive states.

In contrast, NAM-induced occupation of discrete solution states upon binding characterised receptor conformations which were non-functional with respect to G protein coupling. We implicated unique conformations in the intracellular side of transmembrane helix 6 in reducing G protein activation.

Our investigations into distinct equilibria changes and occupation of additional conformational states provide insight into the role of dynamics in GPCR allostery.



Backbone resonance assignment of large intrinsically disordered proteins

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Resonance assignment is the primary move of any NMR-based study of proteins. Here we present an easy method for backbone resonance assignment, applicable to intrinsically disordered proteins (IDPs), even as large as 75 kDa.

Aims

The development of a procedure for backbone resonance assignment for large IDPs.

Methods

Because of high dynamics of IDPs and the similar chemical environment of their residues, IDPs spectra are very crowded. To alleviate this problem high-dimensional spectra (like 4D and 5D) can be used.[1]. Processing of such high-dimensional spectra relies on sparse multidimensional Fourier transform (SMFT)[2], where we use the peak positions of the basis spectrum (e.g. HNCO) to calculate 2D cross-sections of 4D or 5D spectra. Finding the sequential connectivities using the cross-sections is very convenient and can be performed automatically by the TSAR[3,4] (Tool for SMFT-based Assignment of Resonances) program.

To assign exceptionally large IDPs we used the following 5D pulse sequences: HNcaCONH[3], HabCab-CONH[3], hacaCONcaCONH[3], hacaCONcoCONH[5], hNCOcanCONH, haCACOncaCONH (the two latter ones yet unpublished).

Results

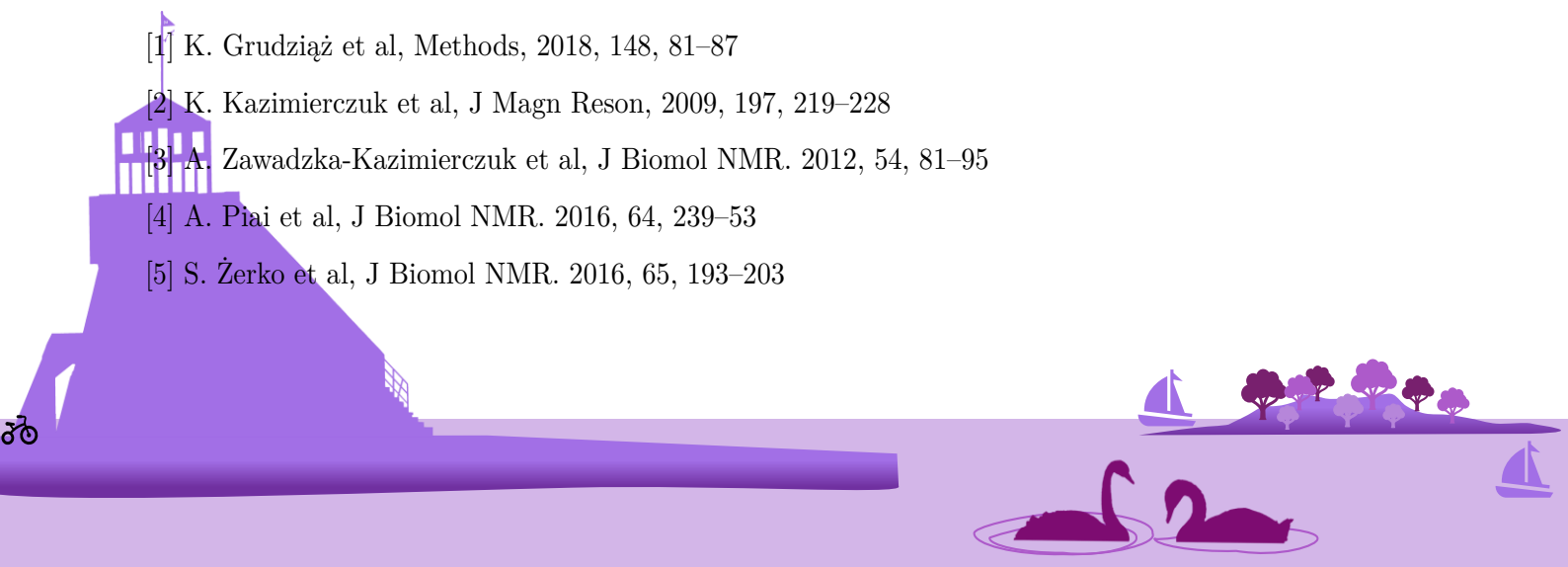
Breast cancer type 1 susceptibility protein (BRCA1) is a large protein (1863 residues). It includes four folded domains, but its majority (83%) is disordered. The studied 679-residue fragment (residues 679-1357) was fully unstructured. Using the above approach we assigned 77% of non-proline residues.

Conclusions

The proposed procedure allows us to gain backbone resonance assignment even for large IDPs, in an easy and reliable way.

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Unraveling the side-chain dynamics of the protein kinase p38 γ through Ultrafast High-Resolution Relaxometry (UHRR)

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Introduction: 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 nanosecond motions [1]. In this work, we introduce ultrafast high-resolution relaxometry as a new method to probe low ps to high us motions in the protein kinase p38 γ . The regulation of p38 γ is expected to rely on allosteric pathways [2].

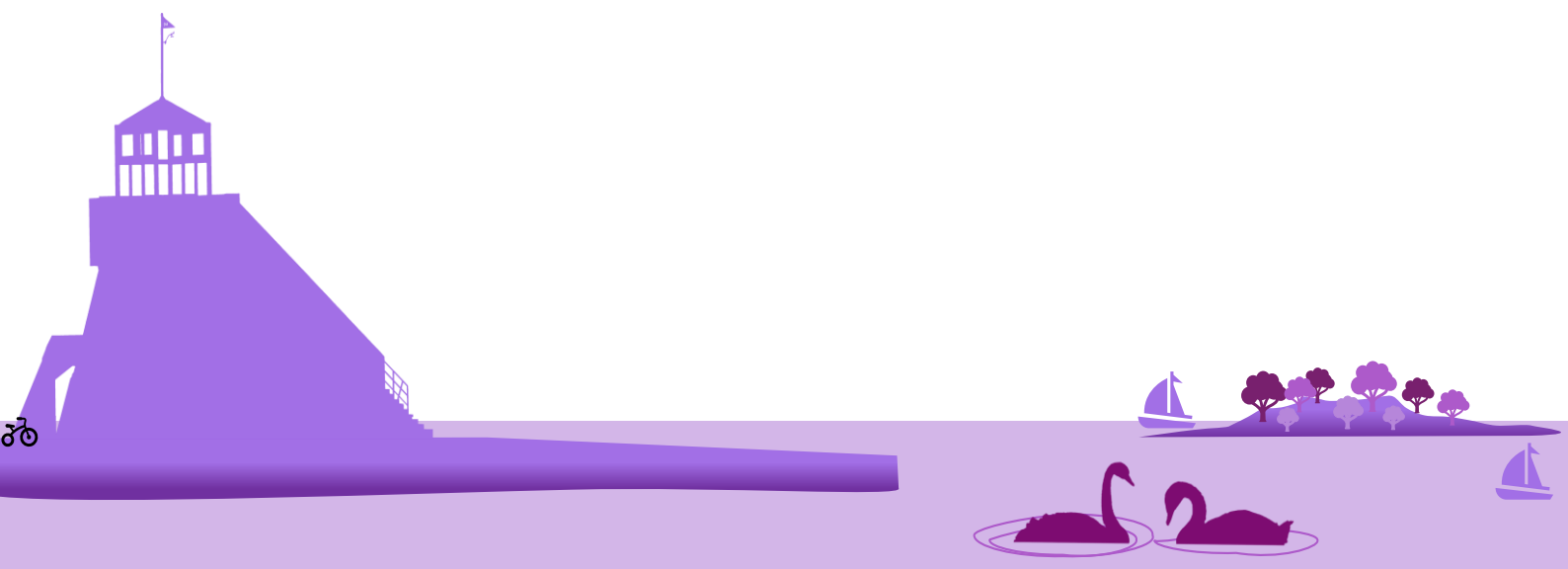
Aims: Our objective is to better understand allosteric pathways in p38 γ and, in particular, the role of nanosecond dynamics of its side-chains with NMR relaxation and molecular dynamics simulations.

Methods: We combine extensive high-field relaxation measurements on ¹³C 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. A new prototype was used to accelerate field cycling from 14 T down to 100 uT in as little as 80 ms.

Results: 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 assigned methyl resonances in p38 γ and have measured relaxation rates at over 28 magnetic fields covering nearly 6 orders of magnitude. Our analysis of relaxation with the extended model-free approach allows the determination of nanosecond motions even in the presence of chemical exchange.

Conclusion: UHRR is a new technique which allows to study the dynamics of large proteins over a broad range of motions (low ps to high us). In this work, we successfully apply this technique to fully characterize the dynamics of p38 γ .

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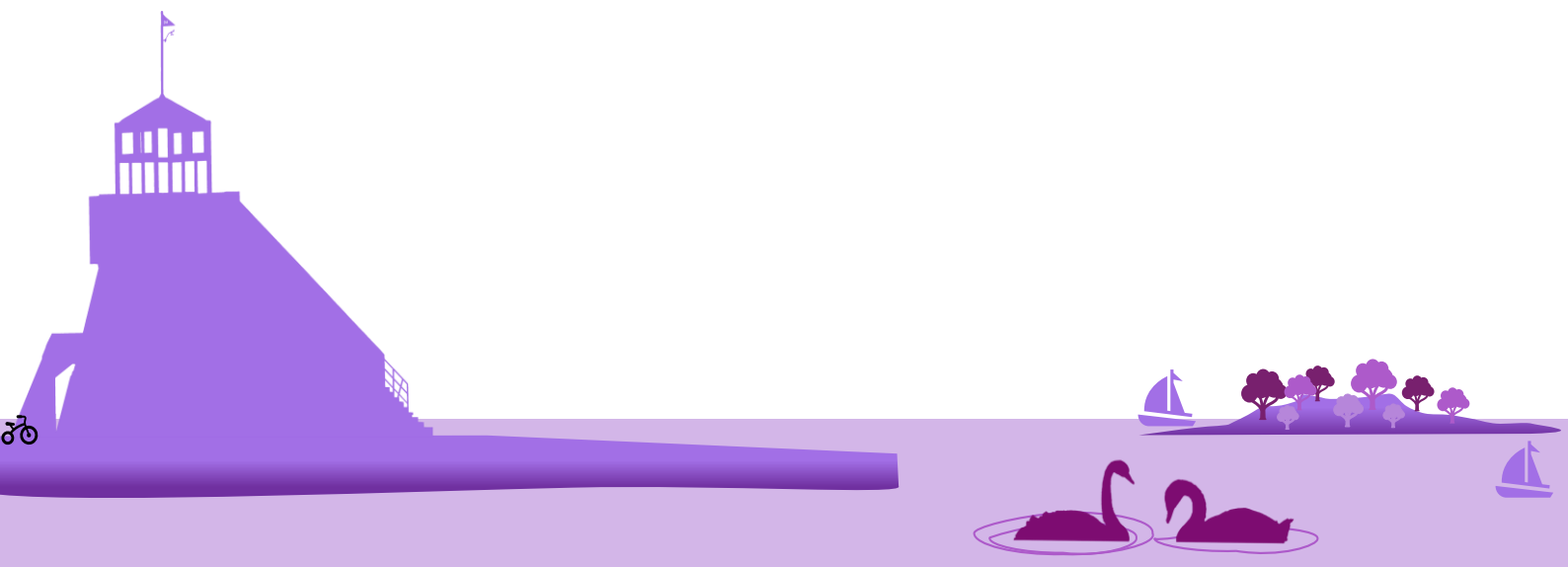
The disordered multi-functional protein Disabled-2 and its role in clathrin mediated endocytosis

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The disordered protein Disabled-2 (Dab2) is a multi-functional protein taking on roles in among others clathrin mediated endocytosis (CME) and platelet signaling. In CME, Dab2 functions as an adaptor protein, linking clathrin to both the membrane and the receptor that needs to be internalized. The early stages of this process requires the formation of an intricate network with many other adaptor proteins. One of these, Eps15, is the earliest arriving protein at the endocytic pit. We use solution NMR to study the network formed between the disordered part of Dab2 and the three folded EH domains of Eps15. Our results show a highly dynamic and multivalent network between the EH domains, Eps15's own IDR, and Dab2, which further leads to Dab2 being recruited into liquid-like condensates of Eps15. Additionally, the ~600 residue long disordered region of Dab2 contains multiple (putative) phosphorylation sites, proposed to regulate the intracellular location of Dab2 and thereby also its function. We study how these phosphorylation events affect the dynamics of Dab2 and its interactions with its partner proteins thereby gaining insights into how the multi-functionality of Dab2 is regulated.



Structural divergence in the dynamics of endocytic FCHO homologs

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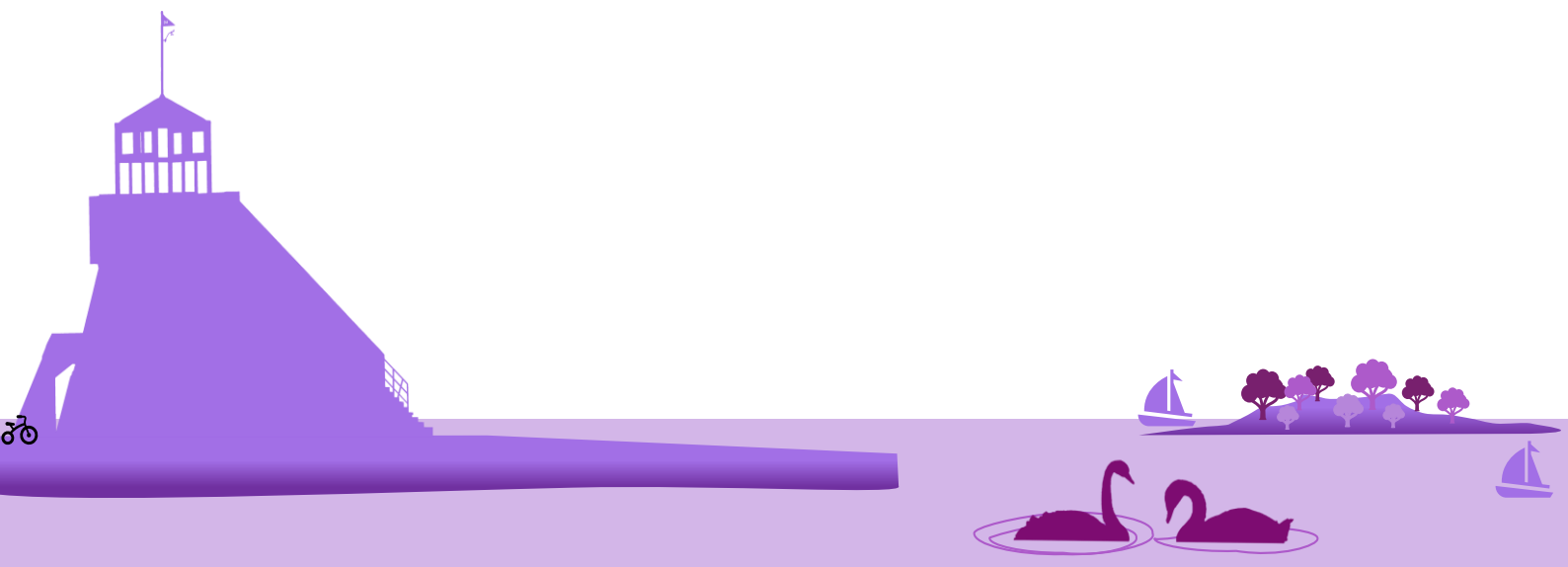
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Clathrin-mediated endocytosis (CME), the process by which cells internalize cargo through vesicles, relies on a complex sorting mechanism that operates with high spatiotemporal precision. To accomplish this, a diverse array of proteins containing a combination of folded domains and disordered regions work together, establishing a dynamic network of interactions. Due to the wide range of cargoes being internalized and the selectivity required, evolution has generated various homologs of the same protein, each participating in different endocytic pathways. A clear example is the endocytic initiator FCHO1/2, which exists as two homologs that initiate the uptake of different cargoes in distinct tissues. Although both homologs share a similar domain architecture, with well-conserved folded domains, their long intrinsically disordered regions differ substantially in sequence. How does this divergence influence the distinct biological functions of these homologs? How does this evolutionary difference affect the structure and dynamics of the FCHO disordered regions?

In this study, we investigated the structural dynamics of the intrinsically disordered domains in FCHO1 and FCHO2 proteins using NMR spectroscopy. Surprisingly, NMR revealed the presence of transient secondary structure elements with different levels of structure in FCHO1, which were completely absent in the FCHO2 homolog. These structured elements lead to different dynamic properties that we assessed by spin relaxation experiments. Notably, the structural and dynamic differences observed between the two homologs also translate into different recognition patterns for key FCHO partners involved in the initiation of CME.

Overall, our structural study characterizes the role of evolutionary divergence between endocytic homologs, providing insights into cargo selectivity and the molecular decisions that govern endocytosis.



Multisite phosphorylation of Dishevelled in Wnt signal transduction

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Wnt signalling has 3 core components: the receptor FZD, the downstream effector Dishevelled (DVL) and the regulator Casein kinase 1 (CK1), which phosphorylates DVL after Wnt pathway activation. Although the basic principles of Wnt signal transduction are known, the mechanistic aspects of DVL phosphorylation remain elusive.

We aim to answer the following: What DVL sites get phosphorylated? Mechanism of phosphorylation? Function of phosphorylation?

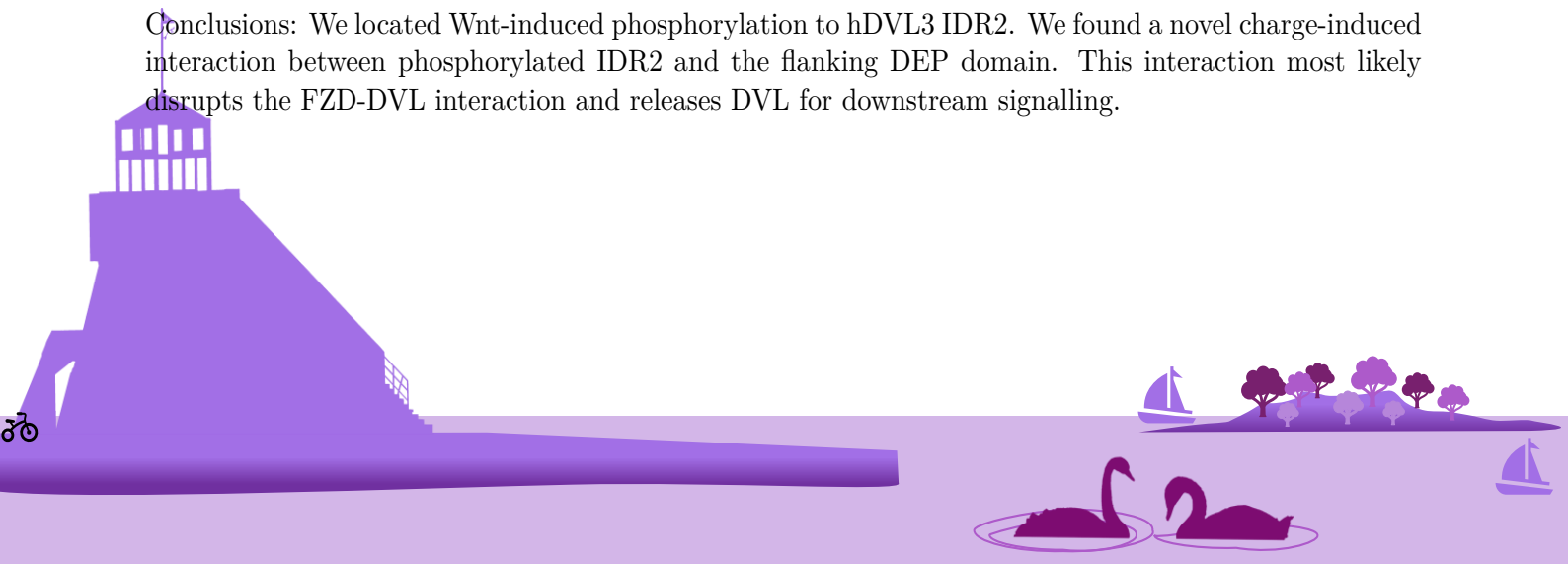
Our work is multidisciplinary with the following methods: Cell-based assays, NMR, MS and HDX.

Results: Our analysis of Wnt-induced phosphorylation of DVL located the phosphorylation in the second intrinsically disordered region (IDR2) of hDVL3. We purified IDR2 either alone or together with flanking structured domains PDZ and DEP and phosphorylated them in vitro by CK1 δ or ϵ . We found up to 20 serine (S) or threonine (T) residues of IDR2 to be phosphorylated. We analysed the multisite phosphorylation by real-time NMR and discovered 3 S/T clusters that differ markedly in their rates. The phosphorylation pattern was confirmed in PDZ-IDR2-DEP by using ^{15}N serine-selective labelling and $^{15}\text{N}/^{13}\text{C}$ threonine-selective labelling.

Next, we found out by chemical shift perturbation that the phosphorylated IDR2 interacts with the flanking DEP domain but not with the PDZ domain. This was further confirmed by HDX. The presence of 500 mM KCl abrogated the interaction, disrupting charge-driven interaction between negatively charged phosphate groups and positively charged DEP.

We confirmed the importance of the phosphorylation for Wnt signal transduction in cells by S/T mutants and analysed their ability to bind DEP. Alanine mutant could do neither of these.

Conclusions: We located Wnt-induced phosphorylation to hDVL3 IDR2. We found a novel charge-induced interaction between phosphorylated IDR2 and the flanking DEP domain. This interaction most likely disrupts the FZD-DVL interaction and releases DVL for downstream signalling.



NMR characterization of intrinsically disordered RNA polymerase regions introducing diversity into bacterial transcription

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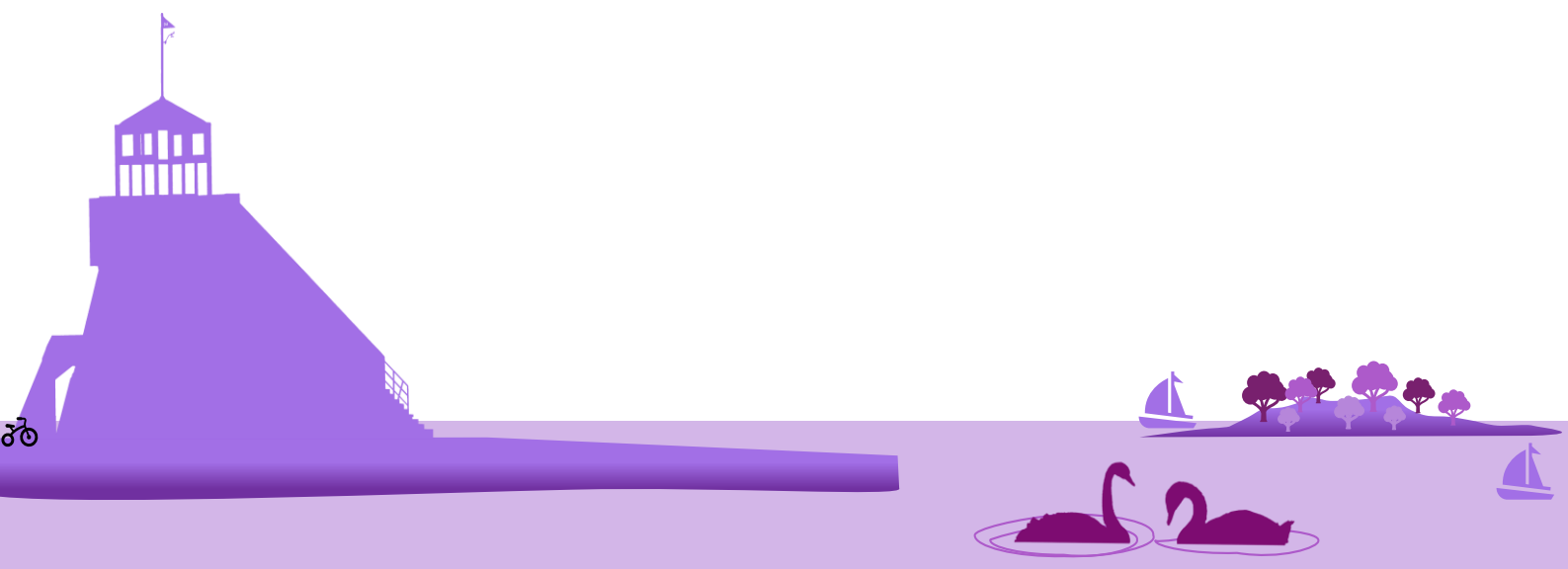
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Whereas the use of NMR in structure determination of well-ordered proteins is limited, NMR is the method of choice in structural characterization of intrinsically disordered regions (IDR) of proteins and in studies of protein dynamics. IDR are more frequent in Eukaryotes, but play important roles also in bacteria. We have previously solved structures and described dynamics of ordered N-terminal domains of delta subunit and of sigma A factor of RNA polymerase (RNAP) of *Bacillus subtilis*, and characterized functionally important conformational behavior of C-terminal IDR of the delta subunit. The aim of the so far unpublished study presented here was to describe structural features and dynamics of IDRs that distinguish RNAP of pathogenic *Staphylococcus aureus* and of a model of Mycobacterial pathogens *Mycobacterium smegmatis* from RNAP of the model Gram-positive bacterium *B. subtilis*. We used advanced non-uniformly sampled assignment and relaxation experiments, paramagnetic relaxation enhancement, small-angle X-ray scattering, and computational methods to characterize the RNAP delta subunit of *S. aureus*. In addition, we applied chemical shift mapping to characterize interactions of disordered N-terminal domains of *M. smegmatis* sigma A with a new transcription factor discovered in one of our laboratories. The results showed a dramatic difference between the conformational behavior of the delta subunits of *S. aureus* and *B. subtilis* that correlates with a lack of an otherwise conserved stretched of lysines in *Staphylococci*. In the case of *M. smegmatis* sigma A, we observed that the N-terminal IDR unique for Mycobacteria is involved in physiologically relevant interactions. In conclusion, our NMR analysis provided insight into molecular basis of specific roles played by IDRs unique for certain bacteria, including serious pathogens. This work was supported by Czech Science Foundation grant No. 25-16037S.



Dynamics of miR-34a in human Argonaute2

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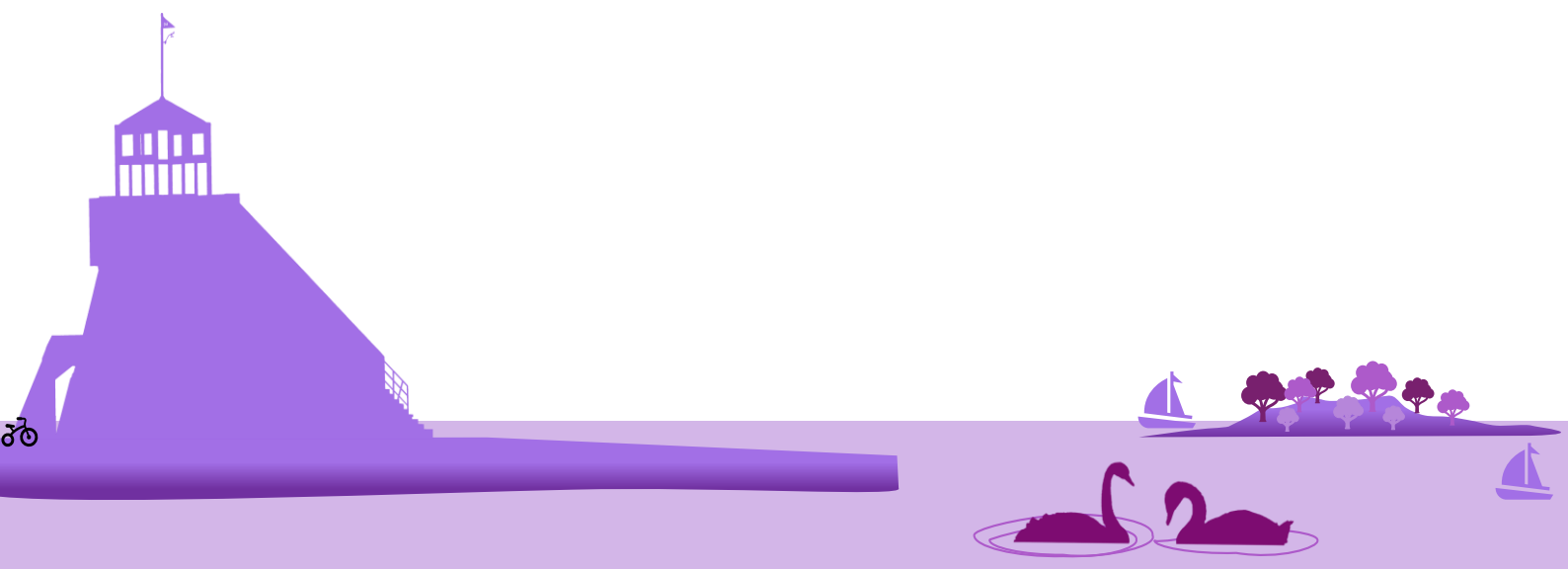
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The mechanism of microRNA (miR) based mRNA regulation relies on the structural understanding of the RNA-induced silencing complex (RISC) and how miR is organized within this complex. The core of the RISC consists of miR and Argonaute2 (Ago2) protein. X-ray crystal structures provided the basic structural information of miR in Ago2, however, due to dynamics, certain regions of miR could not be defined. NMR spectroscopy bridges this gap and provides a complete picture of the structural organisation of miR within Ago2. Due to the large size (110 kDa) of the protein:RNA complex and challenges in obtaining large amounts of sample, conventional solution/solid-state NMR experiments could not be employed. Using dynamic nuclear polarisation (DNP) assisted solid-state NMR and magic angle spinning (MAS) alleviates these shortcomings. Here, the structure organisation and dynamics of miR-34a in human Argonaute2 (hAgo2), using guanosine-specific isotopic labelling and DNP MAS NMR is elucidated. Experiments supported by molecular dynamic simulations revealed that the non-A-form helical conformation of miR-34a increases when incorporated into hAgo2 and further increases upon binding to SIRT1 mRNA compared to the free miR-34a or the free mRNA:miR duplex. A distribution of C2'- and C3'-endo sugar puckering conformers at nucleotide-specific level was observed from the C8 – C1' correlation, revealing trapped dynamics and providing insights into the different conformations upon freezing. These observations provide insights into the molecular dynamic basis of miR-based mRNA regulation while also providing a proof-of-concept that experiments under cryogenic conditions, e.g. at 90K, can trap and with that, reveal frozen dynamic states using methods such as (DNP-enhanced) MAS NMR or Cryo-EM.

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Rapid Interpretation of Protein Backbone Rotation Dynamics Directly from Spin Relaxation Data

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In intrinsically disordered proteins (IDPs), which do not fold into stable 3D structures, understanding their dynamics is crucial for understanding their functions. However, detecting this dynamics is challenging. NMR spin relaxation times are sensitive to motions on the timescale of picoseconds to tens of nanoseconds and can therefore be used to study protein bond dynamics. However, interpreting the measured relaxation times is not straightforward for disordered proteins and molecular assemblies with complex dynamics.

In our work [1], we numerically demonstrate that the effective correlation times of bond rotations, τ_{eff} , can be directly obtained from measured transverse R_2 relaxation rates using a linear relationship, provided that the assumptions behind the Redfield equations hold. These results enable the determination of magnetic-field-independent and intuitively understandable parameter describing the dynamics of bonds in molecules.

We show that, in the case of partially disordered proteins, the dynamics of disordered regions occurs on the timescales of 1-2 ns, independently of the size of the protein or its disordered region. This suggests that the rotations of folded and disordered regions are independent of each other.

[1] J. Phys. Chem. Lett. 2024, 15, 40, 10204–10209.



The mechanism of human hnRNPC1 protein liquid-liquid phase separation driven by its disordered regions

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Human hnRNPC1, a core protein of the 40S ribonucleosome particles in the nucleus consisting of several hnRNP isoforms and pre-mRNA[1], phase separates alone under conditions similar to the cell nucleus enhanced by hnRNPA1 protein or RNA suggesting that liquid-liquid phase separation (LLPS) plays an important role in assembly of the ribonucleosome particle. Our aim was to reveal the molecular mechanism of the hnRNPC1 LLPS to understand its function.

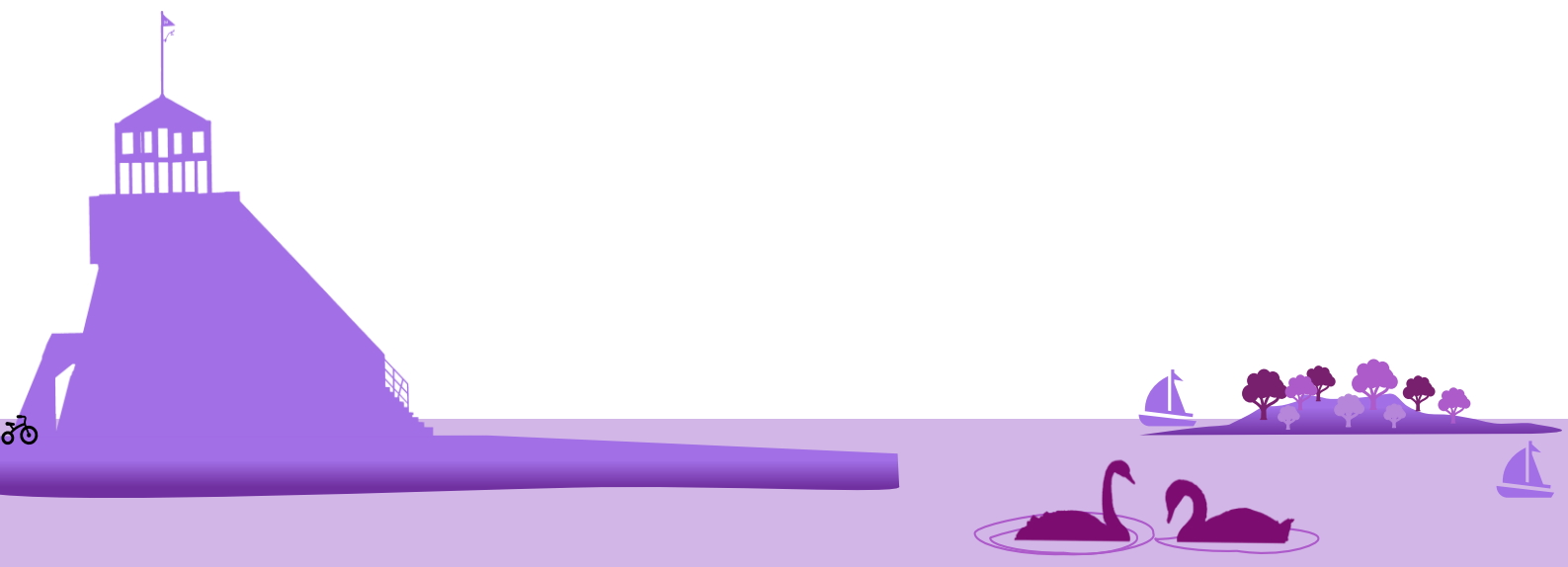
We obtained structural and dynamics information on the interacting disordered regions (IDRs) in dispersed and condensed phase using biphasic samples[2] applying various NMR methods including intramolecular NOE, relaxation measurements, isotopically filtered-edited DOSY combined with the recently introduced LLPS-REDIFINE[3], and ¹⁹F-NMR of labelled RNA. We characterized the reduced molecular motions in droplets and the exchange through the surface of the droplets resulting in a quantitative description of the protein-protein and protein-RNA droplets. We demonstrated how ultra-high field (1.2 GHz) NMR could be exploited for detailed examination of phase separation mechanism, a currently emerging field in biochemistry.

The core interacting regions in hnRNPC1 LLPS are the middle IDR encompassing a tyrosine-rich transient helical motif followed by the basic region (IDR1) and the C-terminal acidic IDR (CTD). The transient helix region induces oligomerization of IDR1 driven by homotypic π - π aromatic interactions. The heterotypic interactions are electrostatic forces between the positively charged basic region and the negatively charged CTD or RNA.

The mechanism of the hnRNPC1 phase separation involves the electrostatic attraction followed by the enhancement of the by π - π interaction based self-assembly driven by the tyrosine-rich transient helix.

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Selectively $^{13}\text{C}/^2\text{H}/^{15}\text{N}$ -Labelling of Arginine and Proline to Probe Protein Dynamics and Interaction by Solution- and MAS-NMR Spectroscopy

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Protein NMR spectroscopy offers a valuable approach to investigate dynamic processes including the quantitative characterization of less populated conformational states. Deuterated samples with isolated ^{13}C - ^1H or ^{15}N - ^1H spin systems are invaluable for obtaining high-sensitivity high-resolution spectra, and probe dynamics quantitatively. We established organic synthesis of Arg and Pro with ^{15}N - ^{13}C - $^1\text{H}_2$ spin systems, and its incorporation into otherwise fully deuterated proteins. Starting from aspartate, we employ commercially available isotopically labelled compounds to synthesize both selectively labelled arginine and proline residues at low costs. The synthetic route diverges at a key intermediate to give arginine in a fully organic synthesis and proline through enzymatic conversion of ornithine. These amino acids are used for cell-based and cell-free protein production. We demonstrate the usefulness of the approach by several solution- and MAS-NMR applications. In a first application, we studied the interaction between ubiquitin and the third Src homology 3 domain of yeast protein Sla1 (Sla1 SH3-3), which highlighted the probes competence to sample rapid dynamics. We used the approach further to probe dynamics in a crystalline protein using MAS NMR. Unlike solution-state NMR, MAS NMR can see a wide range of time scales, which allowed us to quantify nanosecond-to-microsecond dynamics in crystalline ubiquitin.



Investigating the Relaxation Characteristics of ^{23}Na in Variable Dynamics compartments using Diffusion and Relaxation NMR

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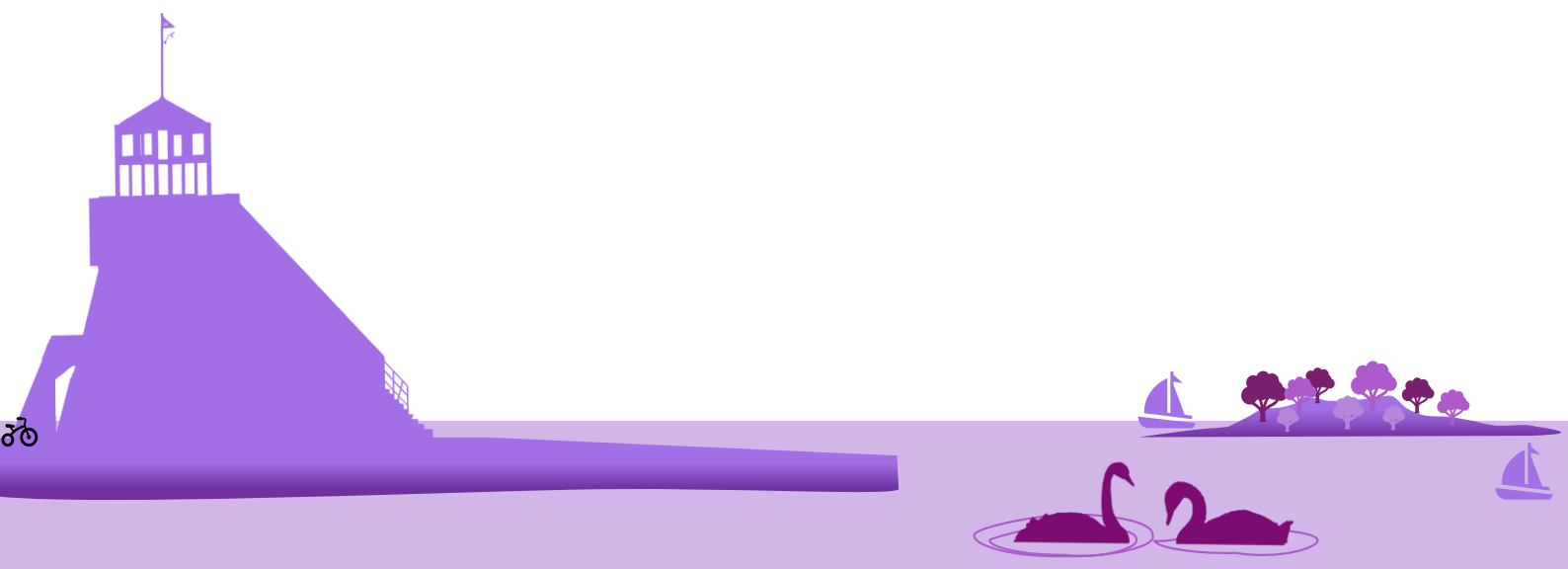
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Sodium ions play a crucial role in biological systems. Sodium MRI is essential to assess brain health since sodium plays a key role in cellular homeostasis and neuronal activity¹. Changes in sodium distribution may indicate neuro diseases. Limitations occur in quantifying intra- and extracellular sodium because ^{23}Na is a quadrupolar $S=3/2$ nucleus, and its relaxation does not reflect the typical protons behavior². Therefore, it would be useful to derive suitable fitting functions to model ^{23}Na relaxation in different dynamics environments. Sodium behavior in solutions containing polymers such as polyethylene glycol (PEG) is of significant interest. PEG is widely used in biological, pharmaceutical, and chemical applications due to its biocompatibility and ability to modulate solution properties. Adding PEG alters the viscosity and molecular dynamics³, which in turn affects the dynamics and relaxation properties of sodium ions. This study aims to define and test models describing sodium relaxation in different dynamic compartments. We investigate ^{23}Na NMR relaxation in aqueous solutions by adding PEG to modify ^{23}Na dynamics. Water solutions with varying PEG-400 concentrations (0%, 20%, 40%, 50%, 60%, 80%, 90%, 100% w/v) were prepared and different quantities of sodium were added (10-140 mM). Using a Bruker Avance-400, Pulsed-Field-Gradient (PFG) NMR was used to quantify diffusion of PEG, water protons and ^{23}Na in each solution. Inversion-recovery and CPMG sequences were used to determine T_1 and T_2 relaxation times. Specific models to fit the relaxation time data of ^{23}Na at different dynamics were tested. Ion-macromolecule interactions, hydration effects, and molecular crowding were analyzed. The findings related to ^{23}Na relaxation in different dynamics of complex aqueous environments may have implications for designing PEG-based formulations in biomedical and industrial applications and for better understanding brain and human tissues ^{23}Na -MRI investigation.



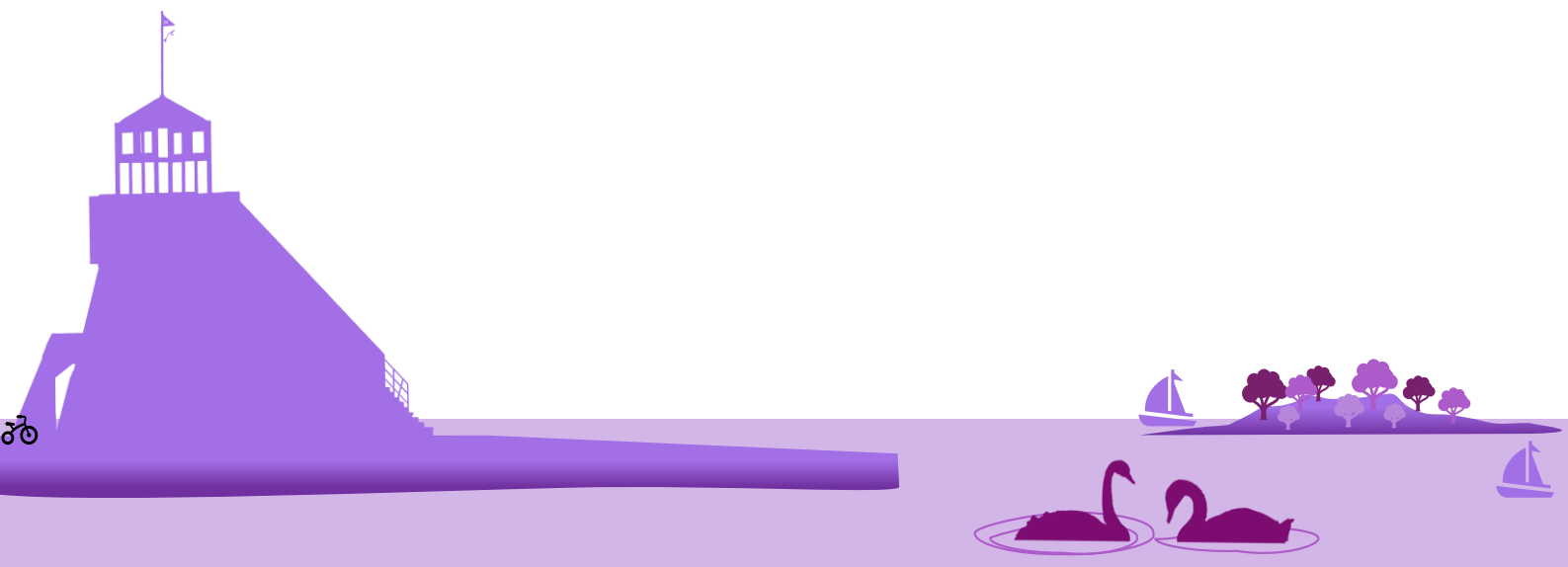
Conformation and dynamics of lutein in Light-Harvesting Complex II

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The most abundant light-harvesting antenna complex of green plants, Light-Harvesting Complex II (LHCII), balances between a light-harvesting and a photoprotective state, where excitations are quenched to prevent photodamage. Despite many research for the last decades, the LHCII antenna switch, involving modified carotenoid-chlorophyll interactions, has not been resolved. We refolded the LHCII apoprotein with ^{13}C isotope-labeled lutein and all other pigments unlabeled, to assess the structures of lutein in LHCII by Magic Angle Spinning (MAS) NMR spectroscopy. Based on ring-current shifts of lutein C18' methyl proton resonances, we could determine the orientation of the lutein molecules that have the ionone e-ring head faced towards the stromal side and b-ring faced towards the lumen. A comparison of LHCII in light-harvesting and quenched states revealed chemical-shift differences that suggest a change in orientation or position of the lutein head groups. The study demonstrates for the first time that lutein, a key chromophore in tuning light harvesting, can adapt adopt different conformations inside the LHCII protein scaffold, associated with specific functional states.



Advanced isotope labelling in mammalian cells enables studying functions and interactions of GPCRs

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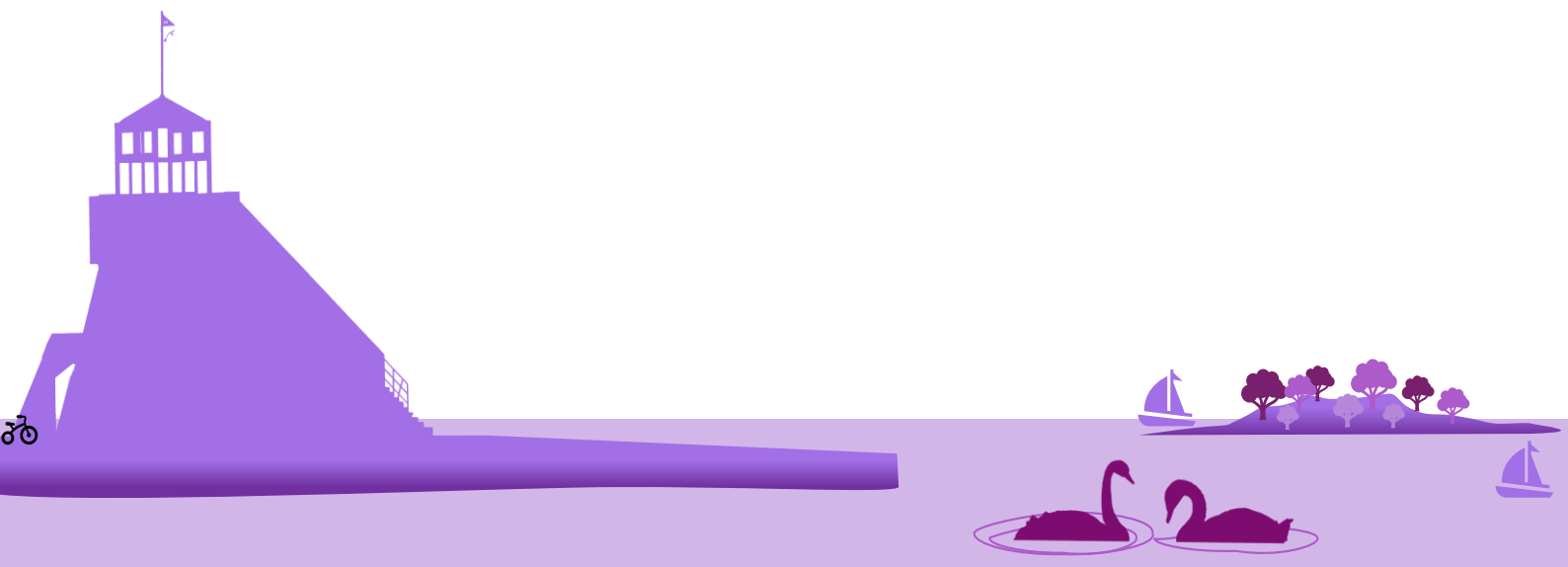
G-protein-coupled receptors (GPCRs) represent the drug targets of more than a third of all FDA-approved drugs. Nevertheless, there is still enormous unexploited therapeutic potential by increasing specificity, and NMR is well suited to study ligand binding, the dynamic equilibria related to GPCR function, and the disordered GPCR regions crucial for phosphorylation and subsequent inactivation.

However, to obtain correctly folded GPCRs for NMR studies, expression in mammalian cell culture is often required, where isotope labelling schemes are not as established as in lower expression systems. We therefore set out to develop simple and cost-effective protocols for uniform and specific labelling in mammalian cells.

Uniform ^{13}C and ^{15}N labelling in HEK 293 cells is achieved using isotopically enriched protein extracts of *Saccharomyces cerevisiae* (^{15}N) and *Ralstonia eutropha* (^{13}C and ^{15}N). These efficient protocols also allow “unlabelling” by addition of excess unlabelled amino acids. In this way, threonine resonances in $[^{13}\text{C}, 1\text{H}]$ and $[^{15}\text{N}, 1\text{H}]$ HSQCs were unambiguously identified.

In a second labelling approach, the popular ILV-labelling strategy was established by identifying suitable precursors for HEK cells. This enables ILV- ^{13}C -methyl labelling with local deuteration, making large proteins expressed in HEK cells amenable to NMR studies.

The ability to express GPCRs in mammalian cell culture allowed functional studies of interactions of GPCRs with other proteins and ligands, as well as establishing a novel assay for determining the affinity of drug-like molecules to a GPCR using a ^{19}F -detected competition assay.



Unraveling the Internal Dynamics of p38 α through Stereospecific Methyl Labeling in Solid-State NMR

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The protein kinase p38 α (MAPK14), a highly conserved 41 kDa enzyme, plays a pivotal role in the MAPK/ERK signaling pathway where it translates external stimuli into cellular responses. It is essential for key processes such as proliferation, differentiation, and cell division. Dysregulation of p38 α has been linked to inflammatory diseases, neurodegenerative disorders, and cancer, making it a promising therapeutic target.

We aim to characterize the effects of two known p38 α -binders—sorafenib, an approved anti-cancer drug known to inhibit multiple kinases, and β -OG, an amphiphilic molecule often used for membrane protein stabilization—on the global and local dynamics of p38 α . To achieve this, we combine NMR of p38 α in either solution or a crystalline environment with a stereospecific isotope-labeling approach that enables analysis of methyl groups in a highly deuterated environment. This technique generates very well-resolved C-H correlations that allow rapid peak assignment and facilitate quantitative comparison between conformational-exchange dynamics in solids and in solution.

To facilitate characterization of p38 α by solid-state NMR, we are establishing a protocol for detecting CHD2 methyl groups in paramagnetically doped p38 α crystals using solid-state NMR. The size-independence of solid-state NMR, as well as slight shifts for the timescales of internal dynamics, offers unique advantages for studying large complexes and may ultimately provide insights into regions of the enzyme that are obscured in solution NMR. By integrating solution and solid-state NMR techniques, our work aims to deepen understanding of p38 α dynamics and interactions at both molecular and systemic levels.



Intracellular loop 3 dependant allosteric effect of PIP2 on the function of the prototypical GPCR b1 adrenergic receptor

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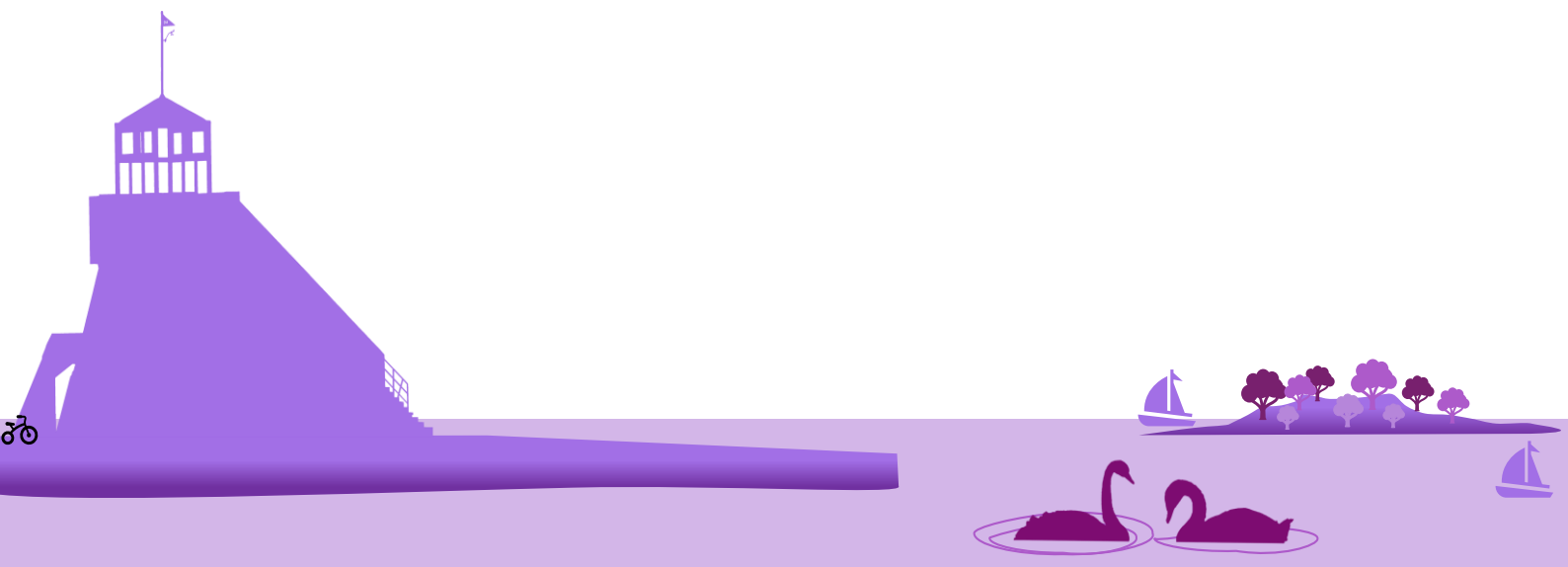
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Class A G protein-coupled receptors (GPCRs) follow a common activation mechanism. Agonists bind to the extracellular side of the receptor causing a shift in the conformational equilibria towards an active state that is competent for binding intracellular binding partners (IBPs), canonically G proteins.

Growing evidence indicates that membrane lipids influence the function of receptors, either through the intrinsic bulk biophysical properties of the bilayer or through binding of specific lipids at allosteric sites. One such lipid, phosphatidylinositol 4,5-bisphosphate (PIP2) has been shown to affect the function of a range of membrane proteins.

Here we use biolayer interferometry (BLI) to show that the presence of PIP2 can modulate the affinity between detergent solubilised b1AR and an engineered version of the canonical IBP, miniGs. Further, we demonstrate that the effect of PIP2 is dependent on the presence of intracellular loop 3 (ICL3) on the receptor construct. ¹H,¹³C HMQC NMR of b1AR labelled with ¹³C-methyl-methionine in the presence of miniGs corroborates the BLI data. HMQC spectra of b1AR bound to the full agonist, isoprenaline, reveal the allosteric effect of PIP2. In the absence of ICL3 PIP2 causes the receptor to adopt a state which is less competent for binding IBPs. In the presence of ICL3 no such PIP2-induced inactivation occurs. Instead, the lipid stabilises a miniGs bound conformation of the loop which is presumably favouring the formation of ternary complex.

This work demonstrates the intricate interplay between cell membrane components and GPCRs, furthering understanding of how cell signalling may be fine-tuned in native environments. Also, it alludes to potential avenues of drug development to target and modify GPCR function.



Probing the backbone and binding sites of light responsive diamagnetic Zinc protoporphyrin and semi-artificial peptide scaffold by MAS NMR.

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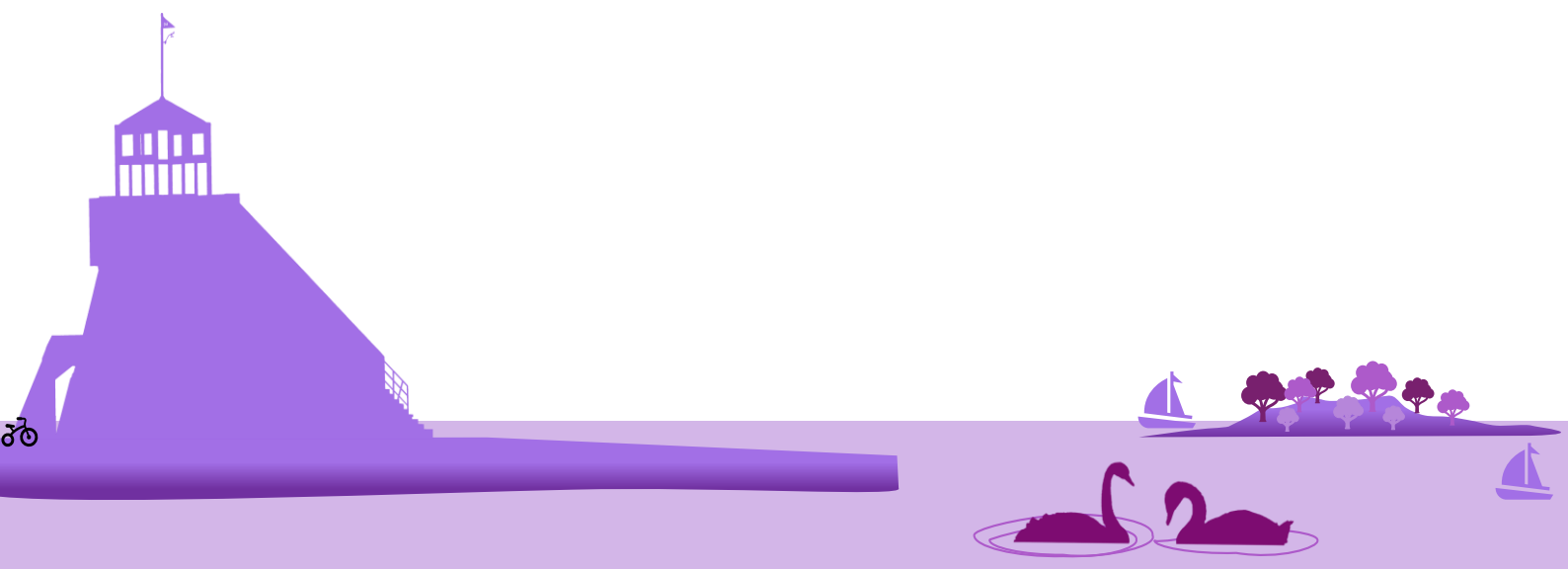
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In nature antenna systems harvest light and transfer the excitonic energy to photosynthetic reaction center, where conversion to chemical energy occurs. One of such antenna systems, the chlorosome, found in photosynthetic green sulfur bacteria *Chlorobaculum tepidum*, has baseplate membrane component. This baseplate is a scaffolding super structure, formed by protein CsmA and bacteriochlorophyll a, Bchl_a. Here, we use truncated CsmA construct with central amphipathic helix, tCsmA (83% hydrophobicity index) and zinc protoporphyrin IX, ZnPP as the chromophore binding partner, to put forth a simple 2D charge separator complex mimicking the natural CsmA-Bchl_a baseplate.

Zinc porphyrins are well known photosensitizers and tend to form self-aggregates in solution. Embedding Zinc porphyrins within binding sites of proteins or small peptides prevents this aggregation. The tCsmA is fused with a novel auto-cleavable fusion tag EDDIE and is recombinantly overexpressed in inclusion bodies in *E. coli*. Both tCsmA and ZnPP are ambipolar and dissolve only in excess alcohol buffered conditions. The tCsmA-ZnPP complex is sedimented into MAS rotor after dialysis of stoichiometric mixture of ZnPP and tCsmA in phosphate buffer.

Here, we report the solid state NMR studies on the uniformly ¹³C,¹⁵N labelled tCsmA-ZnPP at 750MHz and 1200MHz. Our data shows the complex is well preserved in sediment state. We further do homology backbone chemical shift assignments through a series of ¹³C,¹³C DARR spin diffusion and ¹⁵N,¹³C double CP/MAS experiments. This helps in identifying the amino acid spin systems.

Additionally to the ¹³C detected experiments, ¹H detected solid-state NMR under magic angle spinning provides increased possibilities to probe the rigid and flexible regions of tCsmA peptide in ZnPP bound state. These results will provide the basis to understand the interaction mechanisms behind light harvesting ZnPP and tCsmA peptide complex.



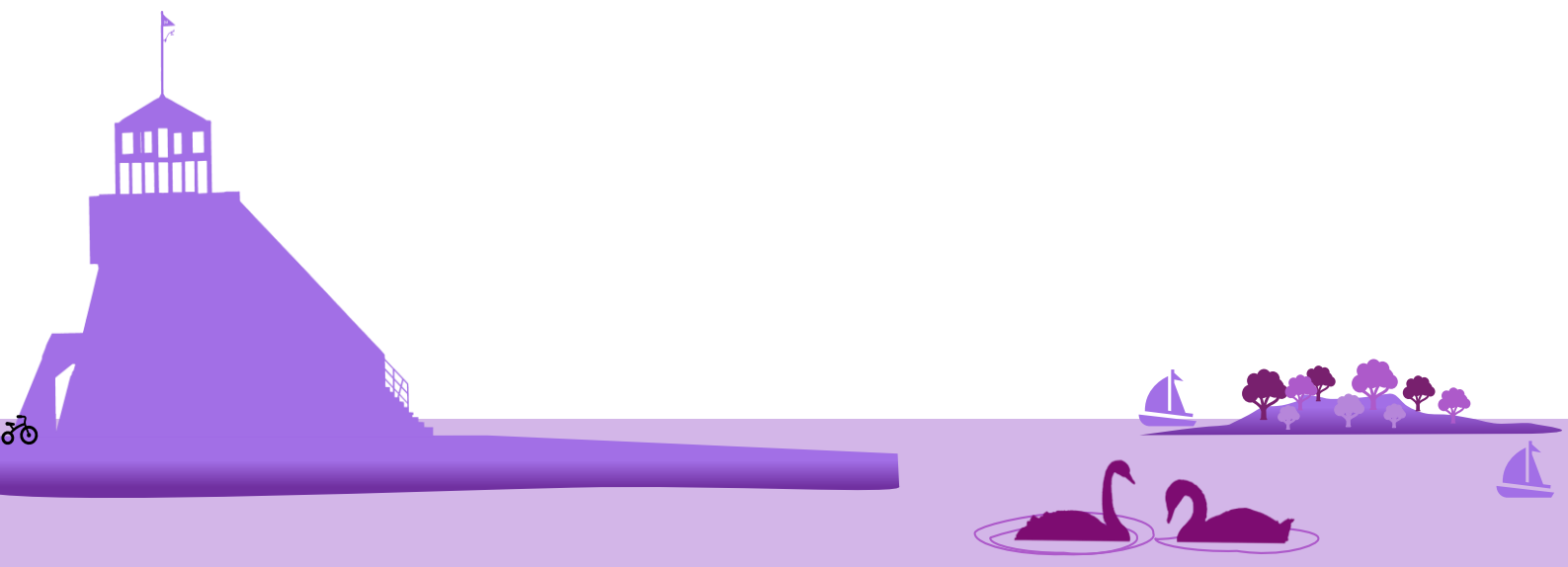
Structural studies with 2D ^{13}C detected Super-Resolution solid state NMR Spectroscopy at moderate MAS

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Structure determination by solid-state NMR spectroscopy is a challenging task given the lower resolution of solid-state NMR data compared to structural studies by solution-state NMR methods. Structure refinement and determination in the solid-state typically requires spin-dilution labelling schemes with deuteration or ^1H detection at fast MAS, the latter requiring specialized equipment and expertise not widely available. Here we demonstrate the experimental implementation of apodization in the indirect dimension through dynamic scan sampling, achieving a 2-fold increase in spectral resolution in 2D spectra at moderate (20 kHz) MAS. The super-resolution method affords greater sensitivity compared to digitally apodized data. Through the super-resolution method, long-range ^{13}C - ^{13}C contacts become tractable in 2D solid-state at moderate MAS without resorting to ^1H detection methods making structural studies by solid-state more broadly achievable with commonly available equipment and expertise. We use these restraints for structure refinement of the viral capsid protein AP205 utilizing a non-crystalline fully protonated U- ^{13}C , ^{15}N -labelled sample. The reduced peak overlap enables automated assignment methods at these spinning speeds, further expediting the structure determination process in the solid-state.



A Deeper Understanding of APLF's Role in the NHEJ Pathway Through Intramolecular Interactions Revealed by NMR

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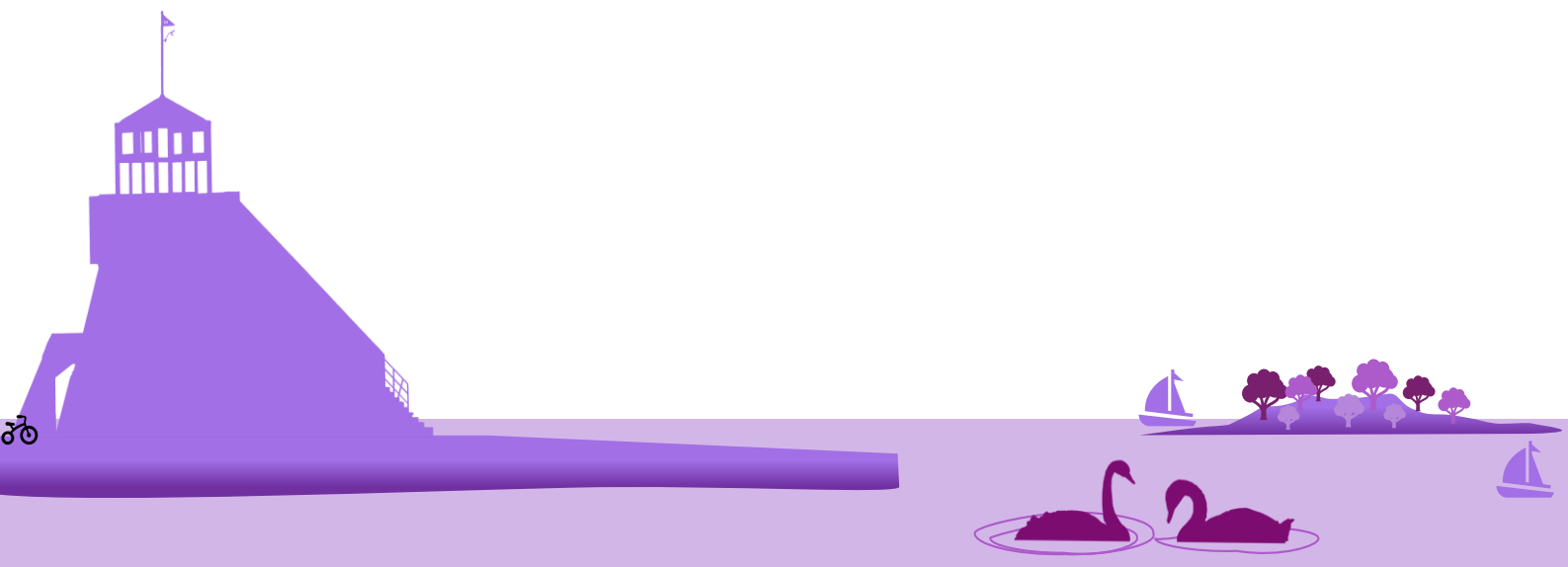
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Non-homologous end-joining (NHEJ) is the primary pathway for repairing DNA double-strand breaks (DSBs) in eukaryotes. NHEJ involves numerous proteins, many of which contain intrinsically disordered regions (IDRs), making NMR an ideal tool for their study. Aprataxin and PNKP-Like Factor (APLF) is an NHEJ protein characterized by extensive disorder and comprising four functional regions: the fork-head-associated domain (FHA), the middle domain (MID), and the C-terminal acidic domain (CTAD). The FHA binds to the X4L4 ligase complex via XRCC4, while the MID interacts with the Ku heterodimer, the initial responder to DSBs. APLF is described as a scaffolding protein that stabilizes the NHEJ complex, tethers DNA synapsis, and promotes ligation. However, the exact mechanisms and underlying dynamics remain unclear.

We investigated the interactions of APLF with other NHEJ proteins using NMR titrations. We further characterized the exposed interactions using advanced NMR techniques such as CPMG relaxation dispersion and CEST experiments. Leveraging NMR's atomic resolution, we identified some binding sites and designed specific mutants. To complement our NMR approach, we employed single-molecule magnetic tweezers experiments.

Our findings describe two key intramolecular interactions: the binding of the CTAD to the FHA, and the homodimerization of the FHA. The CTAD binds to the FHA near its XRCC4 binding site, suggesting a potential competition between these partners. Additionally, the CTAD competes with DNA for binding to the Ku complex. Point mutations introduced in the FHA revealed a putative allosteric regulation mechanism between the CTAD binding site and the FHA dimerization interface.

This network of weak interactions may help better understand the role of APLF in the NHEJ pathway. While some findings may suggest how APLF can stabilise DNA synapsis with only Ku, others could provide insights into the dynamics and regulations of APLF in the assembly of the NHEJ complex.



NMR BIOMARKERS OF RADIATION DOSE-RATE EFFECTS

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Background and aims

The molecular mechanisms that regulate distinct biological responses to radiation dose-rates of different magnitudes are to be understood as high dose-rate -'FLASH'- radiation treatment emerges. NMR can follow molecular pathways involved in radiation response. We present herein new biomarkers of radiation dose-rate effects via high-field and Earth-field NMR spectroscopy.

Methods

Samples of glioblastoma U-251 and microglia BV-2 cells were irradiated in different dose-rate regimes. Doses between 0.2-30 Gy were delivered using gamma beams from a Co-60 source in the classical regime (Gy/s) and high-power laser-driven electrons for the ultra-high dose-rate regime (Gy/ns). NMR spectra recorded 2h after irradiation were analyzed in terms of principal components to establish biomarkers. New methods based on relaxation time constants measured in Earth's magnetic field (Terranova MRI) for transverse magnetisation and long-lived spin-order were also introduced for quantifying oxidative effects.

Results

We have identified new NMR biomarkers for radiation dose-rate effects: choline and lactate are consistent main contributors to principal components in irradiated cells. Increases in lactate and decreases in choline concentrations are observed with increasing radiation dose. These results were further confirmed by NMR analyses of samples from in-vivo irradiated animals (zebrafish, mice). High radiation dose-rates biomarkers reflect alleviated oxidative effects, i.e. free-radical recombination on short timescales. Free-radical recombination effects were equally detected via long-lived spin order and NMR relaxation experiments in Earth's magnetic field.

Conclusion

Biological responses to radiation dose-rate can be followed by NMR biomarkers on pathways related to oxidative stress and via NMR relaxation methods.

Thanks to: Research Ministry 'Nucleu', PN-III-P4-ID-PCE-2023-1836 UEFISCDI, EU-PNRR-C9-2022-I8-760110, ELI-RO 39,40/2024 (DRAM, REMILASI_FLASH).



Tracking of transported protons in the light derived pump Bacteriorhodopsin by ssNMR at 1.2 GHz at 100 kHz MAS

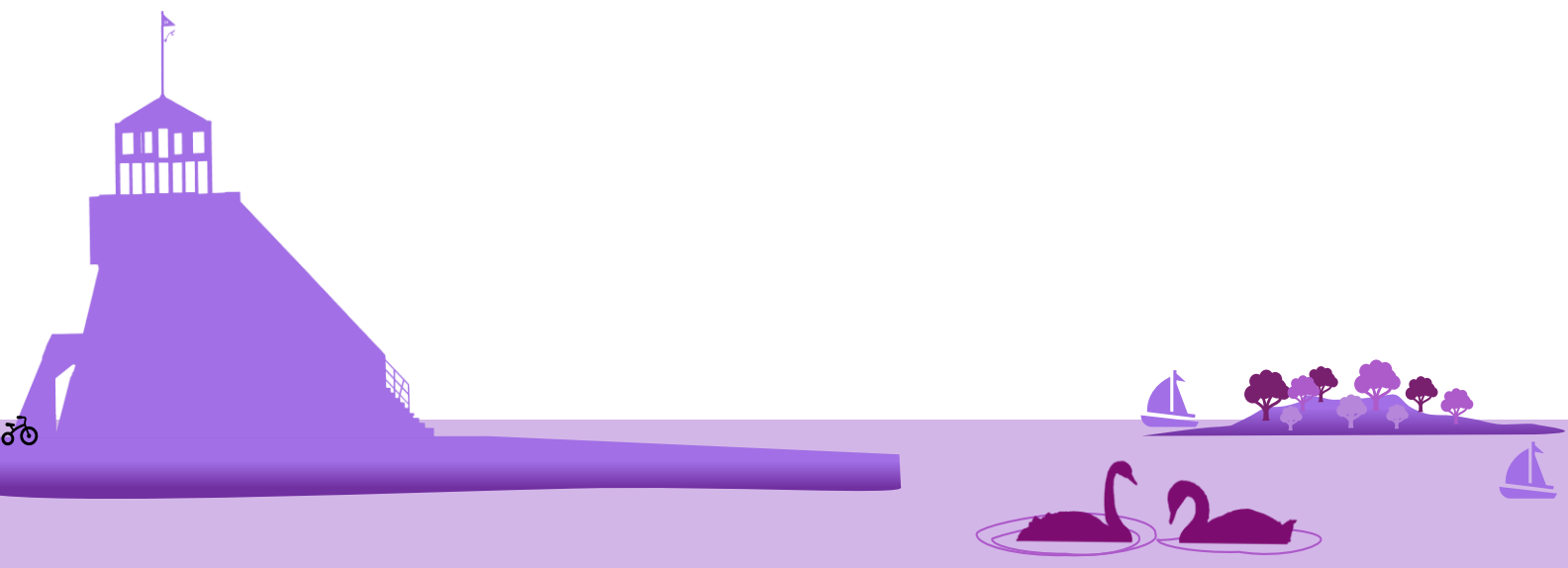
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Bacteriorhodopsin (BR) is a membrane protein (1), which pumps protons from the cytoplasm to the extracellular space. This process is governed by light absorption, involving the retinal Schiff base (RSB) and carboxyl protons of key aspartic acids (D865 and D96). Structures of BR have been solved by X-ray crystallography, electron microscopy, electron crystallography, and solution NMR. Even though structural information has revealed which amino acids are involved in the transport of protons, the pathway and aspects of the mechanism are not fully understood. Previously, a ssNMR study identified proton shuttling between D85 and RSB. In addition, the exchange of D85 and D96 carboxyl protons with water inside the active site was observed (2). By applying 100 kHz magic angle spinning (MAS) on a 1.2 GHz spectrometer, the $^1\text{H}, ^1\text{H}$ -NOESY and $^1\text{H}, ^1\text{H}$ -ROESY experiments allow us to distinguish dipolar and exchange contributions to cross peaks, thus enabling a quantitative view on to the proton flow. In particular, the ratios of exchange between protons of water molecules and RSB, D85 and D96 are accessible. This investigation is set out as a pilot study to the exploration of protein systems where protonation dynamics plays an essential role, such as voltage-gated ion channels, proton channels or pumps of viruses, or those with pH-dependent structural rearrangements.

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Hijacking of the Growth Hormone Receptor by Calmodulin

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The growth hormone receptor (GHR) signals through the JAK/STAT pathway and is mainly known for its role in growth regulation and metabolism. Cellular calcium influx resulting from growth hormone (GH) stimulation suggests a link between GHR and cellular calcium metabolism, both playing crucial roles in pathways related to cancer, obesity and cardiovascular diseases. However, mechanistic models of GHR signal transduction and regulation and the direct link to calcium metabolism are lacking, hindering causal rather than symptomatic treatments of GHR-related disorders. Using cell biology, isothermal titration calorimetry and NMR spectroscopy, we scrutinize the interaction and reveal multiple interaction sites for CaM on GHR, suggesting highly competitive interactions with signaling partners. In addition, we could show a GH-dependent and direct interaction between GHR and CaM in human cells, proving this interaction to be physiologically relevant. This establishes CaM as a novel regulator of GHR signal transduction, linking growth modulations with calcium-oscillations.

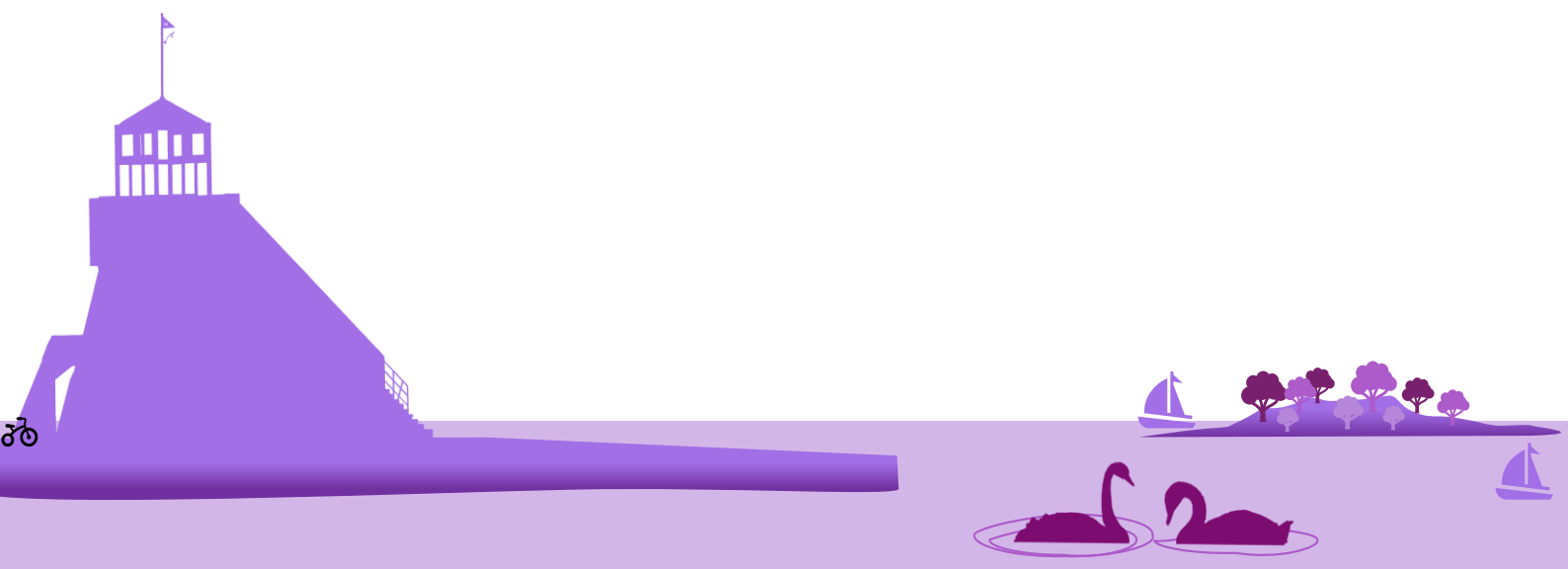
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SNAPS a Protein Backbone Assignment for the AlphaFold

Age: Simple NMR Assignments from Predicted Shifts

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SNAPS is a program that assigns protein backbone shifts by solving the mathematical assignment problem. Its development was spurred by the insight that assignments are often needed for proteins with known structures. While it's hard to predict the values of NH shifts with high accuracy, those of other backbone atoms are predicted with high fidelity by programs such as ShiftX2 and can be used for assignment.

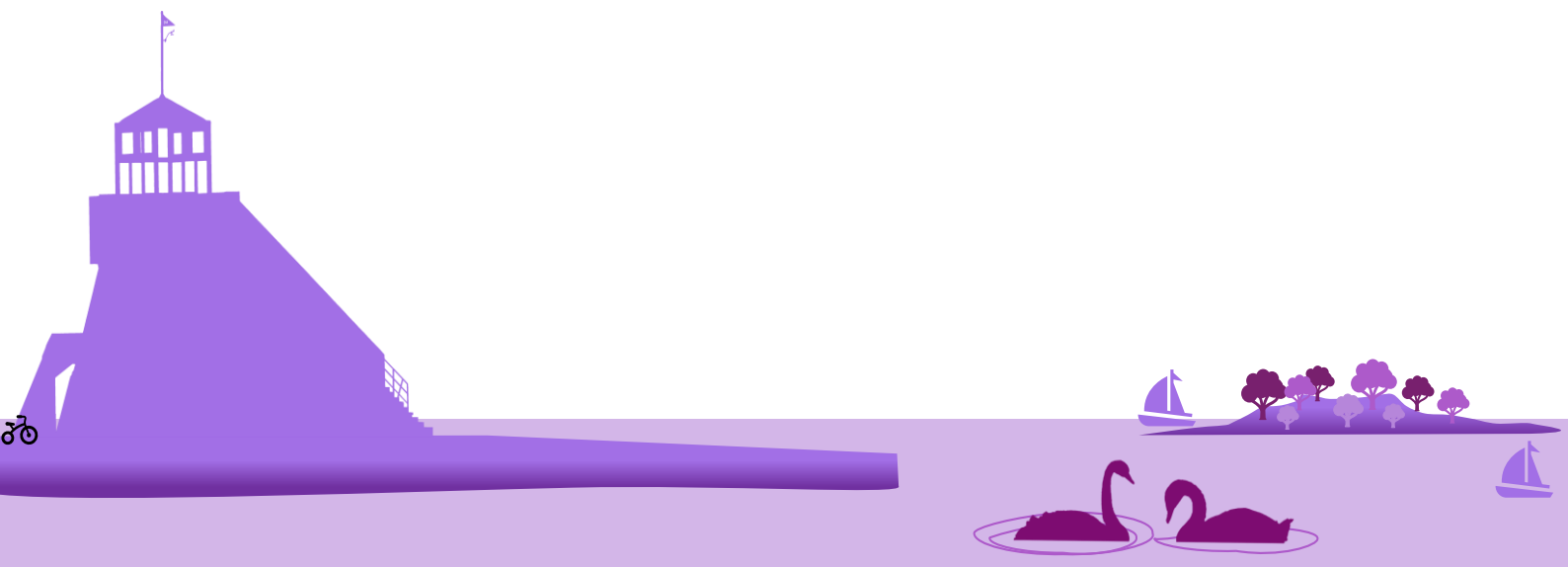
The impact of this approach is magnified by AlphaFold2's high quality structure predictions. An interesting approach using AlphaFold2 is to use assignment by SNAPS to find if disagreements exist between theory and measurement. This is likely to highlight areas with the most interesting biology in a protein due to deviations from the ground state.

SNAPS gives a definitive result which expresses the best fit to measured shifts and other restraints from HADAMAC, RDC & un-labelling data. Current work is aimed at including correlation data into the program from C, C α , C β shifts and pyruvate labelling. In this approach measured shifts from pairs of strips are fitted to those of all predicted pseudo residue pairs weighted by the correlations of shifts between the residues.

Using chemical shifts from a set of 54 proteins SNAPS had an high accuracy the assignment (88% for N, C, C α & C β data) & was robust to missing data for residues, with 80% of the high accuracy assignments remaining after 90% of the residues had been deleted. Analysis of the results for a set of 134 protein with simulated un-labelling for the residues C R N M K Q & H] showed good agreement with the chemical shift assignment data alone.

SNAPS is written using Pandas2 and uses the NMR Exchange Format for data input, output and building high quality test data sets via the NEF-Pipelines library.

SNAPS offers an innovative approach to assignment, exploiting the availability of high quality chemical shift predictions from structures along with other restraints to speed & ease assignment.



Structural and Dynamic Insights into Integrin β Cytoplasmic Domain Interactions with Nascent Focal Adhesion Proteins

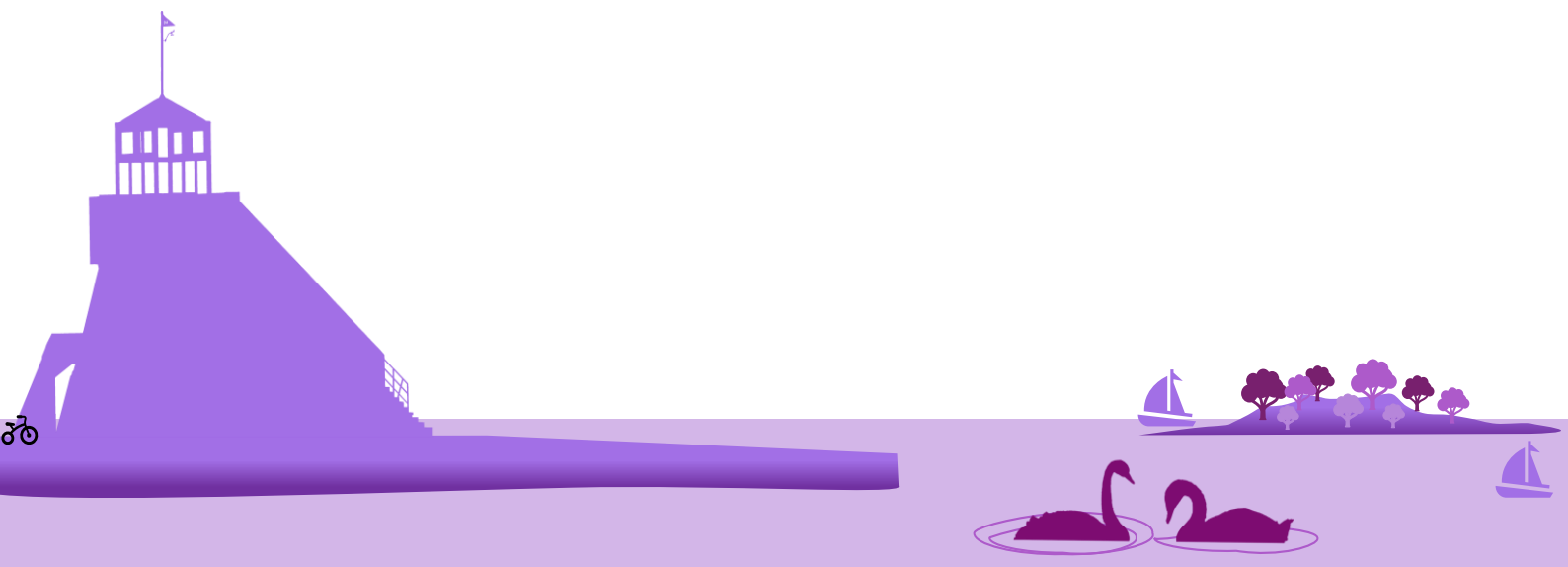
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Focal adhesions (FAs) are specialized adhesive structures that mediate cell adhesion, signaling, and mechanotransduction. These macromolecular complexes link the actin cytoskeleton to the extracellular matrix (ECM) via transmembrane heterodimeric proteins called integrins, supported by a network of cytoplasmic proteins. Nascent focal adhesions (nFAs) are precursor adhesive structures that undergo dynamic assembly and maturation into larger, more stable FAs. Typically composed of 20–60 integrins, these nascent structures form and disassemble within 1–2 minutes, independent of myosin-driven contractility. The β -integrin subunit plays a key role in nFA formation and function, interacting with essential cytoplasmic adaptor proteins such as paxillin, kindlin, and talin. Through interactions with the cytoplasmic domain ("tail") of integrins, these proteins regulate bidirectional signaling and cytoskeletal anchoring. Paxillin, via its LIM domains, and talin and kindlin, through their FERM domains, mediate these interactions, influencing nFA stability and cellular responses.

This study examines the interaction dynamics between the paxillin LIM domains and the cytoplasmic tail of integrin β . Additionally, we explored the binding interactions between talin's FERM head domain and both full-length and truncated integrin β cytoplasmic domains. Our findings reveal that the truncated integrin β cytoplasmic tail retains its ability to bind the talin head domain, supporting the notion that paxillin and talin engage integrin β at distinct, non-overlapping sites. Additionally, we investigated the potential formation of a ternary kindlin-paxillin-integrin complex using paramagnetic NMR spectroscopy. These insights contribute to a deeper understanding of the molecular mechanisms underlying integrin-mediated adhesion and nFA assembly. This knowledge provides a foundation for further studies on integrin-associated signaling pathways and their roles in cell migration.



Towards the catalytic mechanism of ^{13}C and ^{15}N -labelled PcCel45A, Cel45A from the white-rot basidiomycete *Phanerochaete chrysosporium*

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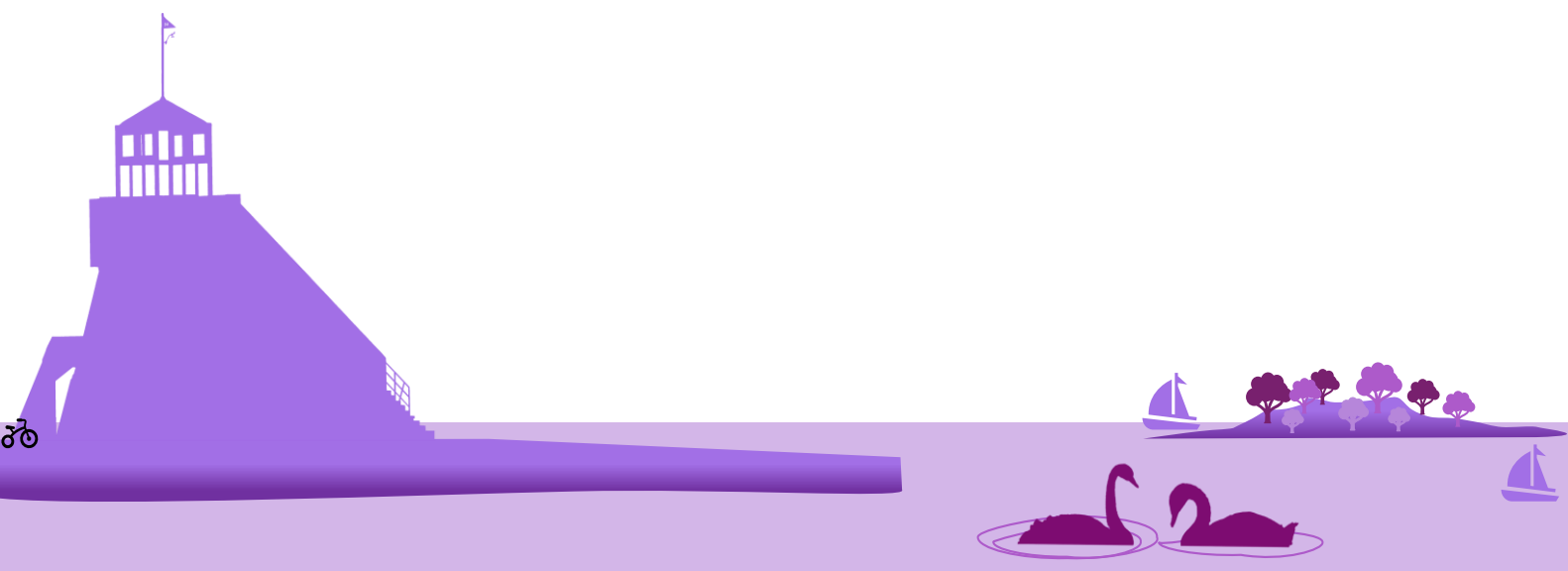
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Cel45A from the white-rot fungus *P. chrysosporium* (PcCel45A) was the first characterized member of subfamily C of Glycoside hydrolase family 45 (GH45) enzymes – inverting endoglucanases which exhibit cellulase, endo-xyloglucanase, or endo- β -1,4-mannanase activity. The C subfamily is unique among all three GH45 subfamilies (A, B, C), due to its uncharacteristic amino acid organization in the active site. GH45 enzymes catalyze the hydrolysis of $\beta(1\rightarrow4)$ linkages in soluble β -glucans via an inverting action mechanism. Two amino acids act as a catalytic acid and base in this mechanism. However, the subfamily C members do not possess the same catalytic base as subfamilies A and B, and yet the action mechanism yields α -anomers from $\beta(1\rightarrow4)$ -linked glucans. As the catalytic base remains undiscovered, the factual hydrolytic mechanism of GH45 subfamily C members is unknown and continues to be of high research interest.

Interestingly, the hydrolytic activities exhibited by subfamily C members are relatively low; furthermore, these proteins possess a close structural resemblance to some non-hydrolytic protein groups, such as expansions and loosenins. Compared to the other subfamilies, subfamily C thus appears distinctively different from GH45 subfamilies A and B concerning the catalytic mechanism; however, subfamily C and B are more alike in the reaction product profile.

To date, the molecular structures of only two GH45 subfamily C enzymes have been experimentally determined – PcCel45A and Cel45A from *G. trabeum*. Both are crystal structures. No structural studies by NMR spectroscopy on PcCel45A or any other GH45 enzyme have been published previously. All structural information regarding this family thus far is derived from X-ray crystallography and neutron crystallography.

To get one step closer to discovering the molecular action mechanism of these enzymes, we had decided to investigate the interaction between PcCel45A and different substrates by NMR spectroscopy.



Tunable Olfactory Peptides for Specific Detection of Chemical Agents

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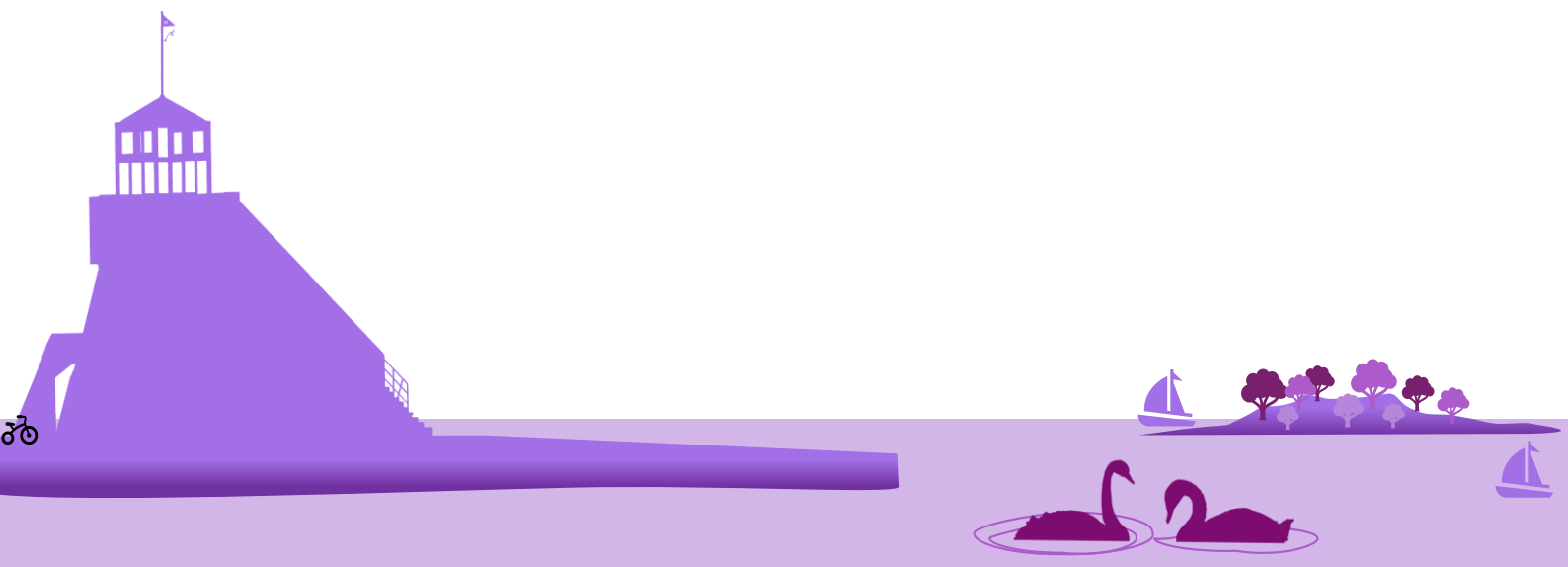
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We present the design and synthesis strategy of artificial peptides inspired by insect olfactory receptors (*Apis mellifera*, *Drosophila melanogaster*, *Solenopsis invicta*) for incorporation into next-generation laser-induced graphene (LIG) sensors targeting chemical warfare agents. These peptides feature a modular design comprising a flexible polyglycine linker, a helicity-inducing solubilizing region, and a sensing domain derived from insect olfactory proteins. The sensing regions are tailored to interact selectively with explosive analytes such as TNT and DNT.

Ten peptide variants were synthesized using microwave-assisted automated solid-phase peptide synthesis (Fmoc chemistry), purified by ion-exchange FPLC, and characterized through ESI-MS and NMR spectroscopy. Structural analysis involved set of 2D NMR experiments, including $^1\text{H}, ^{15}\text{N}$ -HSQC, $^1\text{H}, ^{15}\text{N}/^1\text{H}, ^{13}\text{C}$ -HSQC-TOCSY, and ROESY, conducted at 30 mM peptide at natural isotopic abundance in DMSO-d_6 with 50% non-uniform sampling (16–20 hours total acquisition time). Complementary molecular dynamics (MD) simulations were used to model peptide–analyte interactions, guiding sequence optimization. Ongoing efforts aim to combine MD results with NMR titrations to further validate peptide selectivity and sensitivity toward explosive targets.



Selective targeting of an intrinsically disordered domain by a drug-like small molecule

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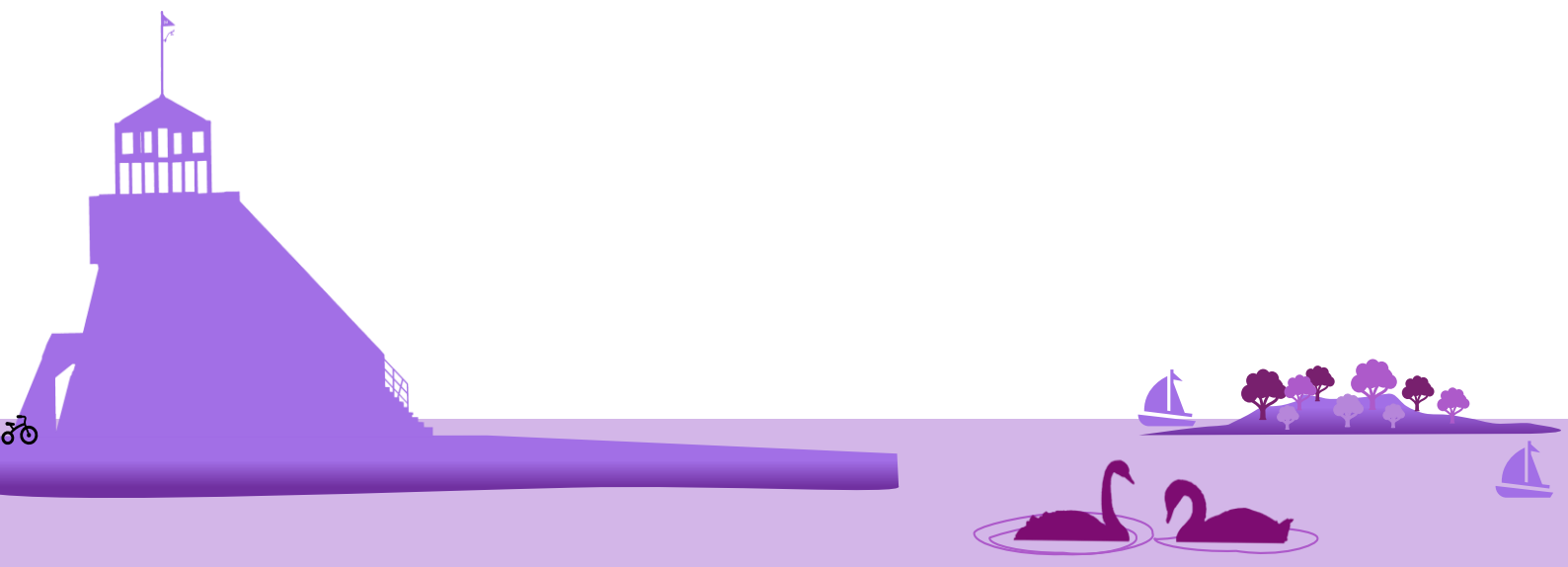
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Intrinsically disordered proteins (IDPs) are attractive therapeutic targets in various diseases, including cancer and neurodegeneration, due to their diverse functions in cells. However, they lack stable secondary and tertiary structures hindering the formation of stable binding sites for small molecules. Although some small molecules targeting IDPs have been reported, the molecular basis of their binding is not well understood. It also remains unclear if the interaction between drug-like small molecules and this class of targets can be sufficiently selective to avoid undesired off-target effects. To address this question, we have studied the interaction between EPI-001, the first drug-like small molecule targeting an IDP evaluated in a clinical trial (NCT02606123), and the activation domain (AD) of the androgen receptor (AR), a therapeutic target for castration resistant prostate cancer.

Using solution NMR we revealed that EPI-001 selectively binds to AR AD and not to other intrinsically disordered ADs of related nuclear hormone receptors sharing similar sequence composition and patterning. The interaction of EPI-001 relies on the formation of a transient binding site in oligomeric AR AD stabilized by aromatic interactions in partially helical motifs. Our data also shows that the network of transient long-range interactions that drive AR AD oligomerization are rewired upon EPI-001 binding. In summary, our findings suggest that it may be possible to target intrinsically disordered proteins selectively.



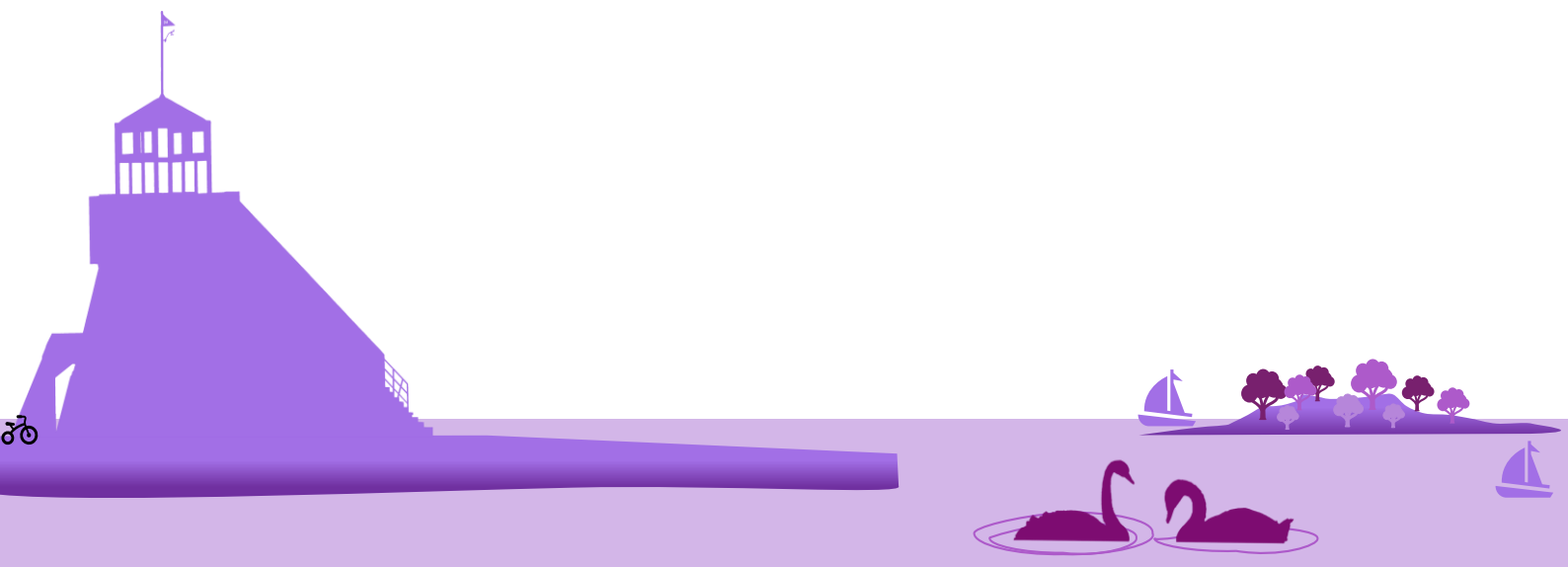
Chemical shift imaging as a tool to study partitioning of small molecules into condensates

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Membrane-less organelles are crucial for eukaryotic cells, as they create distinct compartments with reduced molecular movement and varying concentrations of biomolecules. One mechanism responsible for the formation of these organelles is liquid-liquid phase separation (LLPS). LLPS is often driven by weak, multivalent interactions mediated by intrinsically disordered proteins (IDPs) that feature low complexity domains. Understanding the properties of these condensates is essential for gaining insights into cellular processes and could potentially allow their use as bioreactors. A key characteristic of these condensates is the partitioning coefficient (PC), which measures the distribution of molecules within them. To accurately assess the partitioning of small molecules, we employ NMR chemical shift imaging (CSI) on macroscopic condensed phases. We tested and optimized pulse sequences and parameters using a phase-separating mixture of cold fish gelatin and dextran. Partitioning coefficients of small naturally occurring molecules into a condensed phase produced by synthetic peptides were determined.



Investigating the Binding of f-Elements with Phosphorylated Compounds in Nutritional Components Using NMR Spectroscopy

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Contamination of natural habitats including the food chains by heavy metal f-elements, i.e., lanthanides and actinides is a substantial threat for human life. Nuclear magnetic resonance (NMR) spectroscopy represents a valuable analytical tool for investigating a diverse range of food, feed, and nutritional components. Previous studies have demonstrated a high affinity of f elements, including Uranium (U), Europium (Eu), Yttrium (Y) and Lanthanum (La), for single or multiple nutrients. [1,2]

Among these interactions between (modified) amino acids and f-elements, phosphate moieties have emerged as particularly potent binding sites. These are highly abundant, but not limited to phosphoproteins like the caseins in milk. [3]

³¹P liquid-state NMR is applied to study f-element binding to phosphorylated proteins in extracted milk micelles as well as single phosphorylated amino acids in solution. Furthermore, solid-state NMR is used to analyse f-element-containing precipitates obtained from solutions of the above-mentioned biomolecules.

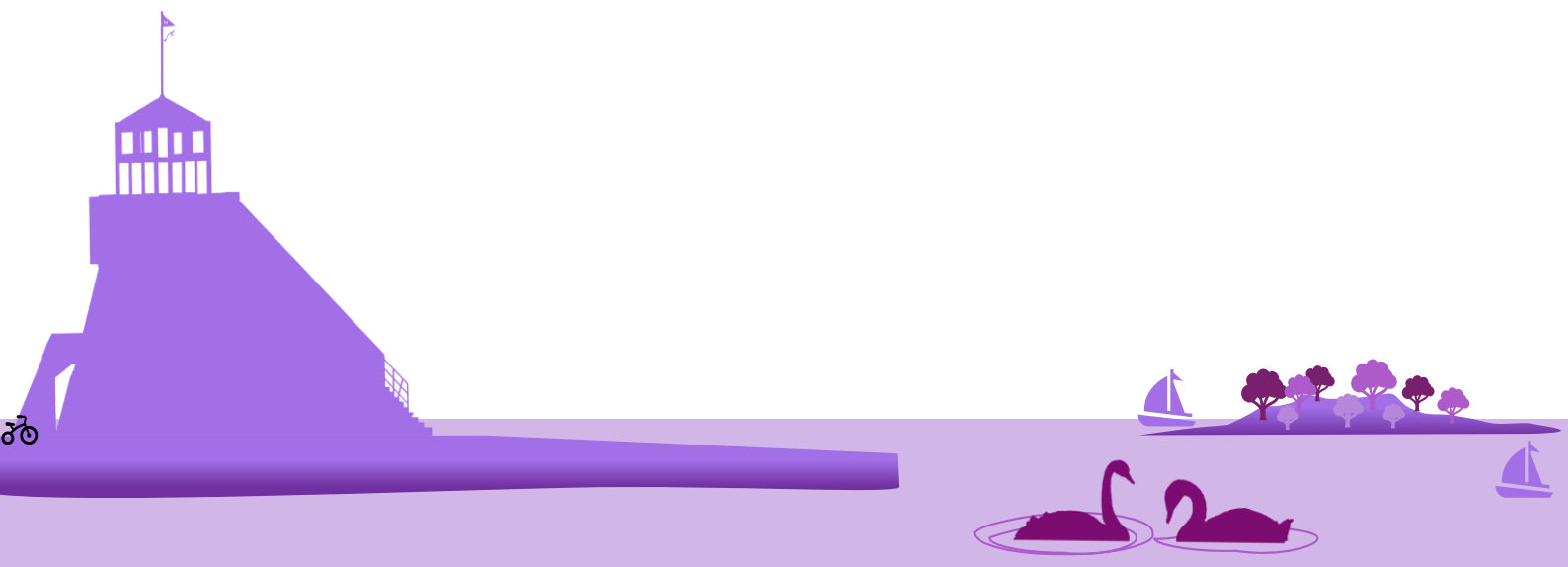
The interactions of Eu³⁺, La³⁺ and Y³⁺ with O Phospho-L-serine were analysed via ³¹P, ¹³C, and ¹H NMR spectroscopy. In combination with Heteronuclear Correlation (HETCOR), the POH and COOH groups could be identified as interaction sites. For easy handling outside of radiation laboratories, Eu³⁺ was selected as a non-radioactive analogue for radioactive f elements. In the solid-state NMR spectra of the precipitates, however, Eu³⁺ caused severe paramagnetic broadening and was thus replaced by the diamagnetic analogues La³⁺ and Y³⁺.

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Electric Field- and ATP-Induced Conformational Changes in Proteins Investigated by MD Simulations and NMR Spectroscopy

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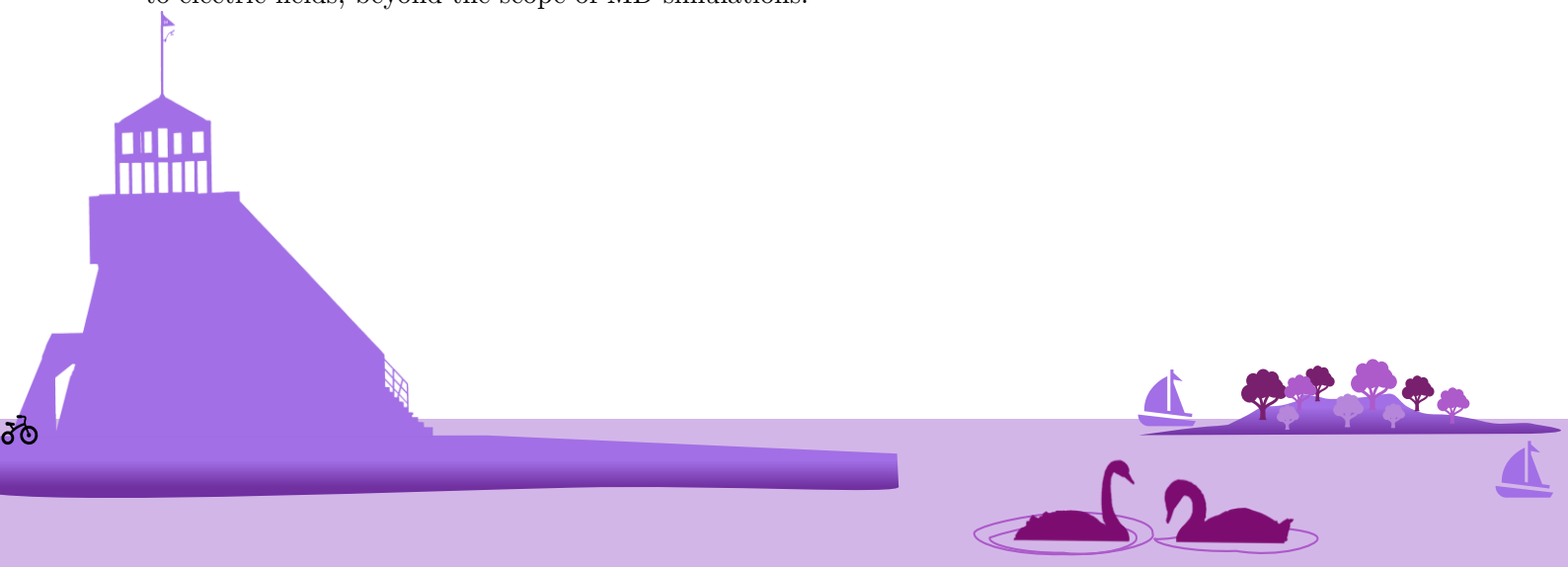
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Protein misfolding and aggregation are associated with neurodegenerative diseases such as Parkinson's disease, in which α -synuclein forms oligomers and fibrils under non-equilibrium rather than static conditions. Recent studies suggest that protein aggregation is significantly influenced by environmental factors such as electric fields and changes in ATP concentration. However, the atomic-level mechanisms remain poorly understood. In this study, we performed MD simulations and NMR spectroscopy to investigate how electric fields and ATP alter protein conformations.

First, to briefly organize the phenomena induced by electric fields, we examined the effects of a uniform electric field on ubiquitin as a model protein. Electric fields reduced the stability of intramolecular hydrogen bonds, particularly located at β -sheet termini, suggesting local structural destabilization. We also analyzed electric dipole moments and found that hydration water molecules near ubiquitin adopt an orientation that is similar to that of ubiquitin, indicating that water reorientation under electric fields contributes to conformational disruption of proteins [Shuto et. al. J. Phys. Chem. B. 2023].

Next, we explored how ATP binding modulates α -synuclein structure. Diffusion NMR and titration experiments showed that the adenine ring of ATP induces a structural elongation through hydrophobic interactions. MD simulations verified the result by revealing a loss of long-range intramolecular contacts and free energy analysis also indicated that the binding of ATP changed the hydration environment. In conclusion, electric fields promote local misfolding, whereas ATP induces conformational elongation of α -synuclein.

Taken together, further work should focus on directly tracing electric field effects on proteins in real-time observations. Accordingly, we have developed E-NMR, a method capable of observing protein responses to electric fields, beyond the scope of MD simulations.



Studies of the isolated LIM3 domain of the protein paxillin in relation to its role in the formation of focal adhesions

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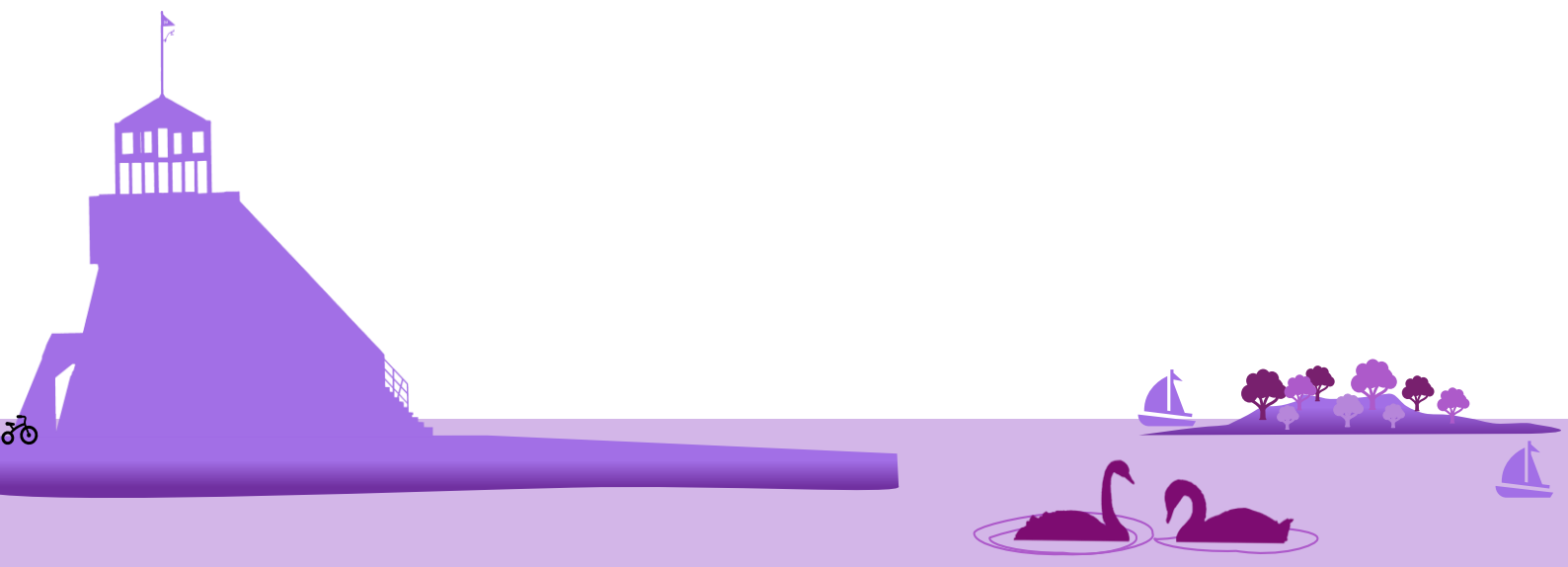
In numerous processes in the organism, the interactions between the cells and their environment play an important role, which are frequently mediated by so-called focal adhesions (FA).[1] These are protein assemblies central components of which are the heterodimeric integrins (Int) and paxillin (Pax).[2] During FA formation the intracellular tail of $\text{int-}\beta$ interacts with the LIM domains of Pax. This interaction has been characterized, recently, showing that the LIM3 domain of Pax has the strongest contribution.[3] To gain a deeper understanding, the interaction between $\text{Int-}\beta 1$ and Pax-LIM3 is being investigated further. We employ NMR spectroscopy to determine the amino acid residues involved in the interactions and to gain information on the 3D structures of heterodimeric complexes between Int and Pax, and ternary complexes with additional binding partners such as talin and kindlin. With the use of paramagnetic lanthanides (Ln^{3+}) and the pseudocontact shifts (PCS) caused by them, a sensitive measure of long-ranging structural information becomes available.[4] For this, the protein $\text{Int-}\beta 1$ was modified by a lanthanoid binding tag (LBT) to complex Ln^{3+} . It turns out that transferring NMR spectroscopic findings from the LIM2/3 tandem construct to the individual LIM3 domain is not trivial. To shed light on this, various LIM3 constructs, and chimeric complexes are being examined regarding their 3D structures and binding properties.

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The BMRB archive of Protein, Nucleic Acid and Metabolite NMR Data

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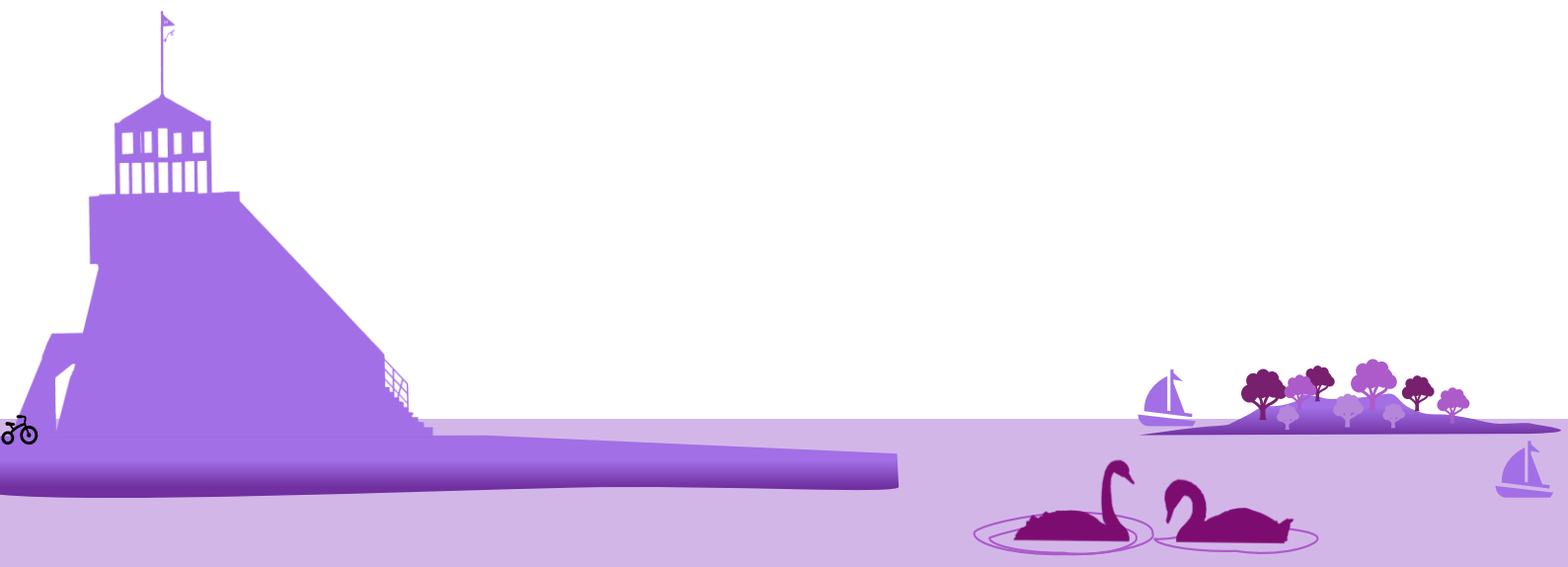
Biological Magnetic Resonance Data Bank [1] (BMRB: <https://bmr.io>) serves the biomolecular NMR community by maintaining a curated archive of primary and derived data and metadata linked to scientific investigations under the “FAIR Principles” (Findable, Accessible, Interoperable, and Reusable)[2]. BMRB is a member and Core Archive of the Worldwide Protein Data Bank (wwPDB: <https://www.wwpdb.org>), which collects coordinate data with assigned chemical shifts and restraints from NMR structural studies via the OneDep (<https://deposit.wwpdb.org>) deposition system. NMR data from other studies are collected by the BMRBdep (<https://deposit.bmr.io>) deposition system.

BMRB holds over 12 million chemical shifts from 16964 macromolecule entries plus several thousand derived parameters (e.g. coupling constants, RDCs, T1/T2 values, heteronuclear and homonuclear NOEs, CSAs and order parameters). Additionally, BMRB maintains a library of carefully curated NMR spectroscopic data of over 1000 small molecules, serving as a reference library for metabolomics research.

The data format used by BMRB is NMR-STAR [3], which is defined by a data dictionary. For programmers, BMRB offers an API [4] and Python libraries (PyNMRSTR [5], PyBMRB [6]) that enable users to develop their own BMRB-based tools for data analysis, visualization, and manipulation of NMR-STAR formatted files. BMRB launched BMRbig (<https://bmrbig.bmr.io>) in 2020 to accommodate the acquisition of diverse data beyond the types currently curated and annotated by BMRB.

Chemical shift stats have been improved by linking experimental data for standard amino acids with secondary structure info fromPDB entries.

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5. <https://pypi.org/project/pynmrstar>
6. <https://pypi.org/project/pybmr>



NMR spectroscopy to study protein assemblies on bacterial membrane

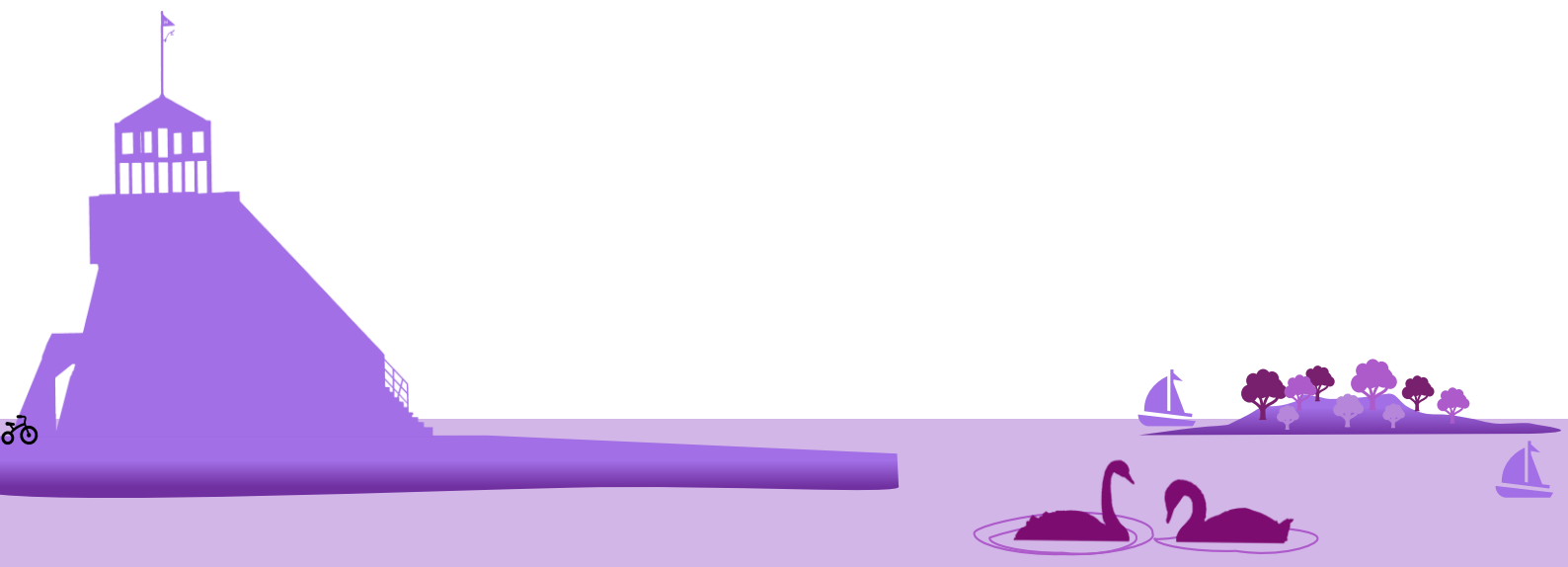
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Antimicrobial resistance in bacteria is an escalating public health threat, making it crucial to identify new therapeutic targets. Understanding the atomic mechanisms behind protein-membrane interactions in bacteria offers a promising strategy to discover novel drug targets. Our research focuses on the ZipA protein, which plays a vital role in bacterial cell division, controlling the initial molecular steps during set-up of the division site in certain bacterial pathogens such as *Escherichia coli*. Using both solution and solid-state Nuclear Magnetic Resonance (NMR) spectroscopy, we investigate the molecular structure of ZipA in a membrane-mimicking environment. By targeting the protein structure in a native-like setting, this study aims to uncover insights into the structures and the interactions with the membrane at the atomic level, providing crucial information for the development of new antimicrobial agents.



Multimodal NMR Approach in Preclinical Studies of Maternal Depression

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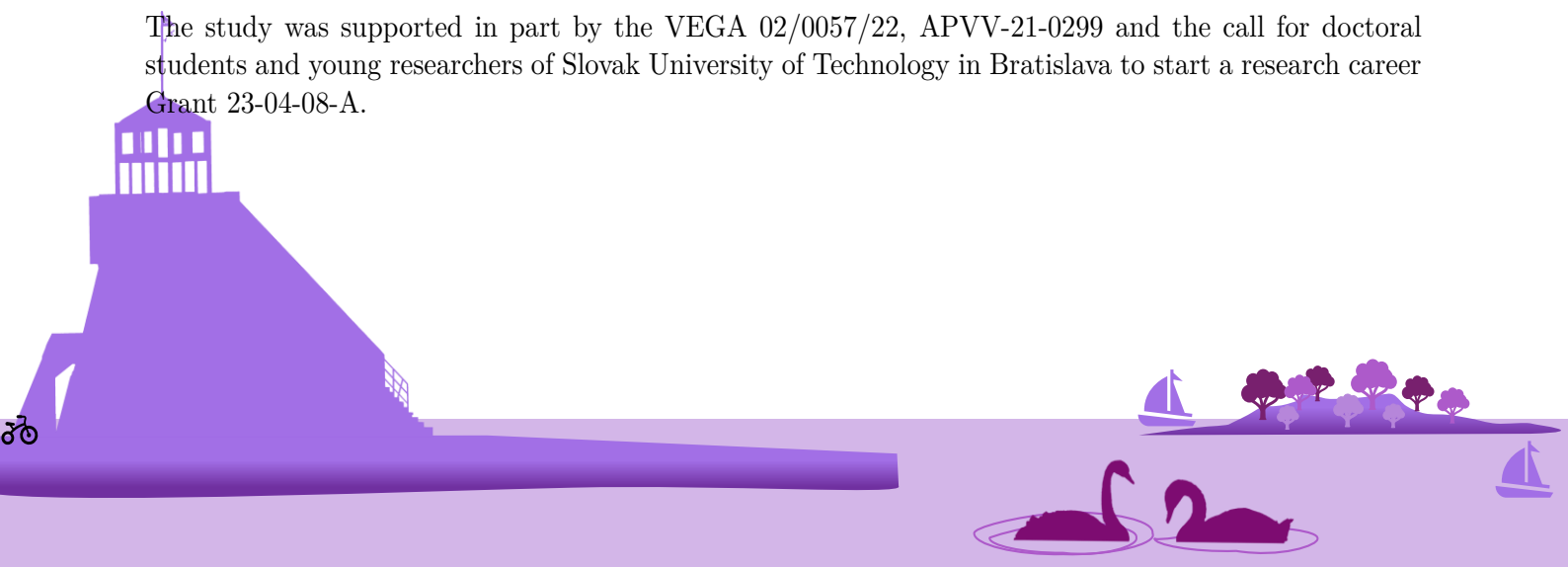
Exposure to depression and to its treatment before pregnancy can induce changes in regional brain structures and neurochemistry in mothers. Some of these changes may be transferred to their offspring or cause additional negative changes. Therefore, the aim of this study is to investigate structural and metabolic changes induced by chronic stress, as a model of maternal depression, in rat dams and their offspring. The effect of antidepressant will be studied subsequently.

In vivo MRI, nonlocalized 31P MRS and localized single voxel 1H MRS were performed at 4.7 T. For structural analysis T2-weighted MRI was used, followed by a volumetric analysis using MRtrix3 and Advanced Normalization Tools. The Waxholm Space atlas v4 was utilized as a reference for quantifying brain regions. 1H MRS was performed using a localization sequence from the hippocampus, followed by automatic quantification by LCModel. 31P MRS was performed using the single pulse sequence and the data were analyzed with jMRUI.

Our in vivo study revealed that chronic stress may have negative effects on the maternal brain, with potential antidepressant-related improvement. Energy metabolism, the GABAergic system in the hippocampus and amygdala volume were affected by stress in rat dams. In contrast, we observed different changes in their offspring. Maternal stress influenced brain development in the offspring, and maternal antidepressant treatment led to changes in brain structures and neurochemistry of N-acetylaspartate levels in hippocampus. These findings highlight the importance of better understanding both treated and untreated maternal depression, and further research is needed to fully clarify its adverse effects in offspring.

Acknowledgments

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siRNA biosynthesis in *D. melanogaster*: NMR studies of biomolecular condensate formation

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RNA interference is a general post-transcriptional gene-regulatory pathway that is guided by small RNAs. In the siRNA pathway of *Drosophila melanogaster*, dsRNAs are processed by a Dcr-2/Loqs-PD protein complex and then loaded onto the Ago2 protein to form the RNA-induced silencing complex.

We have found that both Loqs-PD and Ago2 are located in biomolecular condensates in vivo together with RNA, and display liquid-liquid phase separation in vitro as shown by DOSY experiments. The phase separation of Loqs-PD depends on the RNA binding capability of its dsRBDs and is further modulated by a preceding N-terminal region. An intrinsically disordered region in Ago2 (Ago2IDR) is important for condensate formation in cells and forms condensates in the presence of RNA in vitro.

A combination of NMR spectroscopy and mutational analysis suggests that the Ago2IDR-RNA condensates are fluid and exhibit significant peptide backbone flexibility. They are stabilized by electrostatic and stacking interactions involving arginines and aromatic side chains, as revealed by an extensive NOE network of protein side chain interactions.

Our results indicate that the unique features of the broadly conserved Ago2 IDR drive biomolecular condensate formation, and suggest that phase separation plays a role in siRNA processing in *Drosophila* and hence contributes to an efficient dsRNA-mediated anti-viral defense.



Effect of Fatty Acids and Multivalent Cations on the Dynamics of Bovine Serum Albumin at High Concentrations

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Most investigations of proteins require dilute conditions. In their natural environment, however, most proteins are surrounded by high concentrations of various biomacromolecules. Under such conditions, mobility is reduced, which we analyze using various NMR methods. Depending on the surface charge structure of the proteins, coupling or decoupling between rotation and translation can be observed. Translational diffusion coefficients can be determined by pulsed field gradient NMR, while translational correlation times can be obtained by a combination of $T1\rho$ and $T2$ relaxation times. Bovine serum albumin (BSA), the most abundant protein in bovine blood, shows intermediate coupling. By adding trivalent cations, the intermolecular electrostatic interactions between the proteins can be adjusted, resulting in an interesting phase behaviour involving reentrant condensation and liquid-liquid phase separation.

When comparing various commercially available BSAs, we found a significant difference in experimental results, which could be divided into two groups with similar behaviour. During the purification process, naturally occurring fatty acids may or may not be removed, depending on the manufacturer. Fatty acid-free BSA samples show a decrease in translational and rotational mobility compared to the fatty acid-free variants at the same concentration. Differences can also be seen in salt-protein interactions, where lower amounts of trivalent cations are required to induce aggregation in fatty acid-free BSA. As many studies use commercially purchased BSA without further purification, the choice of supplier may have an influence on the experimental results that is often overlooked.



Structural basis of dual BACH1 regulation

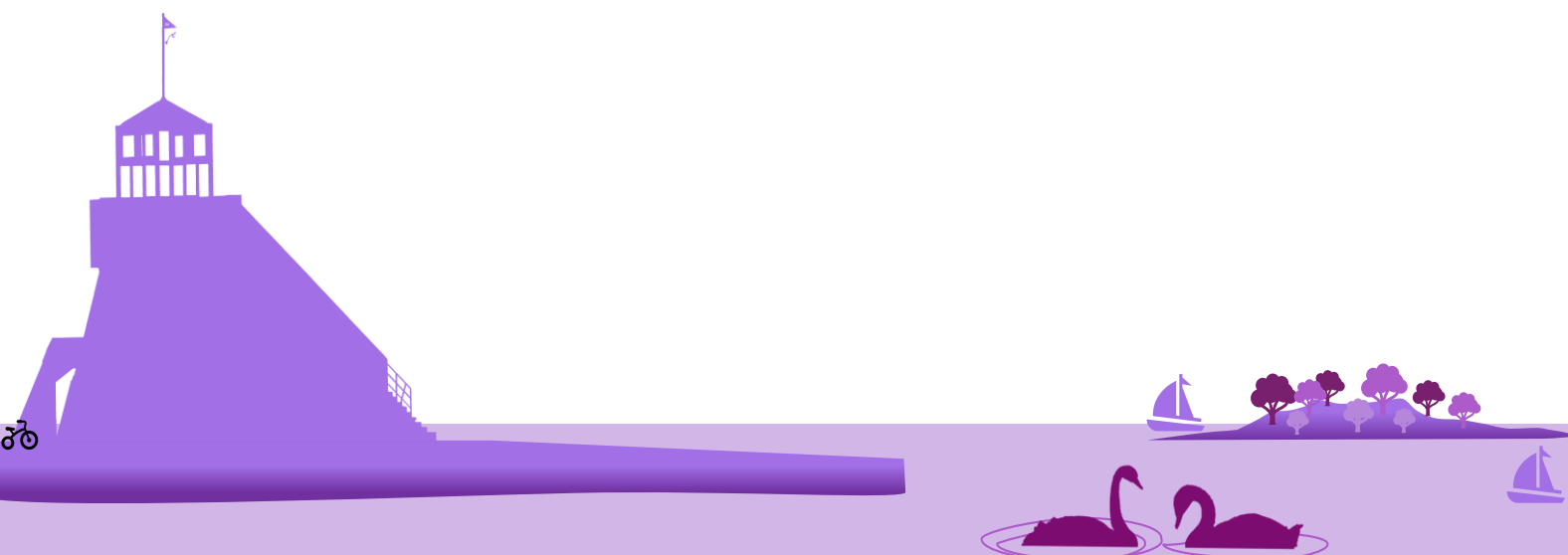
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BTB and CNC homolog 1 (BACH1) is a pivotal transcriptional regulator of the cellular oxidative stress response and a pro-metastatic oncogene. The post-translational stability of BACH1 is meticulously controlled by distinct F-box ubiquitin ligases, including SCFFBXO22 and SCFFBXL17. However, the mechanism by which these ligases recognize BACH1 under oxidative stress remains unclear. In our study, we uncover a structural switch in FBXO22 that governs the recognition of a three-dimensional degron within the BACH1 BTB domain, elucidating its specificity for dimeric BACH1. We demonstrate how cancer-associated mutations in FBXO22 influence the binding and ubiquitylation of BACH1. Furthermore, we show that cancer-related mutations or cysteine modifications destabilize the BTB domain, redirecting BACH1 to FBXL17, where it is recognized as a monomer. These findings shed light on a ligase switch mechanism that facilitates the post-translational regulation of BACH1 by complementary ligases, depending on the stability of its BTB domain. Our results provide mechanistic insights into the regulation of the oxidative stress response and may inspire therapeutic strategies targeting oxidative stress-related disorders and cancer. This work highlights the power of NMR through an integrated approach combining biomolecular NMR, cryo-EM, X-ray crystallography, computational methods, and complementary biophysical and biochemical techniques [1].

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MRI as a method for the quantitative analysis of live electroactive biofilms

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Aims: In microbial electrochemical systems (MES), biofilms grow as catalysts on the electrodes to exchange electrons. MRI is used to reveal the interplay of structure (density, distribution), transport (diffusion, flow), and environment (pH, metabolites) of the biofilms in order to gain a deeper understanding of MES, enable the development of more precise models and ultimately optimized electrode structures.

Methods: Biofilms are growing on porous carbon anodes after inoculation of electroactive microorganisms (*Shewanella oneidensis* MR-1, *Geobacter sulfurreducens*) in special designed MR-compatible bioreactors fitting into a custom-designed RF-coil. MR measurements are done at 7T (Bruker 70/20, AVIII, PV5.1). Growth is followed in operando by 3D T2- and ADC-mapping under monitoring of current production [1]. Microenvironmental conditions in- and outside of the biofilm are measured by Iopamidol-CEST [2,3] which are obtained on a 5mm slice orthogonal to the electrode (resolution: 100µm orthogonal, 1mm parallel to the electrode). pH-maps are calibrated by reference samples inside the bioreactor.

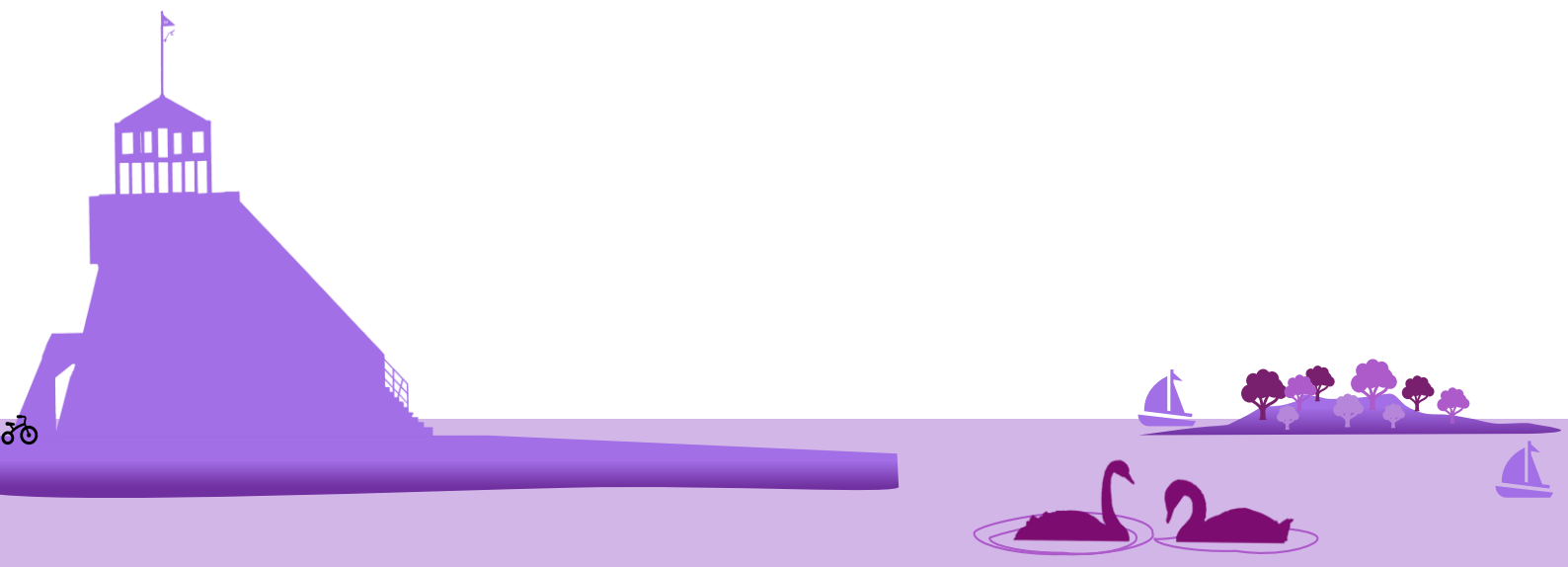
Results: In operando MRI revealed spatially heterogeneous biofilm colonization. The bacterial strain influences colonization patterns: *S. oneidensis* forms localized high density colonies near the electrode-medium interface, while *G. sulfurreducens* produces dense biofilms spanning the entire electrode. Inside the biofilm diffusion is reduced and a correlation between T2 and ADC observed. Also, a pH gradient with increasing acidity further from the fresh medium source is measured. Compared to fresh medium, the older medium behind the electrode exhibits lower pH.

Conclusions: These measurements demonstrated the capabilities of MRI to characterize MES – its benefits will become apparent when applied to comparatively investigate different electrode designs and elucidate their underlying limiting processes.

1. DOI: [10.1039/d2ra01162j](https://doi.org/10.1039/d2ra01162j)

2. DOI: [10.1002/mrm.20441](https://doi.org/10.1002/mrm.20441)

3. DOI: [10.1002/mrm.22608](https://doi.org/10.1002/mrm.22608)



Disordered proteins and fuzzy complexes: structural and dynamical characterization by NMR spectroscopy

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Intrinsically disordered proteins and protein regions (IDPs/IDRs) play important roles in diverse biological processes, they can bind to multiple targets, however they lack a stable secondary structure. To develop potent protein-protein inhibitors, these interactions have to be revealed in detail. Focusing on the disordered N-terminus of the tumor suppressor p53 protein,^{1,2} we performed an in-depth structural and dynamical characterization of the fuzzy complexes formed in interaction with members of the metastasis-associated S100 protein family³. The mobile p53(1-100) region contains the two transactivation domains (TAD1, TAD2) and a proline rich region (PRD). Peak assignment for all residues was obtained from a combination of ¹HN-detected and ¹Hα-detected approaches.^{2,4}

Upon interaction with S100P and S100A4 proteins, we proved the disorder-to-order transition of the TAD1 and TAD2 segments, as highlighted by the variation of Cα, Cβ secondary chemical shift data.⁵ Careful monitoring of the complex formation highlighted the steps of the helix formation in the TAD1 region, which is highly dependent on the interaction partner. Further on, we monitored how phosphorylation -the most frequent posttranslational modification for p53 TAD - affects the binding of S100P to p53(1-60).

A detailed backbone dynamics as a function of the B0 magnetic field and temperature reveals the flexibility of free p53(1-60) and shows significant increase in R2 and hetnoe values in TAD1 and TAD2 regions upon binding to S100P. Reduced spectral density analysis shows that low frequency motions become more frequent in the complex.

The revealed changes at atomic level in binding and binding affinity help us in the development of protein-protein interaction inhibitors for this system.

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Transient order in IDPs using statistics

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NMR chemical shifts and the calculated secondary chemical shifts (SCSs) are the most important atomic-scale variables reporting on the secondary structural propensities of intrinsically disordered proteins (IDPs) and it is becoming a cornerstone for the possible targeting of IDPs and drug development. The SCS can be calculated for a given atom type, as the difference between the measured values and the random coil chemical shift values (RCCSs). Thus, for the i -th position in the aminoacid sequence:

$$SCSi = \delta mi - RCCSi$$

However, finding the proper RCCS values is an important issue especially in IDP studies, where RCCS prediction requires precaution.

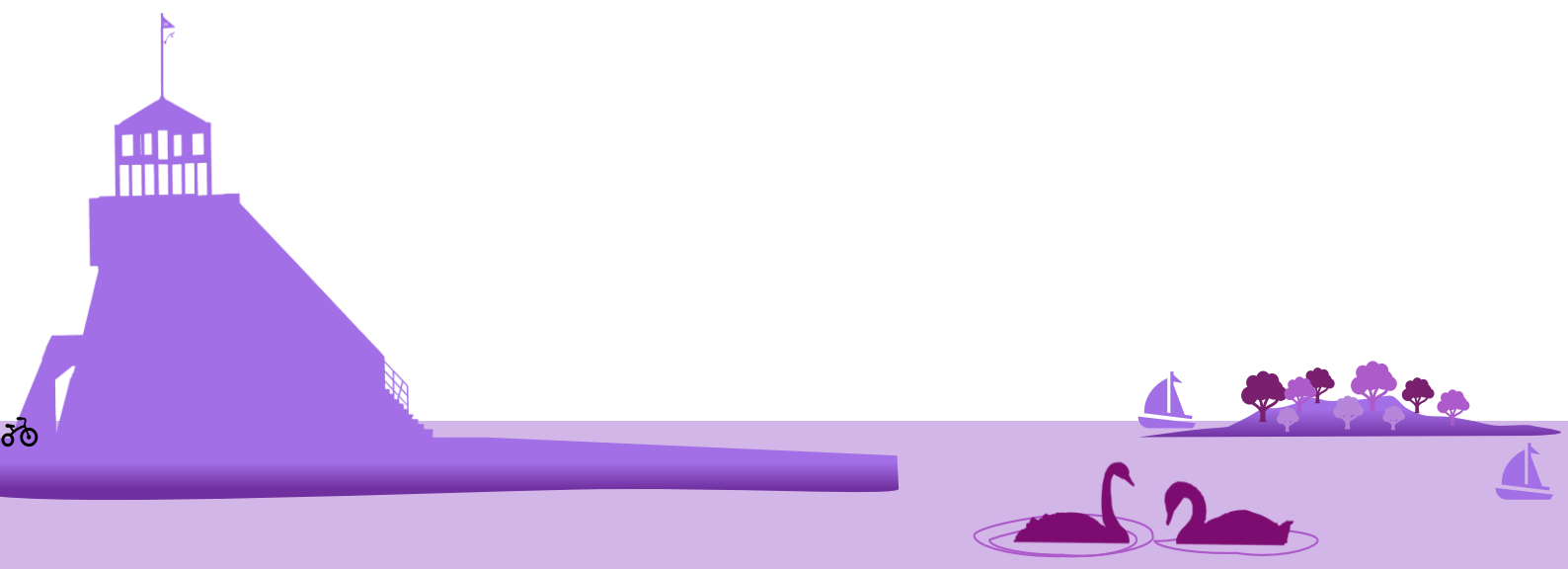
Using a set of benchmark IDPs we performed a visual and statistical comparison of selected RCCS predictors, demonstrating how the choice of any single RCCS predictor might affect the structural conclusions. We introduce and suggest the use of the very sensitive SRD-CRRN analysis coupled with post-hoc complemented ANOVA, that can detect statistically significant differences even in the case of a folded protein with well-characterized structure.¹ Further on, we propose the chemical shift discordance ratio (DR) for prefiltering RCCS predictors based on self-consistency.

Finally, the Structural Propensity Identification by t-statistics (SPIT) approach can be used for extracting maximum information from SCS data by using multiple RCCS predictors simultaneously. This way SCS patterns indicating structural propensities can be clearly distinguished from the “noise”.²

We show the application of these methods focusing on the $C\alpha$, and $H\alpha$ environments, but the presented techniques can be easily extended to various atom types. Thus, discordance ratios can be calculated for the $C\alpha$ - $C\beta$, $C\alpha$ - C' or C' - $H\alpha$ pairs.

These methods may open a new pathway to more rigorous and thus more sound identification of IDP secondary structural propensities based on NMR spectroscopy.

¹Kovács D., Bodor A. RSC Adv, 2023; ²Kovács D., Bodor A. under review



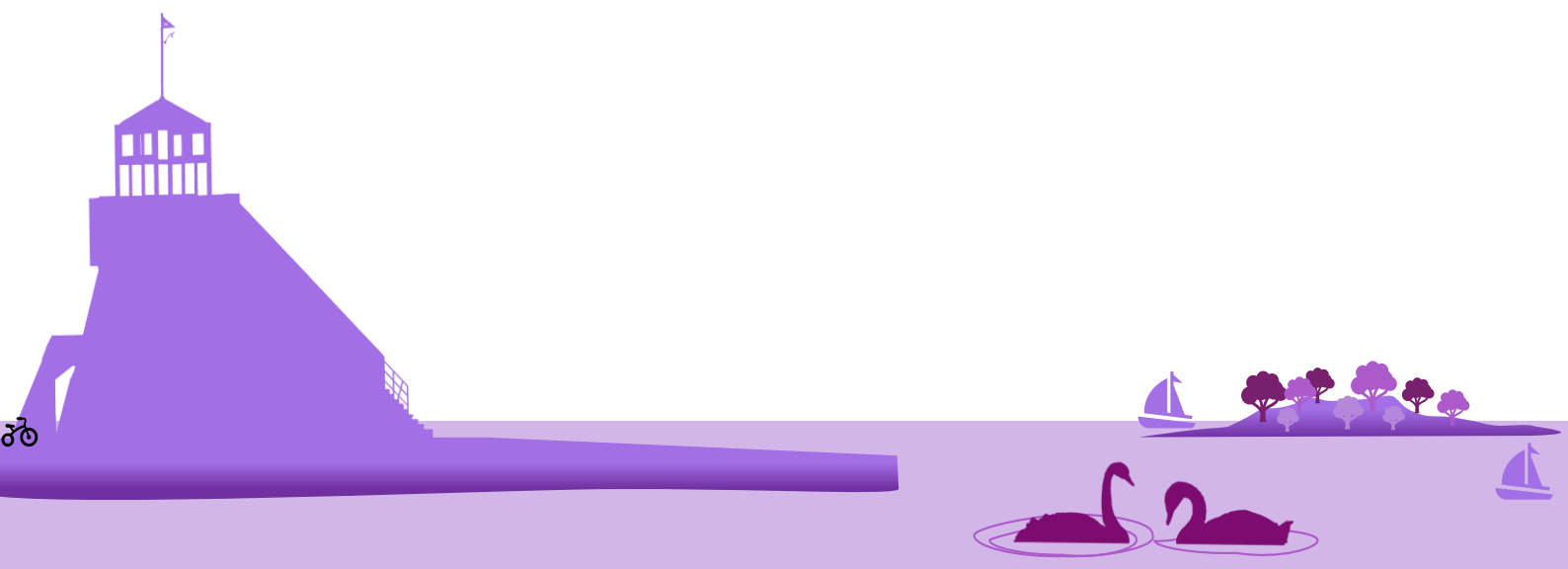
Solid-State NMR Unravels Solvent-Guided Self-Assembly in a Pro–Val–Pro–Val Tetrapeptide

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Short peptides can form surprisingly complex architectures, especially when influenced by subtle environmental conditions. In this work, we investigate the self-assembly behaviour of a linear tetrapeptide Pro–Val–Pro–Val (PVPV), which exhibits solvent-dependent polymorphism. Crystallization from aqueous and water–methanol environments yields either a dense triclinic structure or a nanoporous tetragonal framework. Using an integrated approach combining X-ray diffraction, advanced solid-state NMR, and computational modelling, we reveal how water content, packing symmetry, and local conformational disorder govern the formation of these architectures. Solid-state NMR played a pivotal role in characterizing these systems. ^{13}C CP/MAS spectra clearly distinguish amorphous, hydrated, and crystalline forms, indicating transformations triggered by environmental humidity. High-resolution 2D and 3D proton-detected NMR techniques on isotopically labelled samples enabled full signal assignment, even in the complex $Z' = 4$ triclinic phase. NMR chemical shift tensors (CSTs) provided detailed insights into hydrogen bonding and backbone geometry, exposing subtle conformational differences inaccessible by diffraction alone. Importantly, the failure of static DFT-based chemical shift predictions to capture experimental values underscores the limitations of conventional modelling for systems with flexible backbones and disordered hydration. The combination of experimental NMR and theoretical approaches highlights PVPV as a benchmark system for testing new methodologies in NMR crystallography. This study emphasizes the unique capacity of solid-state NMR to resolve molecular details in different hydrated forms of peptide systems and its indispensable role in understanding hierarchical self-assembly driven by solvent interactions.



NMR study of amyloid-beta self-assembly modulation by lanthanide-containing polyoxometalates.

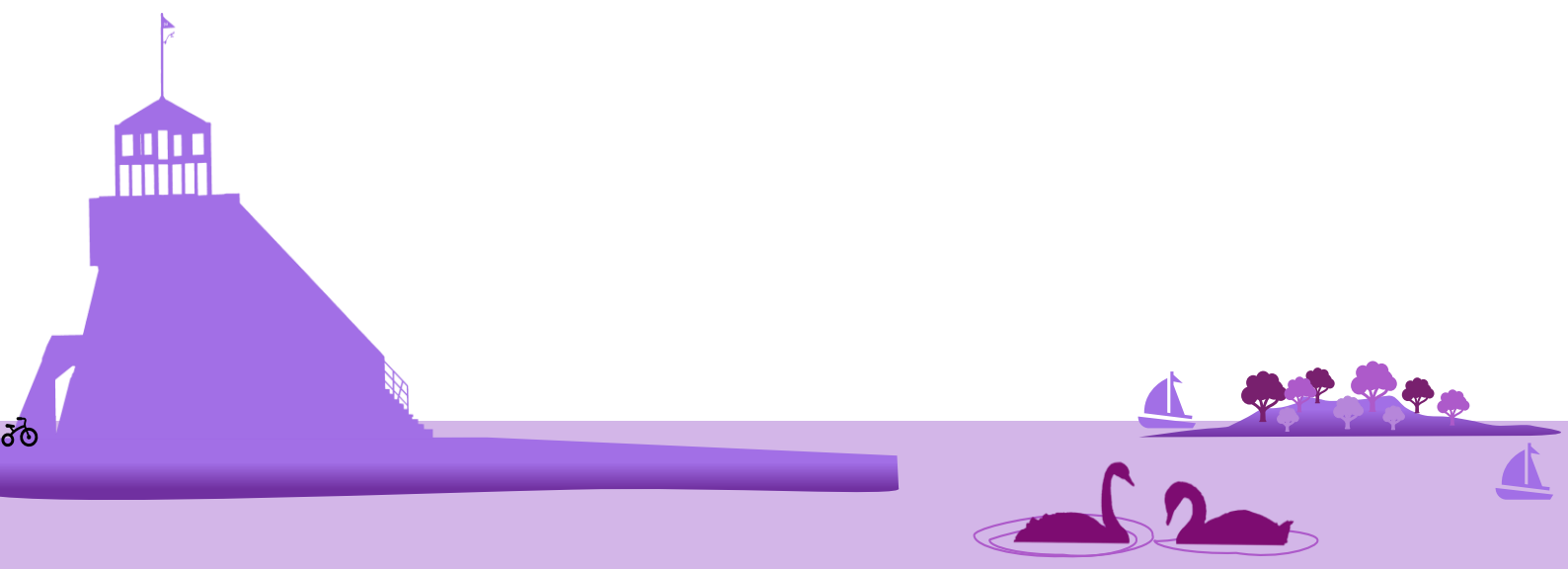
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Amyloid beta (Abeta) aggregation is a hallmark of Alzheimer's disease. Several peptide isoforms can self-assemble in highly toxic oligomeric species, before aggregating as beta-stranded fibrils which can in term lead to the formation of amyloid plaques. Controlling the fibrillization process is thus a promising strategy to slow down the disease progression. Polyoxometalates (POMs) have been investigated recently as potential inhibitors of Abeta aggregation. They are clusters of early transition metals bound to oxygen that can adopt different types of structures (Wells-Dawson, Keggin), and which can be substituted with transition metals or lanthanides for increasing target binding. Lanthanides (Ln^{3+}) are rare f-block elements that have gained interest lately as contrasting agents and potential therapeutic elements. Here we report interactions between a Keggin-type POM complexed to lanthanides ($\text{Na}_6\text{-}\alpha\text{-}[\text{SiW}_{11}\text{O}_{39}\text{Ln}^{3+}]$) and a 28-residue truncated form of Abeta peptide (Ab28) by solution NMR. 1D- ^1H and 2D TOCSY spectra were acquired in presence of different ratios of several POMs- Ln^{3+} (La, Eu, Gd, Yb, Lu), and show similar interaction sites on the peptide, mostly involving carboxylate groups in the N-terminus part, but with different apparent affinities, suggesting different coordination modes based on their respective ionic radii. Moreover, ThT fluorescence assays monitoring the aggregation of Ab28 show different modulation effects depending on the nature of the lanthanide. Additionally, we witnessed different effects at different peptide/POM- Ln^{3+} ratios, implying a complex mechanism in the tuning of Abeta self-assembly. These data suggest that these polyanionic compounds can play various roles in the fibrillization process depending on the molecular mechanisms of the interaction. Our results are a step forward in understanding the tuning of supramolecular self-assembly by polyanion molecules in the context of finding promising agents against Alzheimer's disease.



Newton's cradle-like allosteric mechanism explains regulatory RsmE RNA binding

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In the bacterial Csr/Rsm system, non-coding RNAs activate mRNA translation by removing homodimeric Csr/Rsm proteins from the ribosome-binding sites of mRNAs. In *Pseudomonas protegens*, each RsmZ ncRNA sequesters up to five RsmE dimers sequentially and specifically within a narrow affinity range, functioning as a 'protein sponge'. Although the RsmE binding cascade is cooperative, binding of the highest affinity stem-loop RNA in RsmZ (SL2) reduces RNA binding affinity at the second site by 10- to 30-fold. This unusual negative cooperativity may facilitate RsmE release from tightly bound mRNA for handover to the non-coding RNA, yet the underlying mechanisms remain unclear. Using Isothermal Titration Calorimetry, NMR spectroscopy and Molecular Dynamic simulations, we show that the initial binding event increases conformational entropy at the empty site, partially unfolding the C-terminal helix. Moreover, we reveal an allosteric mechanism coupling RNA binding at the first site to conformational changes at the second site, explaining the reduced affinity of the second binding event. The anti-parallel β -sheets in the RsmE dimer facilitate communication between sites, with H-bond constriction at the bound site and relaxation at the empty site, resembling a Newton's cradle.



NMR study on lipid induced gamma synuclein amyloid fibrils

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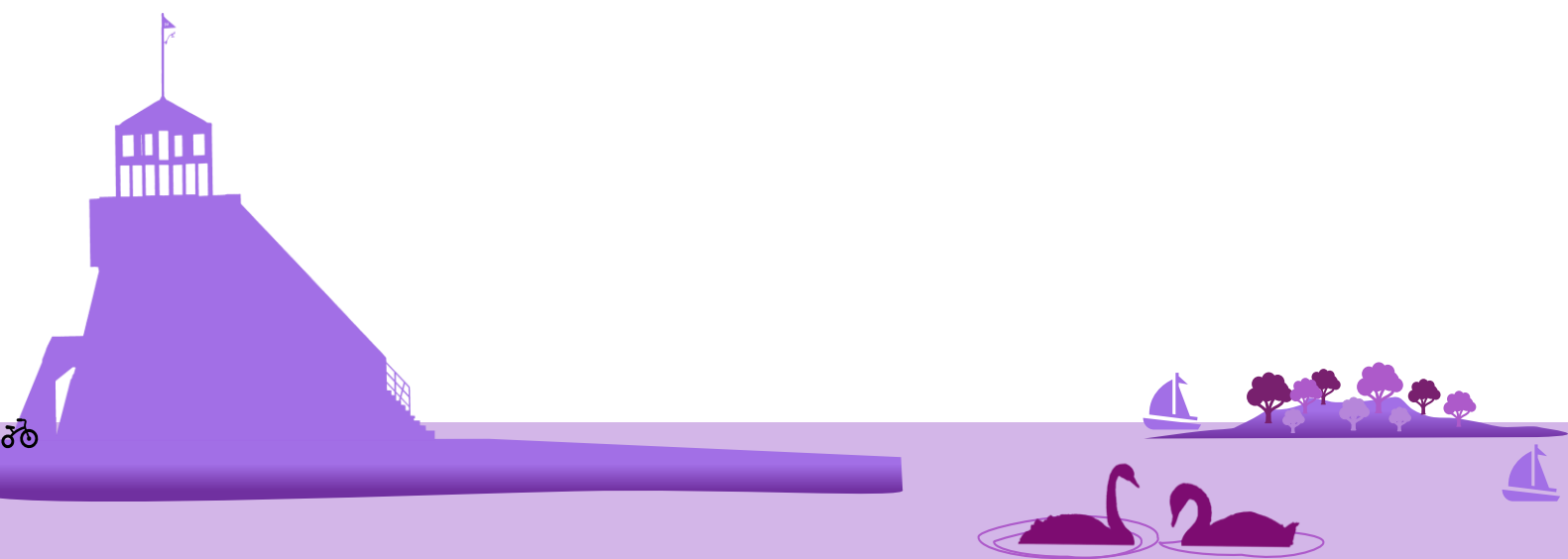
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The synuclein family comprises neuronal proteins that are intrinsically disordered and known to interact with lipid membranes. A hallmark of synucleinopathies is the interaction of α -synuclein (α Syn) with membranes and the formation of amyloid fibrils via toxic oligomers¹⁻³ has been studied in structural perspectives. Although γ -Synuclein (γ Syn), which also has increased cytotoxicity depending on membrane-binding⁴, is less well characterized. Here, we investigated the formation of γ Syn amyloid in the absence of lipids and under lipidic conditions using solution nuclear magnetic (NMR), and fast MAS proton-detected solid-state NMR techniques⁵ support with cryo-EM⁶. In lipidic condition, γ Syn shows extended interactions with lipid at both N- and C- terminal regions. The involvement of this region, which shares only 33% similarity with the corresponding region of α Syn, suggests that γ Syn may adopt a distinct aggregate conformation potentially involving differences in the aggregation mechanism compared to α Syn.

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The Hateful Five Percent: cis-Pro47 as the Trigger of the p53–BAX Activation

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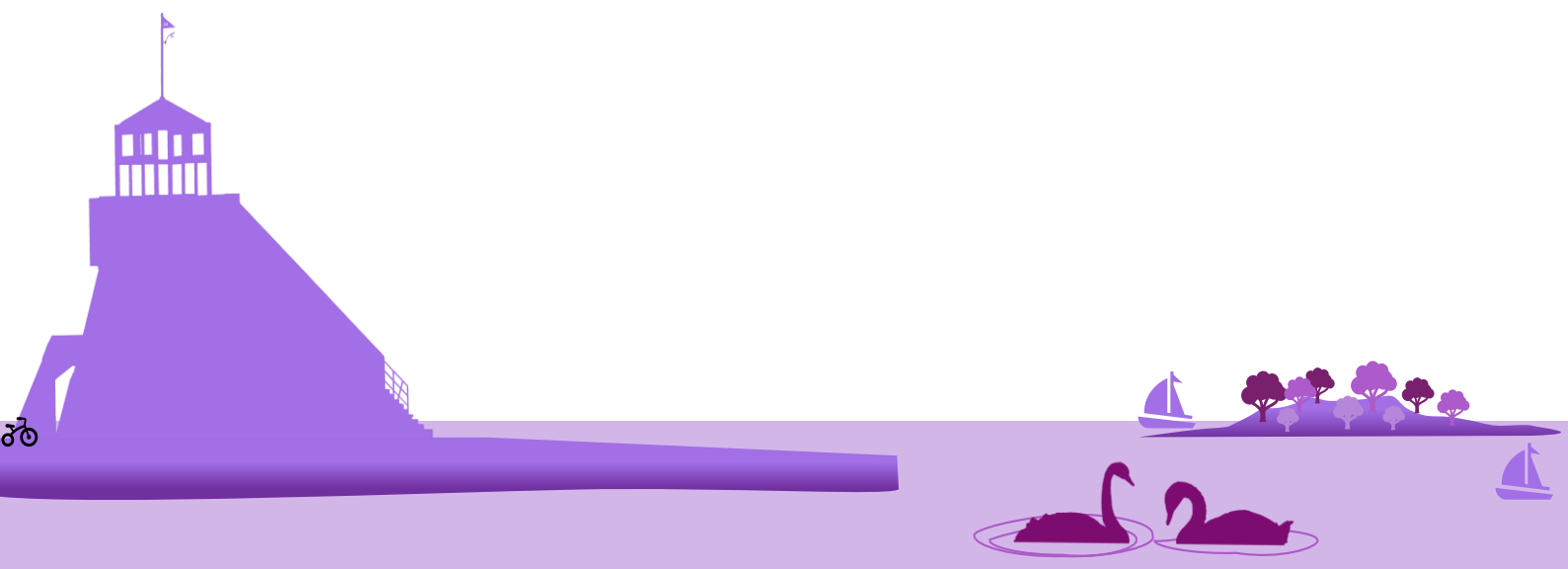
One activation route of the pro-apoptotic BAX is its interaction with the intrinsically disordered trans-activation domain (TAD) of p53. A possible trigger for BAX activation can be the P46 proline cis/trans isomerization in the p53 TAD. Based on our previous results, the cis-P46 conformer is present in the solution in ~5.2%. Our goal was to structurally characterize this interaction.

First, we established a cost-effective bacterial system for the expression of isotopically labeled, full-length BAX at yields of 3–4 mg/L, suitable for NMR studies. PFG-NMR measurements helped elucidating the time-dependent dimerization of BAX, and emphasized the importance of DTT, TCEP - overcoming disulphide bond, the dimer formation.^{4,5} Working with ¹⁵N labeled BAX backbone assignment is performed via classical 3D TOCSY-HSQC and NOESY-HSQC measurements. Interaction with p53(1-60) was monitored from the p53 side. Chemical shift mapping experiments (¹H,¹⁵N HSQC and ¹H,¹³C SHACA-HSQC), revealed line broadening and intensity loss for the residues in both TAD1 and TAD2 regions.

Fluorescence polarization measurements showed weak binding ($K_d > 250 \mu\text{M}$), and ITC experiments confirmed a transient, disorder-driven interaction.⁵

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Optical control of the ABC transporter MsbA by photoswitchable lipids and proteorhodopsin

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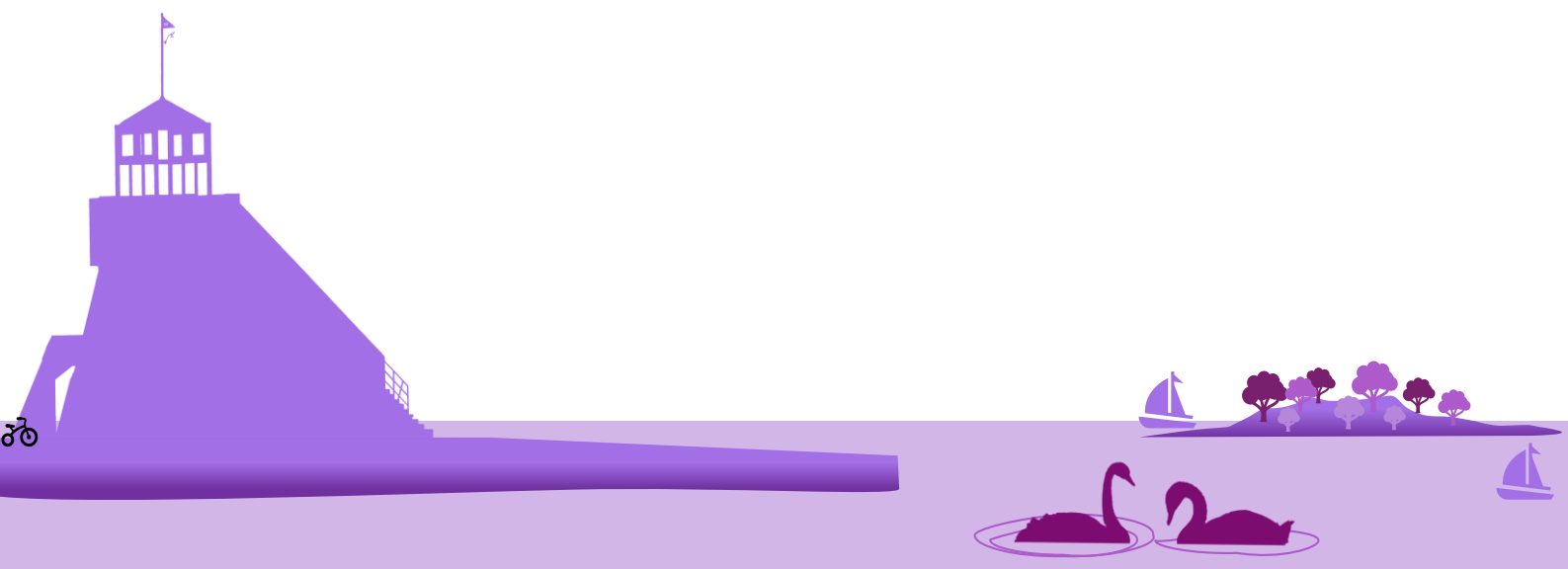
Biochemical processes localized in the cellular membrane are modulated by membrane fluidity and membrane potential [1]. Hence, modulating the properties of the cellular membrane is a key variable in studying biological processes. For example, the membrane environment and pH influences the conformation dynamics of the lipid A flippase in Gram-negative bacteria—MsbA[2]. We therefore employ a combination of high-resolution solid-state NMR spectroscopy and optically tunable liposomes via UV-switchable lipids and a light dependent H⁺ pump—proteorhodopsin to study the light modulation of MsbA. Tuning membrane fluidity by UV-light has been demonstrated in liposomes containing photoswitchable and conventional lipids [3]. In our current work, we show the incorporation of MsbA into photoliposomes and therefore the regulation of MsbA via UV-light. We show through ¹³C-detected 2D-NCA spectra of ¹³C, ¹⁵N-Lys-MsbA and D₂O quenching experiments that protein dynamics is modulated upon photoswitching. We have also explored lipid headgroup dynamics through ³¹P-CSA analysis upon light switching by rotor-synchronized spin-echo experiments. Furthermore, we show a decrease basal ATPase activity in MsbA through an increase in lipid dynamics by real-time ³¹P-MAS NMR. Similarly, incorporating proteorhodopsin and MsbA in an inside-out fashion allows the generation of a light activable pH gradient directly within the MAS rotor. Real-time ³¹P-MAS NMR demonstrates a light-dependent ATPase activity.

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Development of magnetic resonance in Latvia (MR LATVIA)

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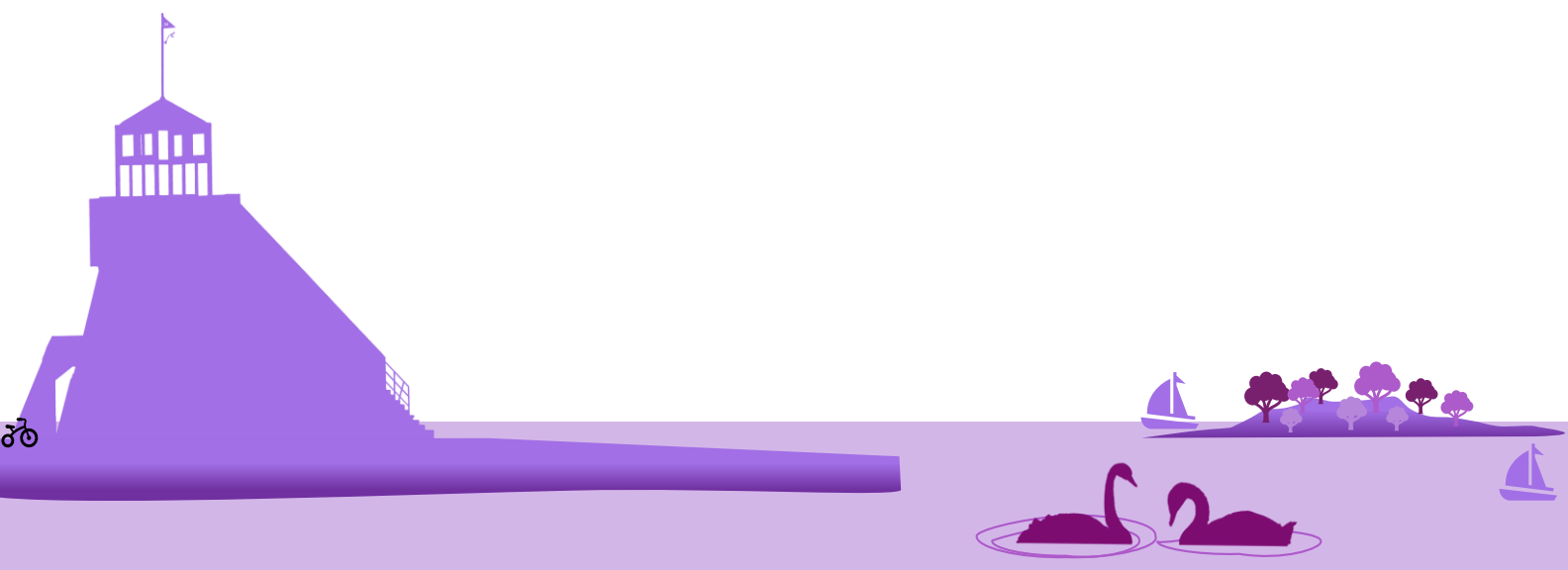
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The MR-LATVIA project aims to establish a regional centre of excellence in NMR spectroscopy at the Latvian Institute of Organic Synthesis (LIOS) in Riga. LIOS is a leading research institute in the Baltic region, with longstanding expertise in medicinal chemistry and pharmaceutical development. Thanks to previous investments, LIOS already hosts one of the most advanced NMR laboratories in Latvia, equipped with high-end instrumentation for both solution and solid-state NMR, including cryogenically cooled probes for biomolecular solution studies and fast magic-angle spinning probes for high-resolution solid-state NMR. This infrastructure provides a strong foundation for cutting-edge applications in chemical, pharmaceutical, and structural biology research.

By linking LIOS with two major European NMR infrastructures (the Centre de RMN à Très Hauts Champs in Lyon (CRMN) and the CERM/CIRMMP consortium in Florence) the MR-LATVIA project supports capacity building through joint research, transfer of expertise, and institutional development. MR-LATVIA also addresses broader goals: integrating LIOS into the European NMR infrastructure landscape, promoting access to NMR across the Baltic region, and bridging the research and innovation gap between Latvia and high-performing EU institutions. The initiative contributes to the development of a sustainable, national NMR core facility, open to both academic and industrial users, and serving as a hub for scientific excellence and innovation.

This poster will present the infrastructure, equipment, and expertise available at LIOS, highlight the collaborative and networking activities with CRMN and CERM/CIRMMP, and promote upcoming access opportunities for academic and industrial users across the Baltic region.

This project is funded by European Commission project “MR Latvia” 101160091, under a HORIZON-WIDERA-2023-ACCESS-02 programme.



Domain–domain interactions and dimerization of the human λ -III immunoglobulin light chain FOR005 investigated by NMR spectroscopy

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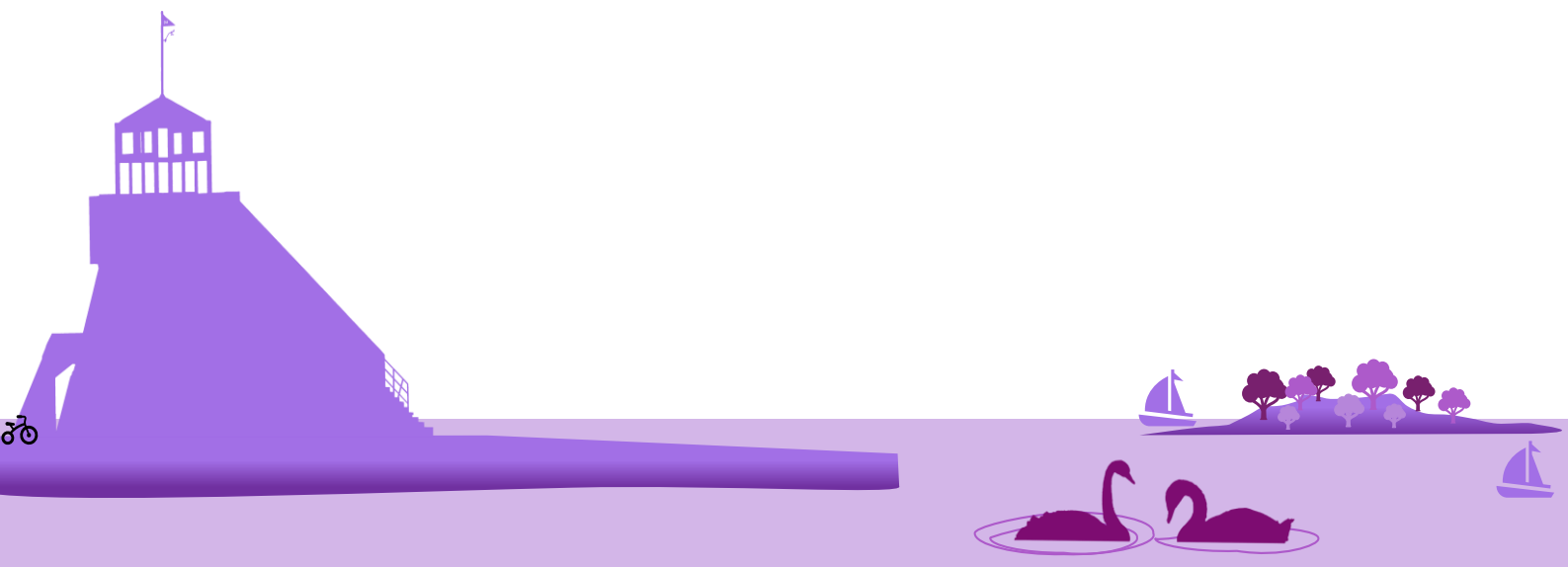
Misfolding of immunoglobulin light chains (LCs) and their deposition as amyloid fibrils are central to systemic AL amyloidosis. We investigated the structural basis of domain–domain interactions and dimerisation in the λ -III light chain FOR005 using solution-state NMR spectroscopy.

Isotopically labeled constructs were prepared for the germline and patient variants of full-length LC, as well as for individual CL and VL domains. Backbone assignments were completed for VL and CL constructs. Concentration dependent HSQCs revealed clear chemical shift changes in the CL domain of the germline variant, consistent with a monomer–dimer equilibrium. In contrast, the patient-derived variant showed no such equilibrium, suggesting a destabilised dimer interface.

To probe the structural origin of this difference, we introduced the patient mutations V136G and C214S into the germline background. Both mutations induced significant chemical shift perturbations in residues located at the CL–CL interface. V136G alone was sufficient to disrupt the hydrophobic core, while C214S modulated solvent exposure and structural packing near the C-terminus. When introduced into the full-length germline LC, these mutations replicated the spectral features of the patient protein.

Fibrillation assays and solid-state NMR confirmed that full-length LC does not readily aggregate under native conditions, but aggregation is strongly induced in the presence of VL fibril seeds. This suggests that partial structural destabilization is required to enable pathogenic conversion.

Together, these findings link specific mutations to loss of native CL dimerisation and to conformational changes that likely represent early misfolding events. NMR provides direct, residue-level insight into the destabilisation mechanisms that underlie amyloidogenic light chain behaviour.



A deep look into structure and interaction of cyclic and linear peptides based on EF-hand motifs with various lanthanides

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Metal-binding proteins are widely common in nature. One notable example is the protein lanmodulin due to its picomolar affinity towards lanthanides. This protein is harboring four EF-hand (helix-loop-helix) binding motifs in which the loop is a 12 residues sequence with a large number of negatively charged amino acids. However, isolated EF-hands do not exhibit as high affinity for lanthanides as when embedded in the intact lanmodulin. Therefore, obtaining a peptide with a good affinity for lanthanides is not a trivial task. In this study, we investigated lanmodulin-derived peptides using paramagnetic NMR to gain deeper insights into their structural properties and lanthanide-binding behavior. While most paramagnetic NMR studies were done on proteins with site-specific paramagnetic labeling employing lanthanide-binding tags (LBTs) or artificial chelators, our approach explores the intrinsic paramagnetic effects of lanthanide-bound lanmodulin-derived peptides. Combining these effects with conventional structure determination methods enables a more detailed characterization of peptide-lanthanide interactions. In this project we focused mainly on elucidating the structures of cyclic peptides using paramagnetic NMR. Due to their size and dynamic properties, conventional NMR approaches provided limited structural information. However, paramagnetic NMR allowed us to identify preferred peptide conformations upon lanthanide binding. We investigated cyclic peptides based on different lanmodulin EF-hands to find out which one would preserve best the 3D structure found in the original lanmodulin. This research is part of a study to obtain a bio-separation platform based on biohybrid technologies capable of separating and isolating lanthanides. This platform will be based on peptide-polymer conjugates, so obtaining peptides with high affinity for lanthanides is one of the main goals at this stage of the project for future applications in bio-based rare-earth element recovery.



Fluoride coordination to Ln(III) complexes: probing anion-anion binding with low and high-Resolution NMR

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Fluoride coordination to metal complexes plays a fundamental role in catalysis, sensing, and bioinorganic chemistry. However, fluoride interactions with metal complexes, particularly with anionic metal species, remain an area of ongoing exploration. In this study, we investigate fluoride binding to linear aminopolycarboxylate complexes of Gd(III) and Y(III), revealing an unusual anion-anion coordination mode. Using a combination of relaxometric and high-resolution ^{19}F NMR experiments, we demonstrate that fluoride displaces inner-sphere water molecules in both mono- and bishydrated complexes, forming ternary adducts with distinct structural and dynamic properties. Notably, while EGTA-based complexes yield simple mononuclear fluoride adducts, the OBETA ligand unexpectedly promotes the formation of a dimeric $[\text{F}\cdot(\text{YOBETA})_2]^{3-}$ complex, where a single fluoride ion bridges two anionic metal chelates. Fluoride exchange dynamics were further explored through ^{19}F NMR line-shape analysis, which indicated a dissociative exchange mechanism for EGTA complexes and an associative pathway for OBETA analogs. Additionally, ^1H relaxometry provided crucial insights into the fluoride ion affinity for Gd(III)-based complexes. Complementary DFT calculations validated the structural models of all studied systems, with particular focus on the $[\text{F}\cdot(\text{YOBETA})_2]^{3-}$ dimer. These calculations were fundamental in accurately reproducing key spectroscopic parameters, such as ^{19}F chemical shifts and JY-F coupling constants. These findings not only provide a deeper understanding of fluoride binding in lanthanide complexes but also highlight how a multi-technique approach enables a solid and detailed characterization of these systems. This comprehensive insight is key to designing next-generation MRI contrast agents and anion-sensitive probes, where fine control over hydration and anion binding can optimize relaxivity and selectivity.



What can we learn about spin-crossover complexes using solid-state NMR?

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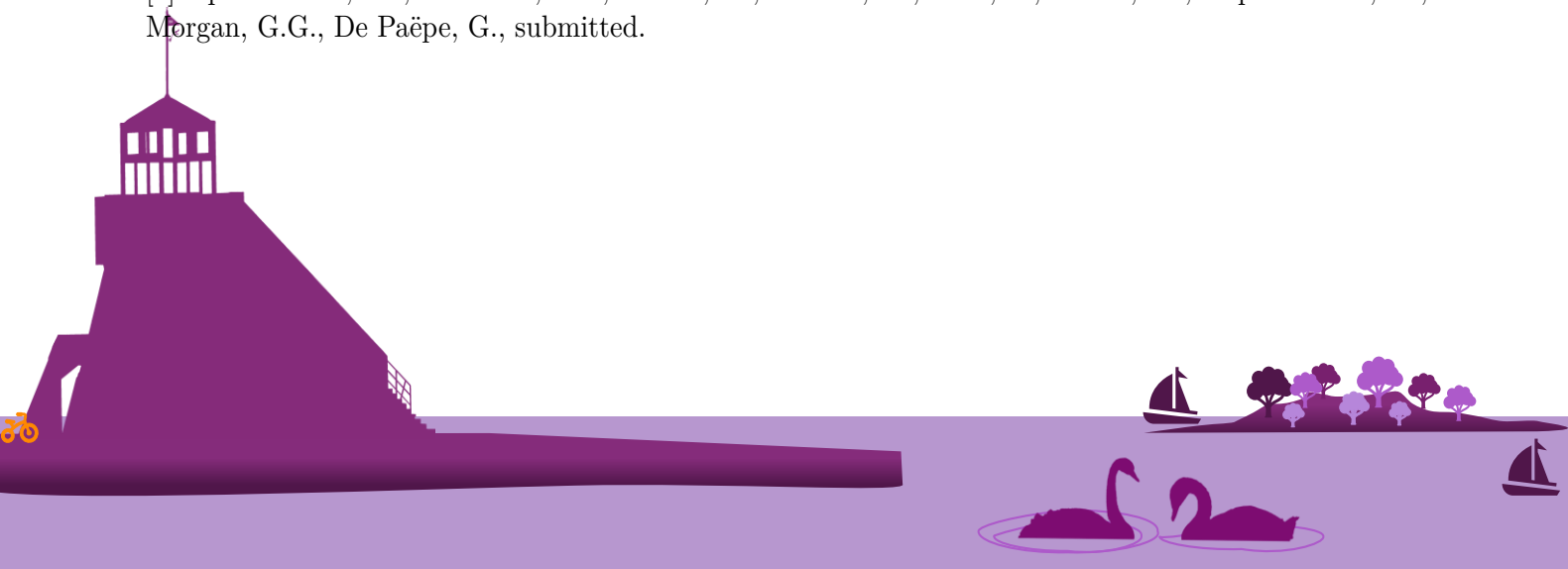
Understanding electron spin transitions is crucial for biochemical processes and molecular spin-control technologies involving transition metal molecules. These transitions are triggered by external stimuli such as temperature, light, pressure, or magnetic fields. The current arsenal of experimental techniques includes macroscopic methods tracking collective molecular behavior and microscopic methods monitoring changes at the metal center or vibrational fingerprints during spin-transition. However, no established technique simultaneously tracks changes in the arrangement and electronic structure of ligands during transitions. Solution NMR can probe electronic changes but has pitfalls such as solvent effects altering spin-state equilibria. Herein by extending the approach to spinning solids, we show that spin transitions can be followed using broadband variable-temperature 2-D MAS solid-state NMR [1], coupled with state-of-the-art quantum chemical calculations of the NMR/EPR parameters [2]. This methodology was initially implemented on a Mn(III) spin-crossover (SCO) complex, exhibiting a narrow hysteresis window near cryogenic temperatures [3]. Subsequently, it is extended to the ultra-fast MAS regime (100 kHz) in a Mn(III) molecular rotor, to probe a partial spin-transition (low-to-high spin) followed by a molecular rotation that forces electron spin-pairing (high-to-low spin) near room temperature. Our findings demonstrate that solid-state NMR is an excellent tool for providing insight into both the subtle changes in the electron spin density distribution in the ligands of SCO molecules as well as the macroscopic cooperative behavior upon such transitions.

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Enhancing the performance of Gd³⁺ chelates as relaxation agents for MRI application

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Concerns over the safety of Gd(III) chelates as MRI contrast agents emerged in the early 2000s following cases of nephrogenic systemic fibrosis (NSF) in patients with impaired kidney function. Although regulations have effectively reduced NSF, safety concerns persist. Additionally, Gd(III) pollution is rising, with elevated levels detected in various aquatic environments, though the long-term impact remains unclear. A viable solution to both issues is to reduce the amount of Gd(III) chelates used in clinical practice while preserving contrast efficiency. This can be achieved by developing novel more efficient, and chemically stable probes.

This study explores two different strategies. First, mono-hydrated α -aryl substituted GdDOTA chelates were synthesized and characterized.[1] These chelates are very efficient MRI contrast agents due to their rapid water exchange kinetics, improved electronic relaxation, and slow tumbling motion in solution. These properties were analyzed by applying a spectroscopic approach based on ¹H NMR relaxometry as a function of the applied magnetic field and temperature, high-resolution ¹⁷O NMR, and ¹H NMR studies of Eu(III) surrogate chelates.

The second approach involved bi-hydrated chiral Gd(III) complexes based on pycnen scaffold.[2] These complexes demonstrated a positive combination of high thermodynamic stability, kinetic inertness and good relaxivity at the clinical magnetic fields, highlighting their potential for MRI applications. In vivo MRI imaging at 3.0 T further revealed their ability to differentiate tumor from normal tissues in a mouse model more effectively than currently used clinical agents.

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Using local techniques to study cathodes materials for Na-ion batteries

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Sodium-ion batteries (NIBs) have emerged as a cost-effective and sustainable alternative to the lithium-ion technology, particularly for large-scale energy storage applications. Amongst the cathodes materials studied for NIBs, $\text{Na}_3\text{V}_2(\text{PO}_4)_2\text{F}_3$ (NVPF) is very promising due to their highly stable polyanionic framework, high energy density and excellent rate capability. On the other hand, Prussian blue analogues (PBA) also represent a promising candidate thanks to its open framework with large channels that allows the reversible insertion of a large panel of cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Sr^{2+} , Ba^{2+}), their sustainability and low cost, their high specific energy and their good rate capabilities.

In this work, I will like to highlight the importance of using local techniques such as Solid-state NMR and Mössbauer spectroscopy to understand the presence of defects in these two cathodes materials and how to correlate the relationship between synthesis-structure-electrochemical properties. More specifically thanks to ^{31}P and ^{23}Na Solid state NMR we were able to identified defects present in the pristine materials (NVPF) and by analyzing a series of NVPF with increase fluorine content, together with DFT we were able to identified and assigned the different peaks we observed in the NMR spectra. In the case of PBA we were able to synthesize and identified a series of rich phases and their morphology composition and defects will be discussed.



Multifunctional lanthanoid tags for future application as PCS and FRET probes

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Understanding the complexity of biological systems is of great importance for the fields of biology, medicine, and pharmacology.

Lanthanoid complexes are versatile tools for studying biological structures and their interactions. Their unique magnetic and electronic properties make them excellent probes for different bioimaging and biosensing techniques such as Förster resonance energy transfer (FRET) spectroscopy, fluorescence microscopy, and as pseudocontact shift tags for NMR applications.

Herein, we report the design of two new cyclen (1,4,7,10-tetrazacyclododecane)-based ligands for future applications as PCS and FRET probes for the investigation of biological macromolecules.

The rigid, highly unsymmetrical ligands offer a binding site for covalent binding to cysteine as well as a chromophore residue with an absorption range up to 380 nm, thus offering the fundamental properties needed for such multifunctional usage.

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



Development of PCTA-Based Lanthanoid Chelating Tags for Pseudocontact Shift NMR Applications

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Polyazamacrocycles, such as tacn, cyclen, cyclam, and pycen, have garnered considerable attention during the recent development of magnetic resonance imaging contrast agents and advanced radiopharmaceuticals. Especially PCTA (2,2',2''-[3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triyl]triacetic acid) has shown great potential owing to its structural stability, high kinetic inertness, and fast complex formation with lanthanoid radioisotopes.

In addition, lanthanoid ions possess unique electronic and magnetic properties, making them highly interesting for paramagnetic NMR applications. Several lanthanoid chelating tags (LCTs) have been developed based on the DOTA framework, allowing rigid attachment to a protein via the reaction of the proteins' cysteine residue with a linker on the corresponding complex. The stereoselective introduction of methyl groups allowed the stabilization of only one conformation.

Inspired by this, we developed two LCTs bearing a PCTA scaffold that can be used for pseudocontact shift applications. In order to suppress dynamic processes, methyl groups were introduced, allowing the observation of only one set of signals on the ubiquitin-S57C mutant. The calculated anisotropic susceptibility parameters almost reached those of known M7-DOTA LCTs, making this scaffold highly interesting for further development



Low Field and Fast Magic Angle Spinning Increases Spectral Resolution in Paramagnetic Materials

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Aims: Paramagnetic materials have garnered significant research attention due to their tuneable composition, size, shape and magnetic properties making them suitable for a wide range of applications, including contrast agents, electromagnetic dampers and isolators, magnetic refrigeration systems and energy storage. Understanding structural features of paramagnetic materials is crucial for improving their performance. Solid-State NMR spectroscopy is a unique approach to investigate local structural environment and dynamic of ordered and/or disordered materials at atomic level. However, NMR spectra of paramagnetic materials are often difficult to interpret because they suffer from low resolution arising from large chemical shifts, signal broadening, and short relaxation times. One strategy to overcome these issues is to use a lower magnetic field strength.

Methods: Paramagnetic cathode materials LiFePO_4 (LFP) and nickel-rich layered oxide $\text{LiNi}_{0.8}\text{Mn}_{0.1}\text{Co}_{0.1}\text{O}_2$ (NMC811) were studied with ^7Li MAS NMR at two magnetic field strengths, 9.4 and 4.7 T. Spinning was performed at 62.5 kHz with 1.3 mm zirconia rotors. Spectra were acquired with a WURST-CPMG pulse sequence.

Results: Fast spinning of LFP and NMC811 cathodes at 62.5 kHz at low magnetic field of 4.25 T results in a narrower distribution of spinning sideband pattern, narrower linewidth of isotropic signals, and therefore enhances spectral resolution significantly compared to the measurements at 9.4 T. Additionally, lower eddy currents are induced at lower magnetic fields. These currents often hinder sample rotation, making sample spinning simpler on our 200 MHz compared to higher fields.

Conclusion: By combining low magnetic field with fast Magic Angle Spinning (MAS) up to 67 kHz, it is possible to increase spectral resolution so that valuable structural information about paramagnetic components can be obtained.



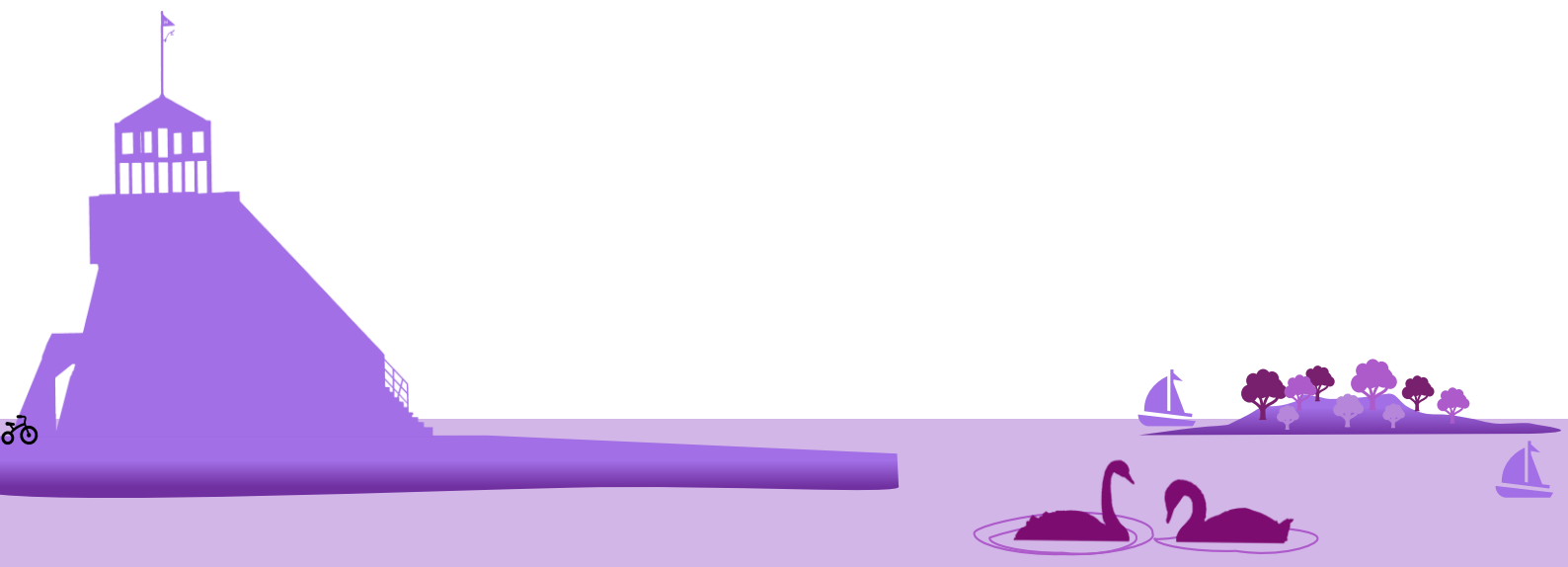
Investigating dynamic processes in molecular charge carriers by means of NMR spectroscopy and differential scanning calorimetry

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Ionic liquids characterized by unique properties such as high thermal stability and low volatility are increasingly popular in literature. Their potential applications in green chemistry include energy storage and usage as green solvents and catalysts. This research involves compounds of ionic liquids, having the same anion with various substituents to cover several lengths of alkyl chains. The experiment aims to investigate the molecular dynamics in selected substances by means of NMR spectroscopy and Differential Scanning Calorimetry. The testing procedure was performed on isotopes of hydrogen atom ^1H and fluorine ^{19}F . The equipment used involved a Bruker CXP 200 MHz Spectrometer. Measurements were carried out in the temperature range 90-400K at a resonance frequency of 200 MHz for ^1H and 187 MHz for ^{19}F . To determine activation parameters of dynamic processes of molecular groups, an approach was used to establish nuclear spin-lattice magnetic relaxation times based on statistical theories of magnetization propagation in a spin system. NMR FIDs signals were collected and the T_1 relaxation times were determined for all temperature ranges. Estimated relaxation times revealed dominant relaxation process related to anisotropic rotation of methyl group accompanying the *trans-gauche* conformational changes of the CH_2 groups for those substances with longer chains. Individual groups motions were observed in higher temperatures. Thermal variability was determined using the differential scanning calorimetry technique. Runs were examined in the temperature range from 150 to 405 K with speed 10 K per minute. From recorded DSC thermograms, characteristic temperatures like T_{onset} , T_{peak} , T_{end} values were estimated for all revealed phases transitions. The study demonstrated that for all substances there is a typical thermal change associated with glass transition at low temperatures, and also for some of studied samples cold crystallisation and subsequent melting process.



Classification and Identification of Facet- and Edge-Specific γ -Al₂O₃ Surface Sites from ¹H/²⁷Al NMR Cross-Signatures and DFT Modelling

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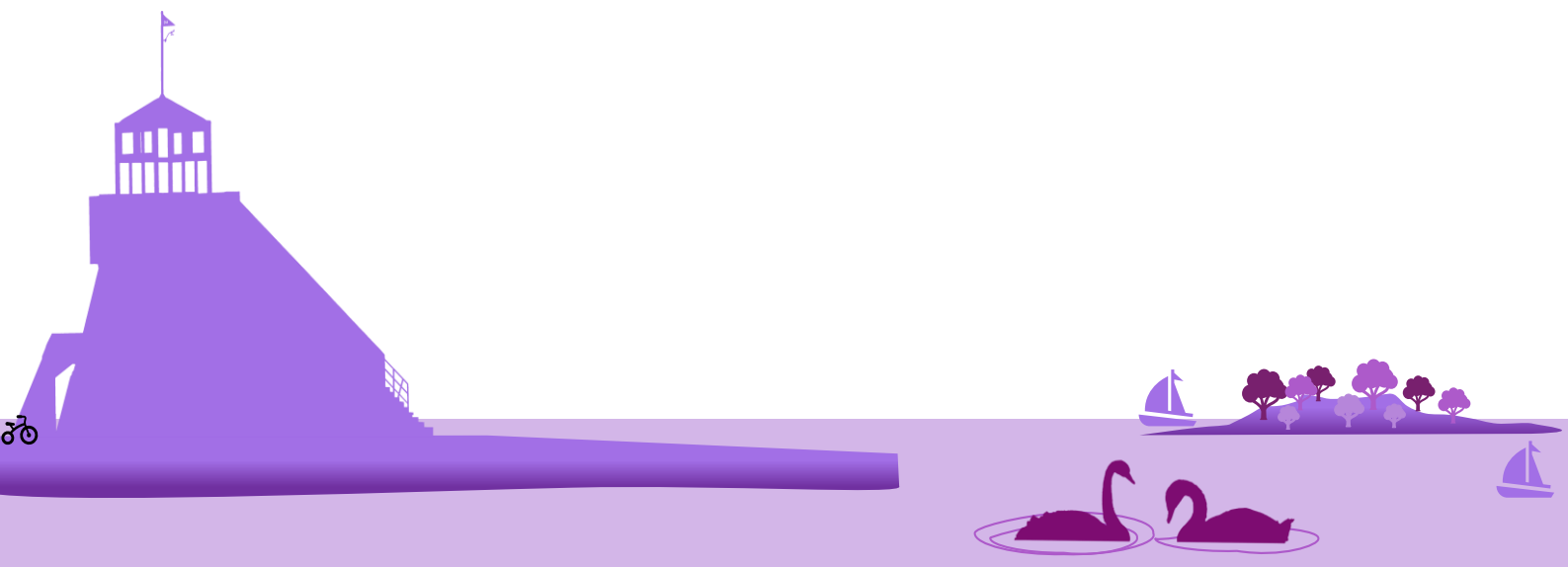
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γ -Al₂O₃ finds applications in industry as catalyst or support for catalytic active phases. The properties of γ -Al₂O₃ have been ascribed to surface sites with varying Al coordination number, acidity and basicity.^{1,2} The presence of specific [i]Al(μ j-OH) has been proposed to be associated with certain facets or edges. In recent years, solid-state NMR (ssNMR) has emerged as a powerful tool because of its high sensitivity to the chemical environment and its potential to resolve the various ¹H, ²⁷Al and ¹⁷O surface sites. Furthermore, two-dimensional ssNMR correlations helped gaining a better understanding of the connectivity of surface species.³⁻⁵ However, Al surface sites are experimentally challenging to detect on de-hydroxylated surfaces, central to catalytic processes, due to expected large CQ values, typically >15 MHz.⁶ Here, we combine surface-specific D-HMQC ²⁷Al{¹H} ssNMR at high field (28.2T) at fast MAS (50kHz) to observe four main distinct families of surface Al-OH sites, including an [4]Al-OH site with an unprecedented CQ approaching 18MHz. We applied this methodology to two γ -Al₂O₃ samples, dehydroxylated at 500° (2.8 OH/nm²), an edge-rich needles shaped γ -Al₂O₃, and the commercially available SBA200. All resulting ²⁷Al-O1H environments are fitted with pairs of (²⁷Al, ¹H) lines allowing us to recover coupled NMR parameters. Comparing the measured NMR signatures (²⁷Al δ iso, CQ and ¹H δ iso) to computed values from a large range of structural DFT models⁷ allowed the identification of facet- and edge-specific Al-OH surface sites in γ -Al₂O₃. The assignments revealed different edge/facet ratio in the studied samples, consistent with their different morphologies. Our findings enabled the derivation of 3D model of γ -Al₂O₃ surface and can serve as a tool to advance the synthesis of materials with specific surface features. This knowledge can be applied to understand the interactions of molecules with γ -Al₂O₃ surface, and to develop more accurate surface models.



Optimally Controlled NMR in Electrochemistry: Surface-Selective Measurements above Conductive Materials

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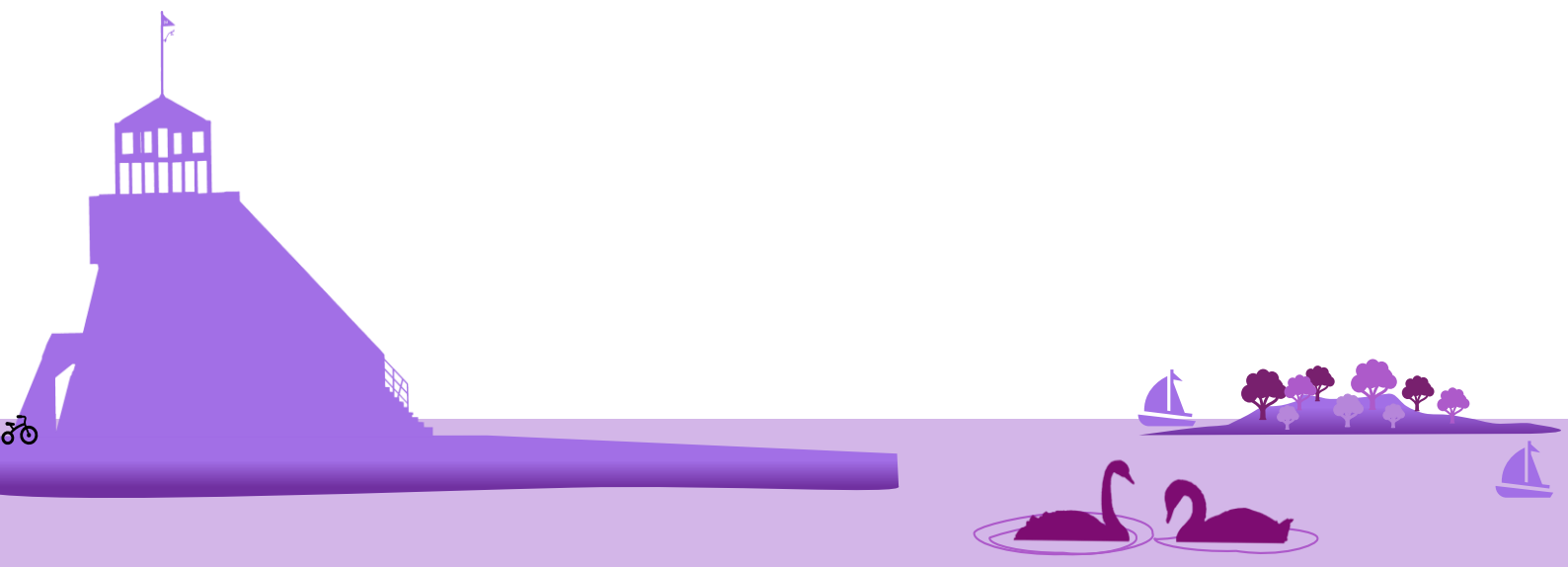
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With the growing importance of nuclear magnetic resonance (NMR) spectroscopy on electrochemical systems, such as in operando electrolysis, complex setups give rise to new experimental challenges that are difficult to address with standard NMR experiments. Particularly, conductive cell components cause magnetic field distortions due to shielding and eddy current effects, leading to reduced resolution, non-quantitative results, and possible artifacts. However, these local distortions can also be selectively addressed using shaped pulses tailored by quantum optimal control (QOC). In our work, we show how finite element method (FEM) simulations accurately predict magnetic field distortions in NMR setups containing conductive materials. We demonstrate how this information can be utilized in QOC workflows to design spatially selective NMR experiments without the need for pulsed field gradients or imaging experiments. FEM simulations indicate that surface selectivity in the range of micrometers can be achieved, suggesting a vast unexplored potential of in operando NMR in electrochemistry, material sciences and even heterogeneous catalysis.



Exploiting ^{17}O Solid-State NMR Spectroscopy of Catalysts and Porous Solids

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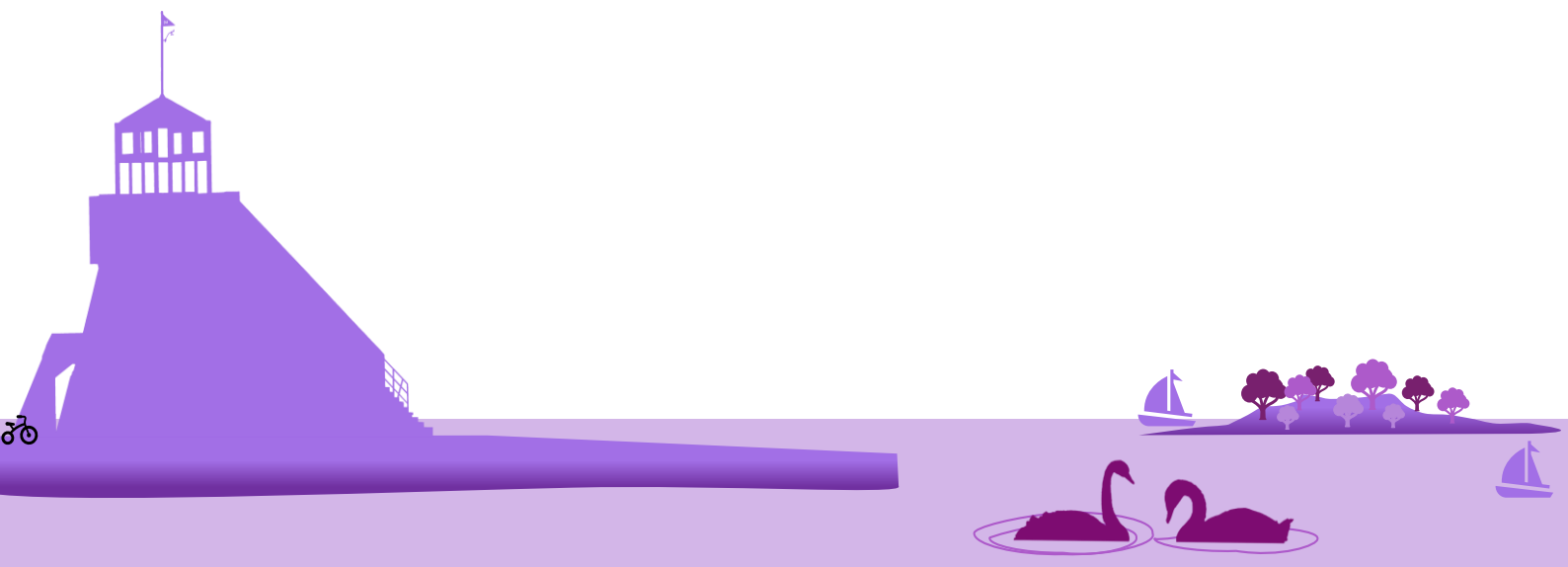
Zeolites are aluminosilicate frameworks with applications in storage, separation and as industrial catalysts.[1] Due to an increasing demand for industrial processes to be performed under milder conditions, there is significant interest in investigating the chemical reactivity of zeolites under aqueous conditions. Zeolites can be challenging materials to characterise owing to the high levels and types of disorder present, but NMR spectroscopy provides insight into local structure, disorder, and chemical reactivity.

Oxygen is a key component of zeolite frameworks, present as Brønsted acid sites in the framework, in the water and some of the guest molecules that fill the pores. However, when considering NMR spectroscopy, ^{17}O has a low natural abundance (0.038%) and isotopic enrichment is usually required to obtain spectra on a reasonable timescale. Recent work has shown that cost-effective and energy-efficient ^{17}O enrichment of zeolites can be achieved at room temperature using a “slurry” with $\text{H}_2^{17}\text{O}(\text{l})$, [2] although the rate and selectivity of the process varies with the type and amount of cations (e.g., H^+ , Na^+ , K^+) present and the timescales can be long (1-100 days).

In this work, alternative methods for efficient room temperature ^{17}O enrichment of zeolites are demonstrated; wetness impregnation and a stirring method. These methods exploit the room-temperature lability of the zeolite framework bonds in the presence of water.[2] We compare the rate, efficiency and selectivity of these methods to those used previously. In addition, we look at the process of back-exchange and its implications for ^{17}O NMR spectroscopy. Ultimately, the structural information and insight into what the chemical reactivity of the framework depends upon will help to better understand these zeolite frameworks and to further develop their use in industrial processes.

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^{95}Mo MAS SSNMR of grafted molecular complexes at ultra-high field (28.2 T)

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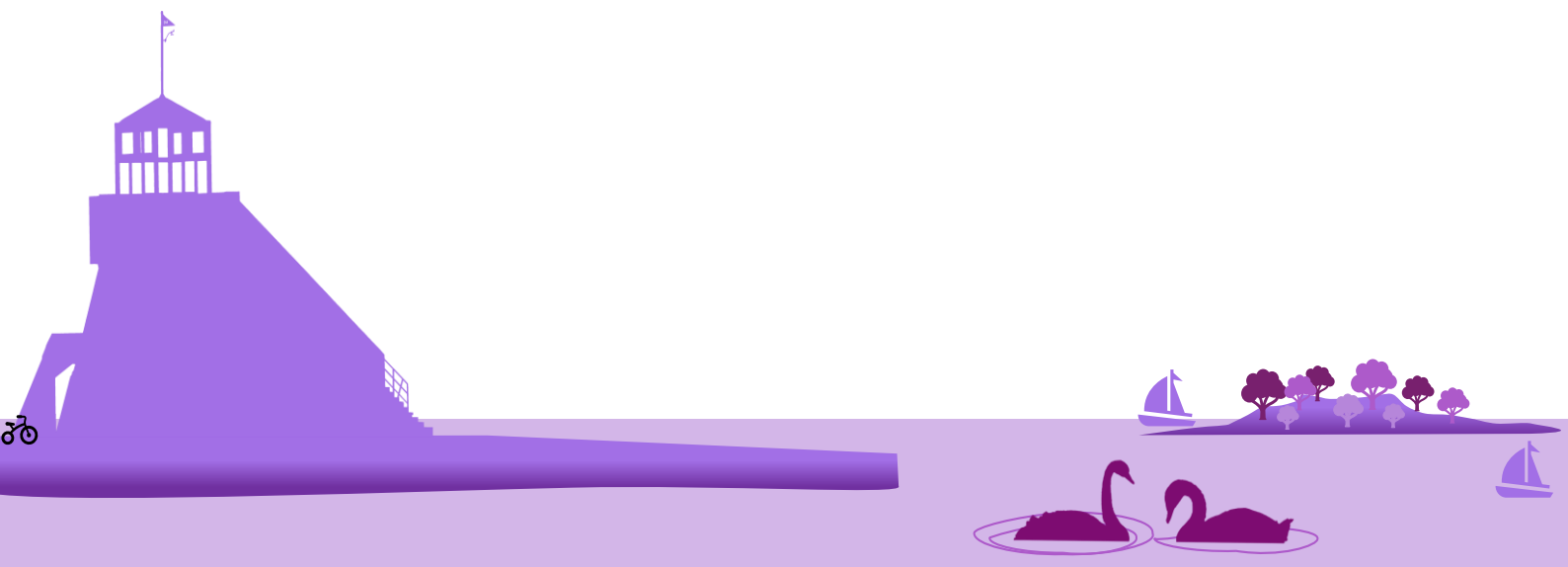
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Olefin metathesis is a key catalytic reaction in industry, often using Mo(VI) complexes derived from Schrock-type homogeneous catalysts. However, heterogenization alters the ligand and metal environments, making atomic-scale structural characterization crucial for understanding catalytic behavior. Solid-state NMR is a powerful tool for probing the local environment of molybdenum in these catalysts, but faces challenges due to the ^{95}Mo (spin 5/2) quadrupolar interaction, low natural abundance (15.7%), weak gyromagnetic ratio, and small magnetic moment, which reduce sensitivity.

To address these issues, we investigated molecular complexes, with mono-oxo and mono/bis-imido functionalities. The use of an ultra-high magnetic field (28.2 T) improves spectral resolution, while an optimized DFS-QCPMG pulse sequence enhances spectral quality. Anisotropic NMR parameters (CQ, δCSA) complement isotropic chemical shifts to characterize the ^{95}Mo environment. Data reveal chemical shifts from -300 to 1000 ppm, CQ from 3 to 8 MHz, and δCSA from 500 to 1500 ppm, paving the way for the study of more complex grafted systems. Periodic DFT calculations (VASP, PBE) confirm the local structure of the complexes, forming a primary database of NMR parameters and enabling further investigation of surface Mo catalyst.

Investigating Mo catalysts grafted onto amorphous silica remains challenging due to their complex local environments, and the low dispersion of Mo atoms on the surface. To overcome these limitations, we synthesized a grafted bis-oxo catalyst, representative of industrial catalyst, via SOMC using ^{95}Mo isotopic enrichment to enhance signal intensity. SSNMR analysis monitors the structural and electronic evolution of Mo during grafting, providing deeper insights into its catalytic properties and facilitating the rational design of next-generation metathesis catalysts.



Degradation Assessment and Conservation Monitoring of Waterlogged Archeological Wood by Multimodal and Multiparametric NMR Methods

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Wood plays a crucial role in cultural heritage, as wooden artifacts preserve records of our past. The anoxic conditions of submerged environments can protect wood for millennia but degradation still occurs. Once excavated, waterlogged wood (WW) rapidly deteriorates, losing mechanical strength unless kept submerged.¹ However, this makes it ideal for NMR analysis. Recently, interest in non-destructive NMR studies on fragile heritage has increased, although some possibilities remain unexplored.^{2,3} From a conservation perspective, WW requires stabilization and when in-situ conservation is unfeasible, consolidation becomes essential to replace water within the wood tissue while reinforcing its structure. Treatment effectiveness depends on consolidants' penetration and distribution, influenced by their properties and the wood's porous structure.⁴ This study combines a multimodal approach using high- and low-field techniques to assess wood degradation and monitor conservation treatments. While MRI and diffusion NMR offer structural and dynamic insights, they lack the resolution to examine the cell wall at the nanometer scale, achievable with NMR cryoporometry.^{5,6} With these methods, we conducted a comprehensive study of wood from the Neolithic submerged village of La Marmotta, assessing degradation after millennia of submersion. Moreover, we explored portable NMR's potential for monitoring the consolidation of WW from the 10th century.⁷ Samples were treated with polyethylene glycol (PEG) solutions at varying concentrations and weights, following standard practices. PEG's use as a consolidant is well-supported, making it ideal for heavily degraded wood.

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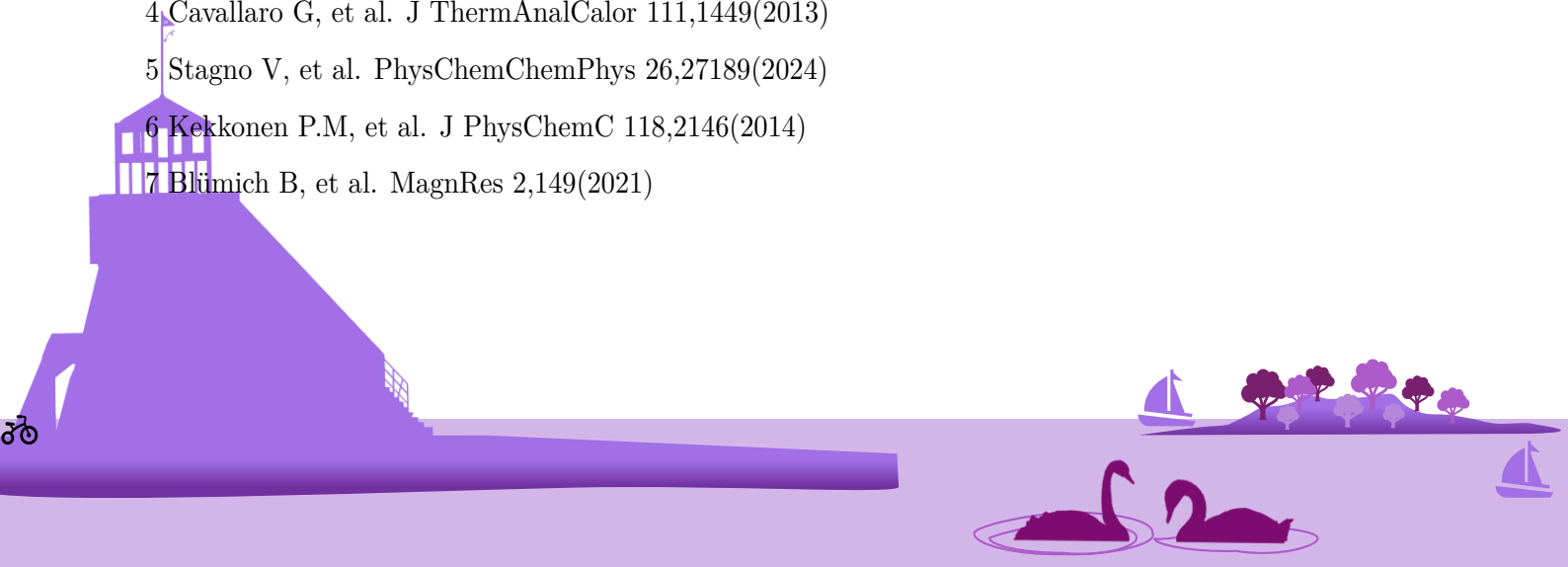
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Close-up of a key step in the elaboration of a new phase change material : Solvent – Cellulose Interactions Studied by Pulse Field Gradient NMR

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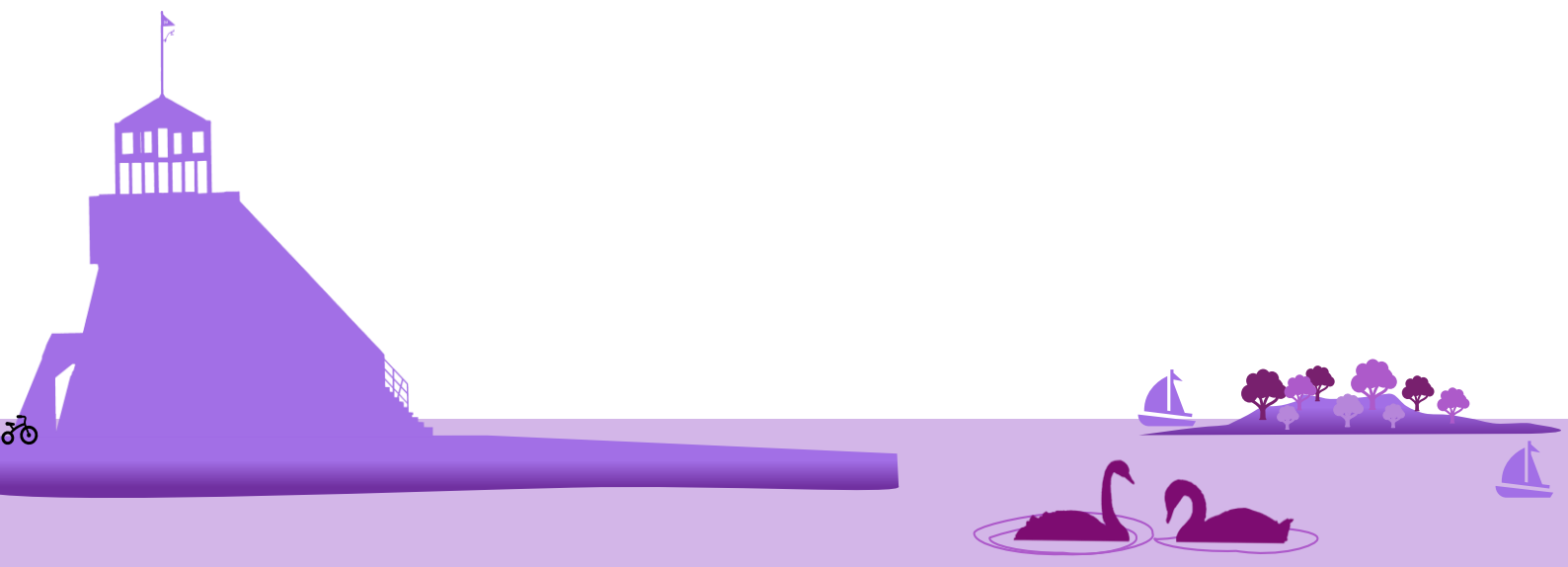
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Since climate change issues lead us to adapt our infrastructures and technologies, one of the key challenges is designing new sustainable and/or bio-sourced materials capable of efficiently storing and releasing thermal energy. Cellulose–Phase Change Materials (PCM) composites are promising candidates. PCMs store and release latent heat when undergoing phase transitions, while cellulose, a bio-sourced material, is already widely used in various domains such as textiles and thermal insulation.

A crucial step in the development of such materials is cellulose dissolution, which is known to be challenging in common organic solvents due to its strong intra- and intermolecular hydrogen bonds. Many newer and greener solvents, such as certain ionic liquids have been reported to dissolve cellulose effectively. Nuclear Magnetic Resonance (NMR) is a powerful tool that provides valuable information on the local structure, dynamics, and molecular diffusion of materials. Though not frequently employed for this purpose, it can successfully assess the efficacy of solvents in dissolving cellulose.

In this work, we studied cellulose dissolution in a green, recyclable solvent (Tetrabutylphosphonium hydroxide) using ^1H and ^{13}C pulse field gradient (PFG) NMR. Key characteristics of the dissolution process were extracted by monitoring the diffusion coefficient of different species at various weight percentages of dissolved cellulose. First, distinct diffusion regimes of the species in solution were identified and linked to the number of bound solvent cations, allowing determination of the solutes' hydrodynamic radii. Second, critical concentrations of dissolution systems were determined, providing essential insights into the dissolution step.

In conclusion, PFG-NMR enabled us to better determine and understand the interactions between solute and solvent in our solution. This bridges the information gathered in the aqueous phase with the next step: assessing the impact of PCM incorporation.



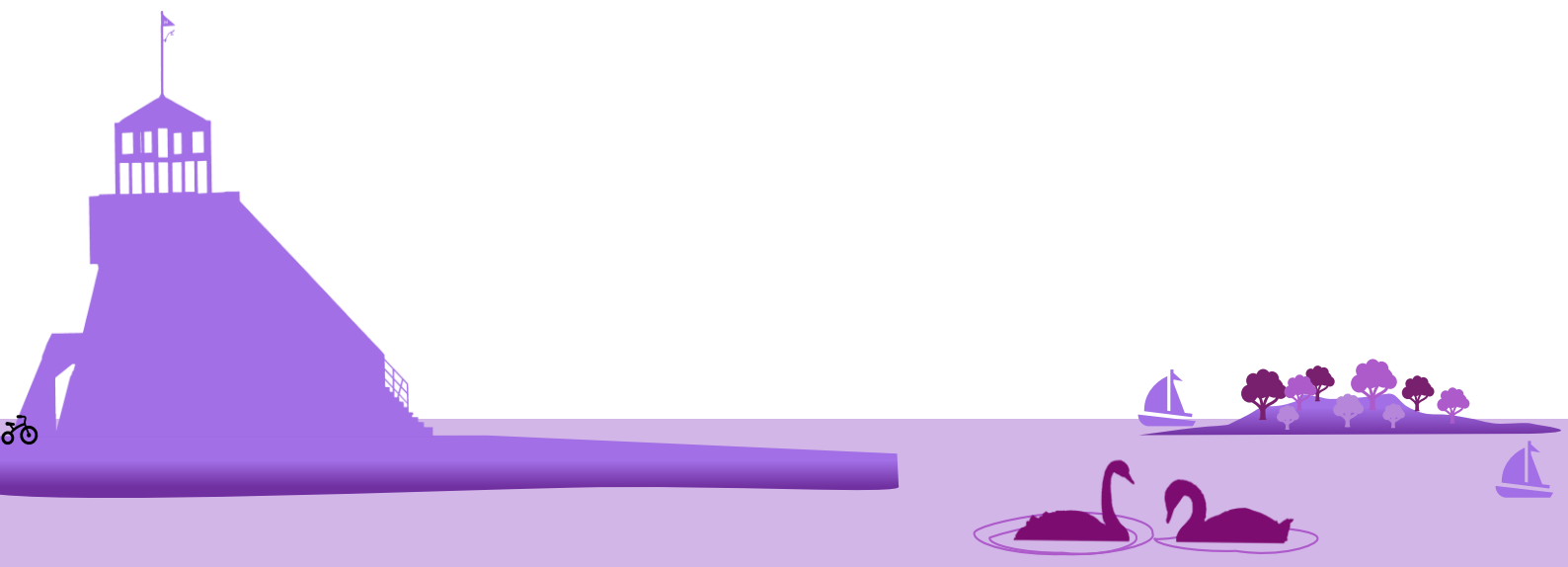
Characterization of Reclaimed Old Wood using 3 point flexure test and Solid-State NMR

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The increasing need for sustainable building and construction methods to preserve the forests longer and reduce carbon dioxide emissions has generated interest in using reclaimed wood from demolished buildings. However, the properties, performance and reliability are poorly understood signifying a research and knowledge gap to effectively use reclaimed wood in new building projects. Therefore, this research uses solid-state nuclear magnetic resonance (NMR) spectroscopy to analyse reclaimed wood samples collected from a building demolition site from three different sections of a 50-year-old building namely: floor, wall, and roof components, to compare them to fresh pine (*Pinus sylvestris*) as an experimental control. The main parameters investigated were hemicellulose degradation markers, lignin-carbohydrate complex (LCC) bonding patterns and cellulose crystallinity index (CI). Mechanical tests were performed to obtain the modulus of elasticity (MOE), and modulus of rupture and density was performed using three-point flexure tests. The results showed that on average reclaimed wood has 15–25% reduction in cellulose and hemicellulose in comparison to fresh wood samples. When comparing reclaimed to fresh wood samples the lignin aromatic signals displayed broadening, showing signs of oxidative degradation and a 11–16% loss of β -O-4 linkages. Hemicellulose acetylation in reclaimed wood increased by a factor of 1.1–1.7 in comparison to fresh wood, correlating with cyclic hygrothermal aging, with roof exhibiting the most degradation and wall samples the least. There is a strong positive correlation ($R^2 = 0.86$) between the MOE, MOR and cellulose CI showing that crystallinity loss affects the mechanical properties of wood. The preserved cellulose, hemicellulose and lignin matrix in the reclaimed wall samples shows a potential for reuse in new building projects, whereas floor and roof wood may be reused in non-structural applications.



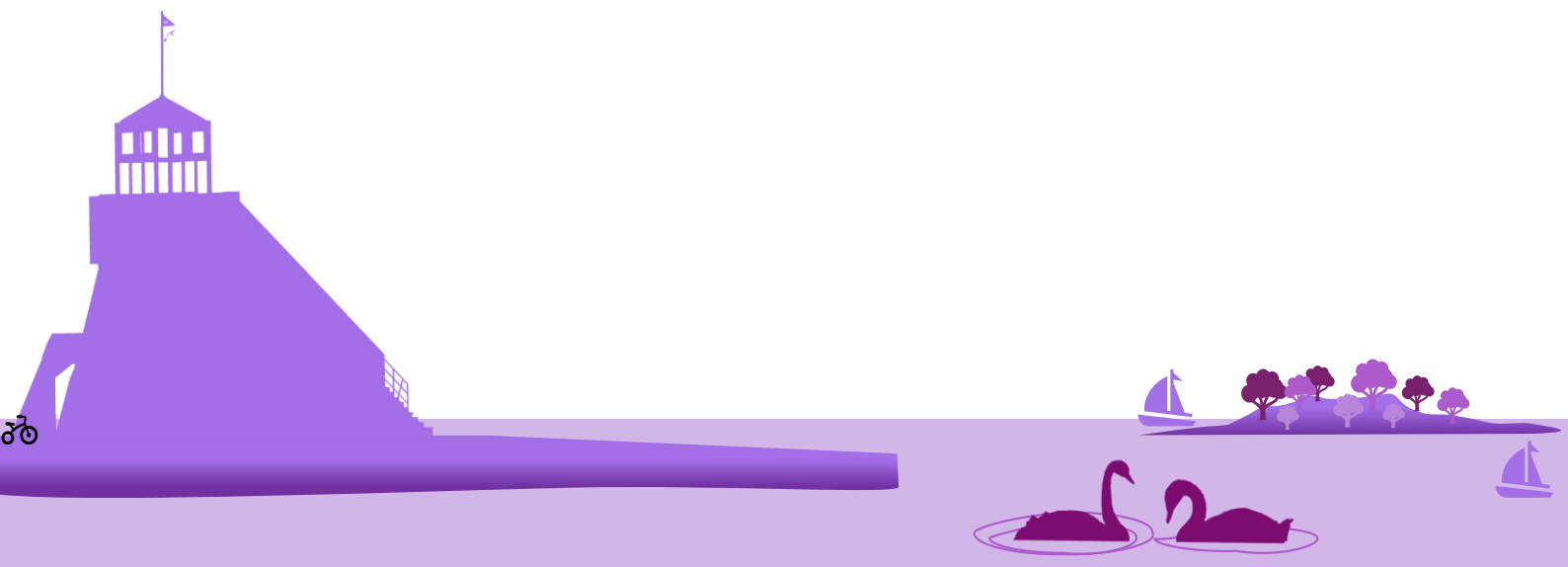
Microstructural and Dynamical Characterization of Polymer Electrolytes for Li-ion Batteries Using Solid-State NMR

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Ionic Conductivity in solid electrolytes represents a significant challenge in the development of efficient all- solid-state batteries along with the interfacial resistance due to imperfect electrode-electrolyte contact and dendrite formation. We decipher the lithium ion (Li^+)-conductivity mechanism in PEO (poly (ethylene oxide)) based SPEs by probing the microscopic properties including segmental dynamics and local structural phases. Here, we understand Li-ion transport through the PEO matrix using advanced solid-state NMR methods in combination with the impedance spectroscopy. We have prepared the PEO films by varying the LiTFSI salt concentration from 2wt% to 30wt% and performed multinuclear (^1H , ^{13}C , ^{19}F , $^6/7\text{Li}$) solid state NMR to gain insights into local phases of the polymer and Li ion dynamics. The inability to observe the cross-polarization in the system and the presence of the J splitting in ^{13}C NMR reveal the liquid-like behaviour of the polymer system. We have also performed variable temperature experiments on ^1H , ^7Li and ^{19}F by varying the temperature from 263.15K to 328.15K. The relationship between T_1 (spin-lattice relaxation time) and temperature provides us information about the correlation time of the polymer backbone and the valuable insights into the local dynamics of the polymer. Additionally, electrochemical characterization and morphological analyses have been conducted to further elucidate the system's properties. Thus, we probe Li-ion affinity to PEO backbone, the phases of PEO, the activation energies of Li motions to gain a comprehensive understanding of Li ion conductivity in the PEO based SPEs system. Our results show the application of advanced NMR techniques to understand the interplay of polymer motion and ion-mobility that is critical in designing next generation SPEs with improved conductivity. In the presentation, we will show key conclusions drawn using the multinuclear NMR, NMR relaxation measurements, and their analysis together on future aspects.



Self-Healing Copper Nanoparticles as Catalyst for Olefins Transfer Hydrogenation Using Ammonia-Borane as Hydrogen Source: A Comprehensive Study about Mechanism(s) and Catalytic Performances

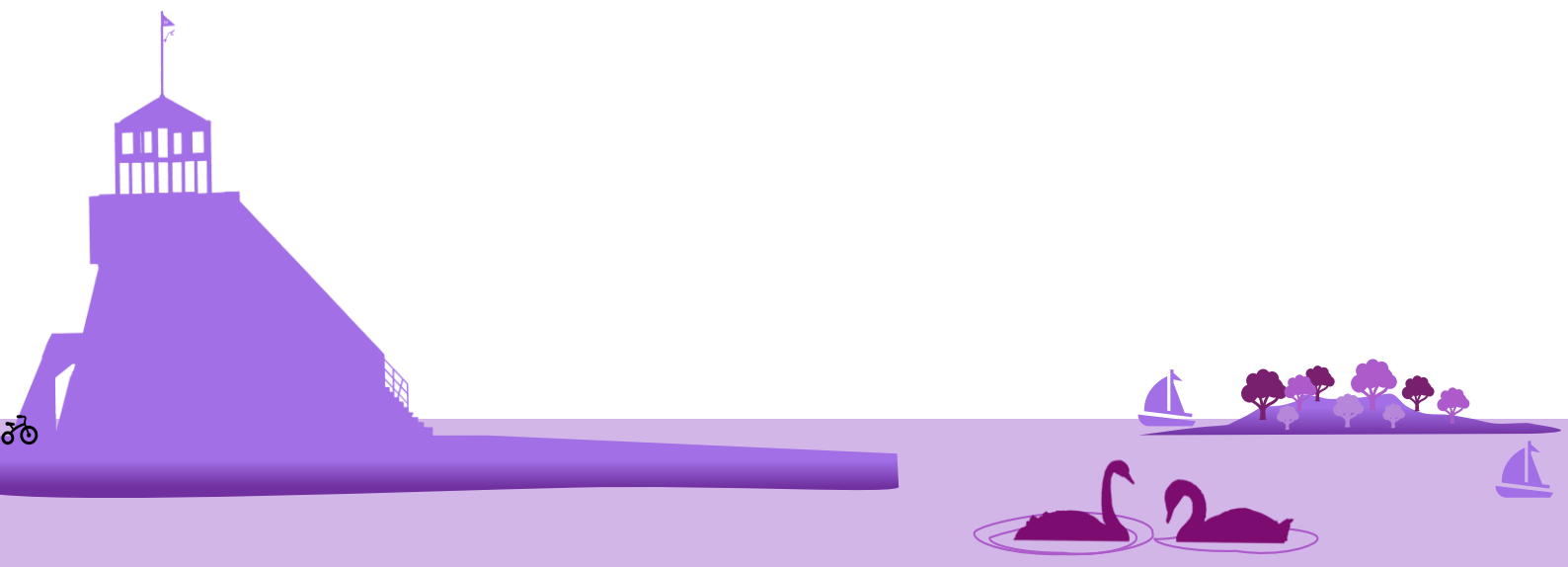
Uboldi, Matteo (1); Vitillo, Jenny (1); Maspero, Angelo (1); Bardelli, Fabrizio (2); Lucarelli, Carlo (1); Schiaroli, Nicola (1)

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In this work, isotope labeling, NMR and Kohn-Sham density function methods (KS-DFT) are combined to investigate the mechanism(s) behind ammonia-borane based transfer hydrogenation (TH) catalyzed by copper nanoparticles (Cu NPs) on olefins, choosing the styrene to ethylbenzene reaction as a case study. Cu NPs are obtained in situ by SION-X ($\text{Cu}_2[(\text{BO})(\text{OH})_2](\text{OH})_3$) reduction with ammonia-borane, acting as hydrogen source and catalyst activator, creating a self-healing catalytic system capable of reducing styrene rapidly under mild conditions, while maintaining quantitative conversion and selectivity. The key role of protic solvents in permitting ammonia-borane decomposition is assessed, and the optimum reaction conditions are established. Recycling experiments demonstrate the stability and reliability of catalytic performance without the need for reduction steps after air exposure between experiments. Isotopic labelling experiments on ammonia-borane (ND_3BH_3 , NH_3BD_3 and ND_3BD_3) with ^1H , ^2H and ^{13}C NMR disclose the simultaneous presence of two radical-based mechanisms, thanks to the kinetic isotopic effect affecting products distribution and spectra. These two mechanisms are named Ntrans and Surftrans. Ntrans, already proposed in the literature, consists in the direct transfer hydrogenation from the N-H to the styrene, while Surftrans is a fully surface-mediated mechanism, and it is here originally proposed. DFT methods (M06-L/def-2-TZPV) are employed to rationalize NMR data. Combining experimental and computational data, the two mechanisms are assessed and refined, indicating a predominance of the Ntrans, while Surftrans is responsible for about 30% of product formation. The radical nature of the mechanisms is also confirmed by experiments using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as radical scavenger.



The use of isotopic enrichment for the study of zeolites and zeoMOFs using solid-state NMR spectroscopy

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Microporous materials are one of the most important classes of materials, with uses ranging from heterogeneous catalysis to gas storage and separation. Zeolites and MOFs are two examples of such materials, and their specific uses are dependent on their structural architecture and chemistry. The ADOR process is an effective way of producing zeolites that would not be feasible through traditional routes.¹ The ADOR process consists of four stages, assembly-disassembly-organization-reassembly. The structure and chemistry of the parent zeolite are an important consideration, with a focus on zeolites with silica-rich layers linked by germanium-rich cubic units.

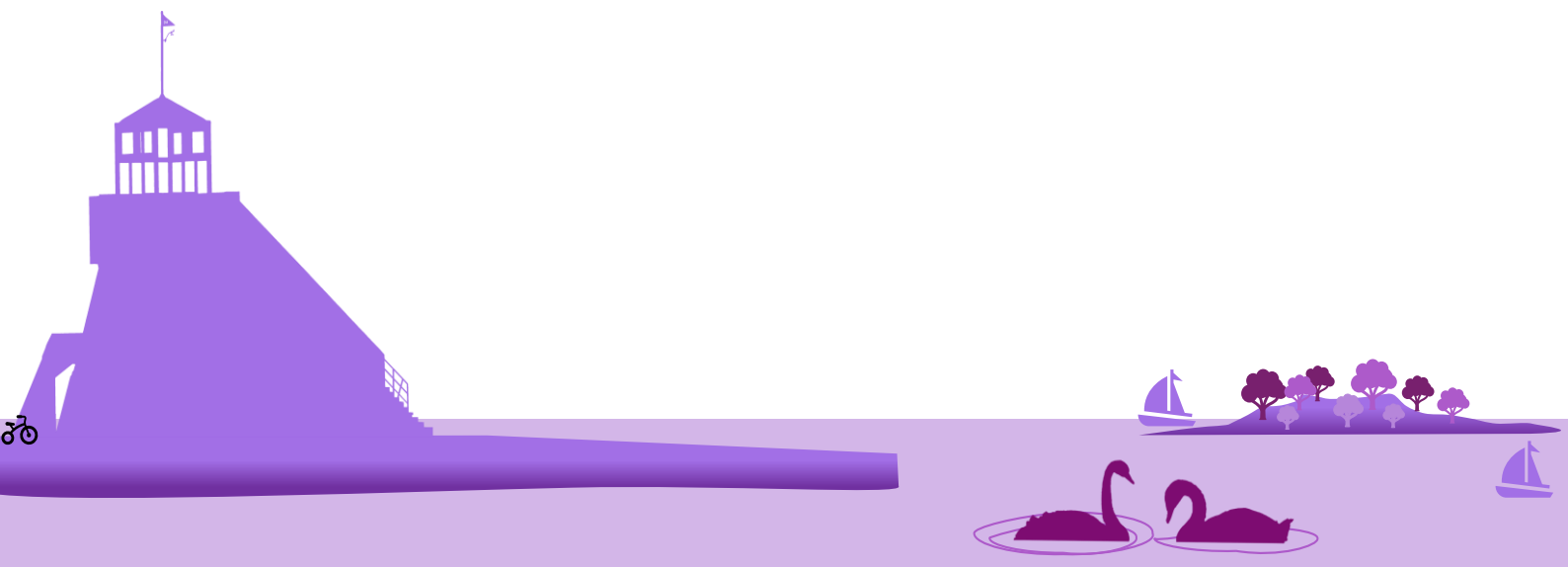
The intermediate IPC-1P, which has a layered structure, can be used to develop a new class of porous materials by growing MOFs in between the layers, these are termed ZeoMOFs. These hybrid materials are envisioned to be used for a range of applications ranging from catalysis to biomedical. The rational design of these structures and precise control of reaction conditions are vital to achieve highly stable and crystalline hybrid materials.

Isotopic enrichment allows for signal enhancement and improvements to acquisition speed. This is particularly useful in the case of in situ measurements where an enhancement in signal allows for acquisition of spectra at shorter intervals.² Isotopic enrichment is necessary for the acquisition of lower abundance nuclei such as ^{17}O where measurements are unfeasible at natural abundance.

Here we have demonstrated how ^{29}Si enrichment allows for the acquisition of in situ NMR measurements to understand the formation of IPC-1P. This can allow for a better understanding of how we can use this layered material to develop novel ZeoMOFs. Additionally, the use of ^{17}O enrichment at various stages of the reaction can allow for the in-depth analysis of the resulting ZeoMOFs.

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Li migration effects on composite LATP-PEO solid electrolytes in solid-state lithium batteries from MAS NMR and impedance spectroscopy

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Next generation high-capacity lithium batteries are foreseen to comprise of Li metal anode and solid electrolyte designed to quench dendrite formation and improve safety against thermal runaway. One of the approaches researched today to improve the performance of solid electrolytes for Li batteries is the use of composite solid polymer electrolytes (SPE).

AIM: While those types of electrolytes were studied extensively, the effect of the inorganic additives on the polymer structure and dynamics and on lithium mobility through the polymer matrix remains unclear, as studies were conducted at lower temperatures due to the high adhesion of the SPE to the Li metal electrode².

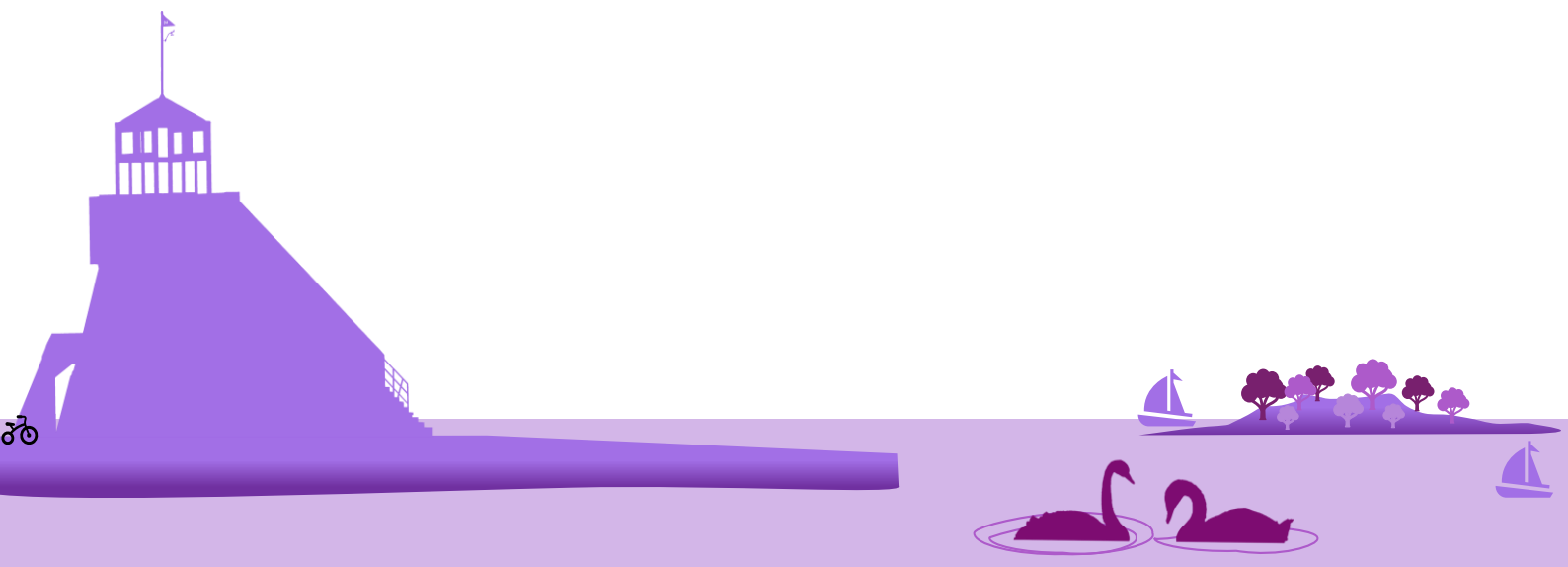
METHODS: Here, Pre-operation and post-mortem characterization of composite SPEs made of polyethylene oxide (PEO) containing LATP ceramic additive in symmetric cells were studied using electrochemical impedance spectroscopy as well as MAS NMR on battery cells^{1,2}.

RESULTS: ¹H-¹³C CPMAS measurements of EIS cycled PEO+LATP show an increase in the polymer crystalline/amorphous ratio but no change in CP dynamics of the two regions and the appearance of a new PEO carbon species. ¹H-⁷Li CPMAS interfacial polarization transfer measurements from polymer ¹H to LATP ⁷Li M1 and M3 sites point to decrease in M1/M3 population and major changes in post-EIS CP dynamics. An overall increase in rotating-frame relaxation for both Li sites and a faster polarization of M3 species and vice versa for M1 are seen.

CONCLUSION: Both bulk polymer and interfacial region in the ceramic particles are affected by ion migration under EIS. The decrease in M1 sites population and preferred interaction of polymer chains with surface Li M3 sites suggest the former sites in the ceramic crystal are more labile. Disorder is developing with ion migration in the LATP boundary shell. Furthermore, the increase in polymer crystallinity may have an adverse effect on electrolyte performance.

(1) 10.1039/c9cp00405j

(2) 10.1149/1945-7111/ad27b8



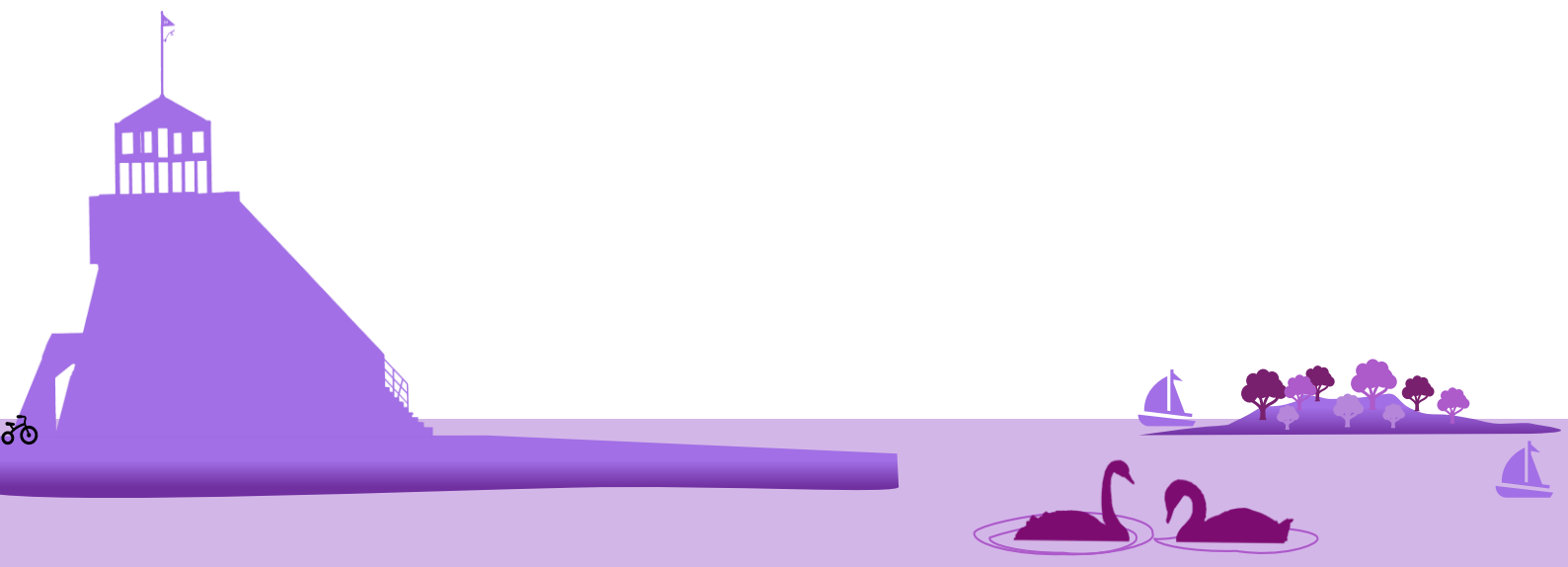
Investigation of Calcium Carbonate Using Solid-State NMR and Dynamic Nuclear Polarization

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Calcium carbonate is one of the most abundant materials on Earth and plays a crucial role in both biological and geological systems. Despite its prevalence, some of its polymorphic forms remain poorly understood. In this study, we demonstrate the potential of solid-state nuclear magnetic resonance (NMR) to gain valuable structural insights into calcium carbonate. Using a combination of density functional theory (DFT) calculations and ^1H and ^{13}C NMR experiments, we show how crystal structures can be refined with improved accuracy. Additionally, we investigate calcium carbonate from the perspective of calcium spins, developing an approach to record high-resolution ^{43}Ca NMR spectra at natural isotopic abundance in less than 12 hours using Dynamic Nuclear Polarization (DNP). This advancement enhances our ability to probe calcium environments in complex carbonate structures, offering new avenues for exploring their structural and dynamic properties.



Combining solution- and solid-state NMR for the structural elucidation and mechanisms of self-assembly of colloidal materials and multicomponent pharmaceuticals

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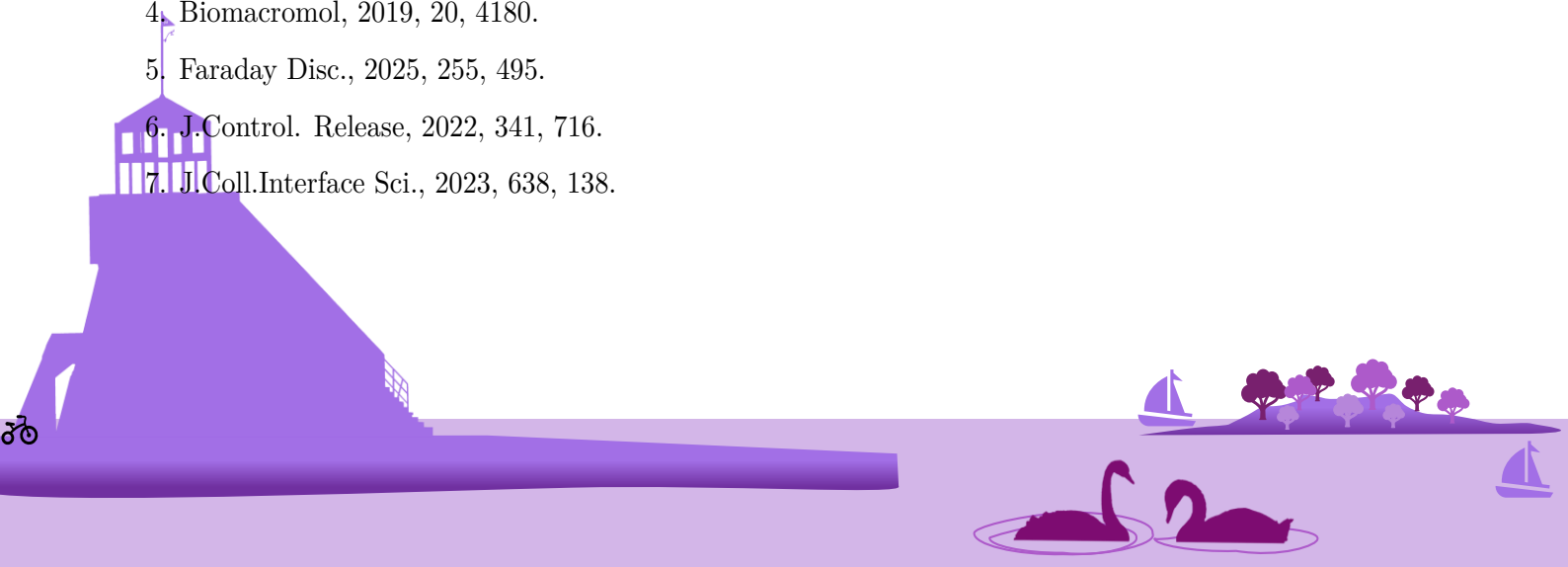
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Combined application of solution and solid-state NMR is valuable for many materials which consist of different structural and dynamic domains (surface/core, organised/amorphous, rigid/mobile) along with diffraction and computation tools. Combined Liquid- And Solid-State In-Situ Crystallisation (CLASSIC) NMR [1] can also be used for in-situ monitoring of crystallisation pathways for pharmaceutical materials. This presentation will focus on summarising advantages and challenges of such approaches using several classes of colloidal and supramolecular materials studied over the years in our groups.

Using carbohydrate-based systems and soft matter [2-6], we studied the organisation of rigid components with different levels of ordering and identified flexible parts of such assemblies using a combination of advanced solid- and solution-state NMR approaches. We have also developed an NMR based tool-kit for elucidation of the mechanisms of partitioning of anti-microbial pharmaceuticals in micellar drug delivery systems. Combination of NMR methods (including the multifrequency-STD NMR) corroborated with molecular dynamics (MD) simulations, enabled us to elucidate intricately detailed patterns of drug/micelle interactions in a single NMR experiment within minutes [7].

Using CLASSIC NMR we gained mechanistic understanding of formation and phase transition in several co-crystal systems [theophylline and benzamide, metronidazole and gallic acid, nicotinamide and isonicotinamide with 3-hydroxybenzoic acid, 2-hydroxybenzoic acid, and 2,3-dihydroxybenzoic acid, of varying stoichiometry]. We proved it to be an effective tool for following the solid-state phase evolution in time, comparable to synchrotron TRIS-PXRD.

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2. Carbohydrate Polym., 2022, 289, 119413.
3. Chem.Eur.J., 2021, 27, 1374.
4. Biomacromol, 2019, 20, 4180.
5. Faraday Disc., 2025, 255, 495.
6. J.Control. Release, 2022, 341, 716.
7. J.Coll.Interface Sci., 2023, 638, 138.



Study of hypoxanthine guest molecules in guanine crystals in fish swim bladder - seeking MAS NMR evidence of dynamic tautomerism suggested by DFT calculations

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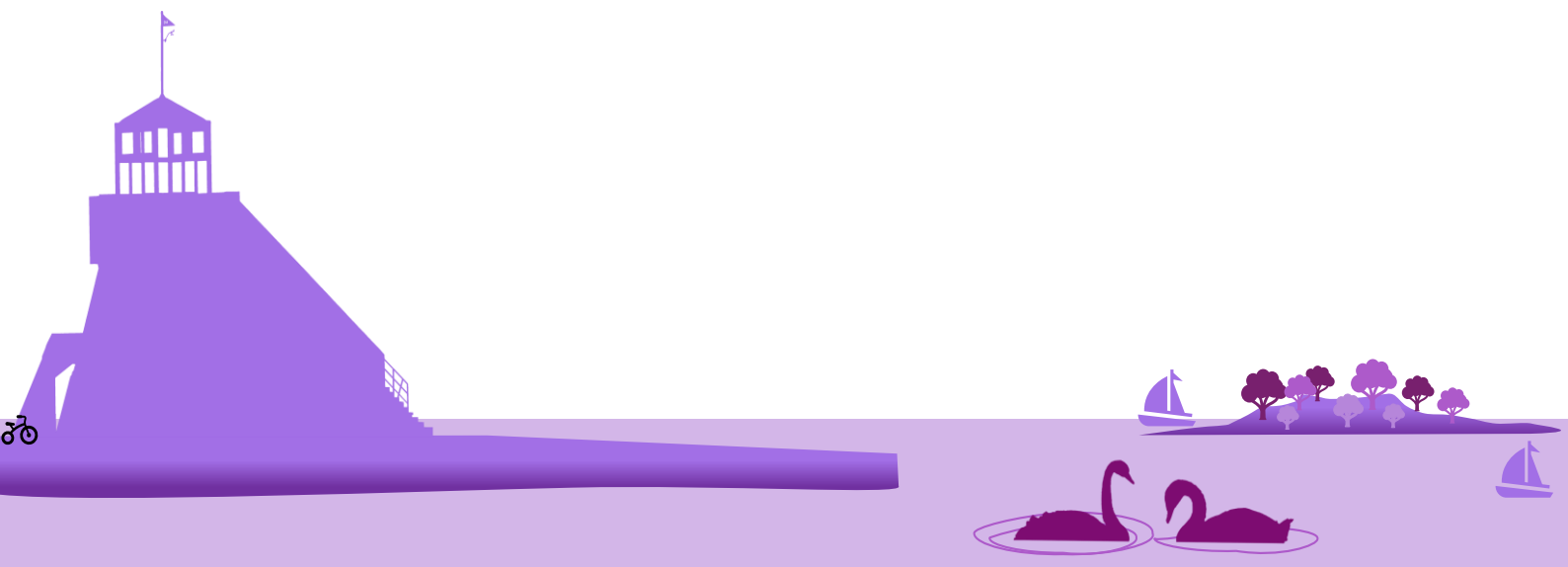
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Guanine crystals are one of many examples of multi-purposing in Nature. Their remarkable photonic properties are famously used to confer traits like iridescence and vision. Another intriguing role they play is in reinforcement of the gas-impermeable inner lining of the buoyancy bladder of fish. We have recently shown that guanine crystals from the swim bladder of *Sardina pilchardus* are hosting hypoxanthine (9%) and water (~7%) molecules inside the crystal, with minimal effect on lattice parameters or crystallite morphology [1]. This ability of guanine crystals to host other molecules enables animals to build physiologically “cheaper” crystals from mixtures of metabolically available purines, without impeding their original functionality. What is, then, the role of these guest molecules in guanine crystals? Are they evolutionary spandrels?

DFT calculations [2] of hypoxanthine carbon chemical shifts were performed considering the eight possible tautomers of the molecule. The computed shifts were inconsistent with measured values in hypoxanthine crystals unless a bimodal combination of keto:enol forms (35:65) in fast exchange was taken. Furthermore, for hypoxanthine as a guest molecule inside swim bladder guanine crystals, a minor population of a third tautomer was necessary to explain experimental chemical shifts, pointing to an effect of confinement in the foreign crystal field on the potential energy and the balance of different tautomers compared to the crystal field of hypoxanthine itself. Cross polarization dynamic measurements provide additional hints on possible dynamic proto-tropism in hypoxanthine as a dopant molecule.

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2. [10.1021/acs.jctc.2c00275](https://doi.org/10.1021/acs.jctc.2c00275)



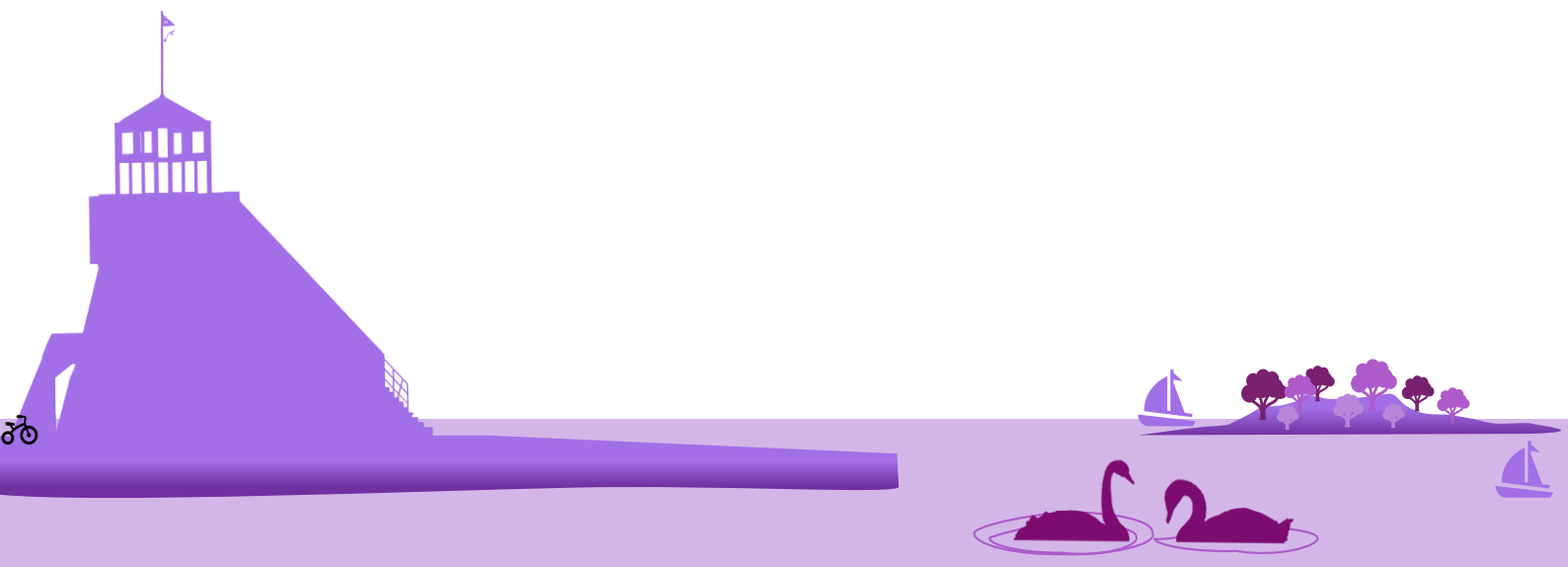
Defect Tolerant Quantum Cutting in Mechanothesized Ytterbium-doped Cesium Lead Chloride Perovskites

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The formation of defects in halide perovskites is inherent in growth conditions and synthesis methods. Point defects are the most common and appear as substitutionals, anti-site occupations, vacancies and interstitials. The presence of defects influences the optoelectronic properties of halide perovskites, such as how charge carriers are trapped or recombined. Halide perovskites are known to be tolerant to the incorporation of impurities and defects. This means that even under fairly relaxed synthesis conditions, for example at room temperature and in air, the defect densities are small. Such densities are comparable to what is achieved under significantly more rigorous conditions for other classes of optoelectronic semiconductors. That being said, as with other types of semiconductors, defects can be purposely introduced into the halide perovskites with the goal of tuning their optoelectronic properties and even adding new ones. One such property is quantum cutting. Quantum cutting is defined as the emission of more than one low-energy photon subsequent to the absorption of one high-energy photon. Halide perovskites, when doped with lanthanide ions, are capable of exhibiting this process, particularly when Yb^{3+} is used as dopant, since only one electronic transition can occur, involving the ground state $2F_{7/2}$ and the excited state $2F_{5/2}$. Solid-state nuclear magnetic resonance spectroscopy (SSNMR) can provide information on how insertion of the Yb^{3+} ion can affect the local environments of the probed nuclei in the perovskite lattice. We investigate how increasing the number of Yb^{3+} dopants as well as mechanochemically induced point defects affects the local magnetic environment and the photoluminescence properties of the doped structure. We find that there is an optimum grinding time to maximize photoluminescence response and using La^{3+} dopants combined with ^{139}La and ^{133}Cs NMR we characterize the local symmetry of distinct vacancy sites that form upon doping.



What's being measured with NMR Probe molecules adsorbed on zeolites? A molecular tightrope walk

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The nature, strength, and spatial distribution of acid sites in zeolites are central to their catalytic performance, yet their characterization remains a formidable challenge. Using trimethylphosphine oxide (TMPO) as a probe for acidity, we combined solid-state NMR and computational modeling to redefine the understanding of zeolite acidity. 2D ^1H - ^{31}P HETCOR combined with *ab initio* molecular dynamics revealed that confinement effects and protonated TMPO dimers – not distinct Brønsted acid strengths – explain ^{31}P NMR resonances in HZSM-5.[1,2] An atomistic view of host–guest dynamics is provided by examining aluminum siting and guest–guest interactions. Extending this approach to external zeolite surfaces, we are able to identify unique SiOH species, pore-mouth Brønsted sites, and tricoordinate Al-Lewis sites, previously undetected.[3] This talk also demonstrates the importance of optimizing TMPO adsorption methods, showing how solvent choice and gas-phase loading influence dimer formation and acid site quantification.[4] Altogether, these insights bridge internal confinement effects and external surface chemistry, offering a unified framework for a better atomic-level understanding of acid site structures in zeolite catalysts.

Acknowledgements

CICECO-10.54499/UIDB/50011/2020-10.54499/UIDP/50011/2020-10.54499/LA/P/0006/2020-FCT/MCTES-PIDDAC). Infrastructure Project 022161 (FEDER-COMPETE2020-POCI/PORL). ERC CoG-GA-865974. FCT-10.54499/2020.00056.CEE-CIND/CP1589/CT0005 and 2020.08520.BD. Spanish Ministry of Science, Innovation and Universities-MU-23-BG22/00145.

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Spontaneous synthesis of Pickering emulsion using dynamic combinatorial chemistry

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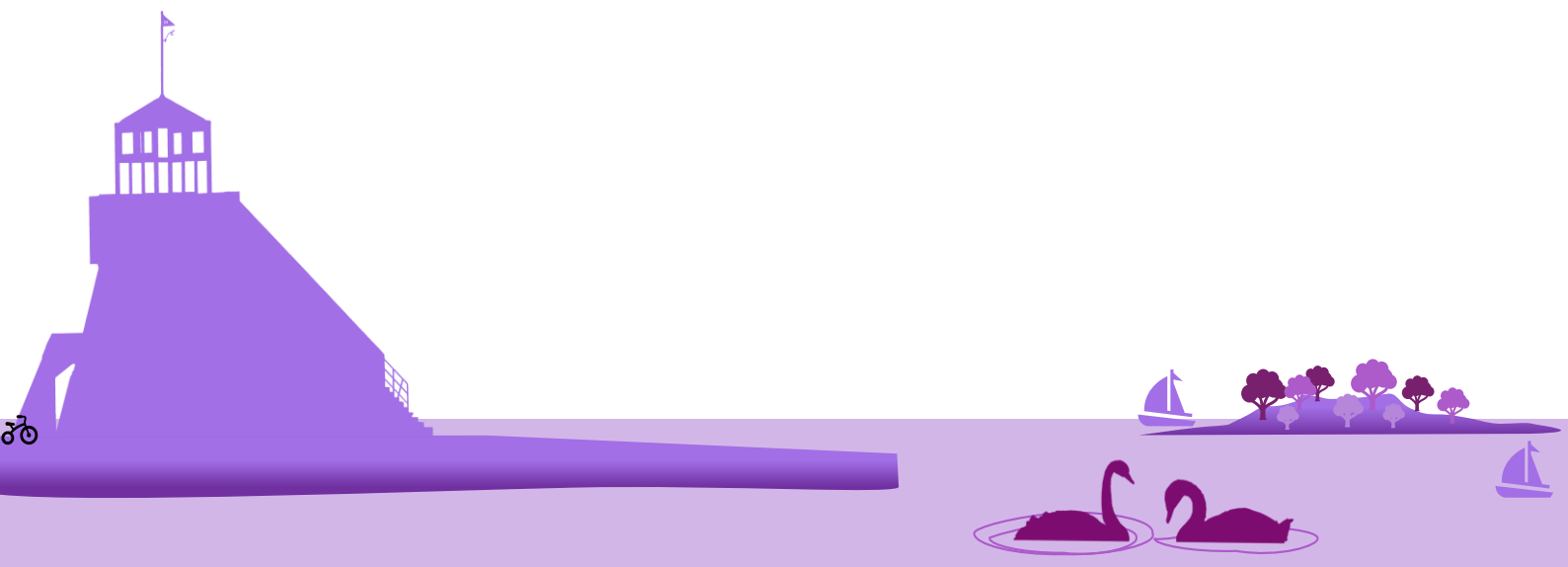
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Conventional method of preparing Pickering emulsion require precise control and synthesis of solid particles prior to the emulsification. Our research utilizes Dynamic Combinatorial Chemistry (DCC) to impart spontaneous selectivity in the formation of solid particles. DCC allows for the generation of a library of potential candidates, which dynamically equilibrate and self-assemble with templates into the most thermodynamically stable configurations. This approach ensures the formation of particles with the lowest free energy, providing optimal stabilization for the emulsions.

In our study, we investigated the effects of DCC on the formation and stability of Pickering emulsions. We prepared water-in-oil emulsions using different concentrations of a benzoic dithiol building block (BY) and cetyltrimethylammonium bromide (CTAB). Our results demonstrated that the addition of BY significantly enhanced the emulsion's stability. Small-angle X-ray scattering (SAXS) fitting results, along with dynamic light scattering (DLS) and transmission electron microscopy (TEM) analyses, confirmed the formation of Pickering emulsions. Additionally, size exclusion chromatography (SEC) and Matrix-assisted laser desorption/ionization (MALDI) analysis verified the formation of larger macrocycles. The application of nuclear magnetic resonance (NMR) elucidated the supramolecular organization inside the solid particles. Rheological analysis revealed that these larger macrocycles resulted in more robust and cohesive interfacial structures, enhancing the mechanical stability of the emulsions.

Our findings demonstrate that DCC enables the spontaneous formation of optimally stable particles. This innovation simplifies the synthesis process and enhances the robustness and functionality of these emulsions in various biomedical applications.



Single scan restricted diffusion NMR analysis

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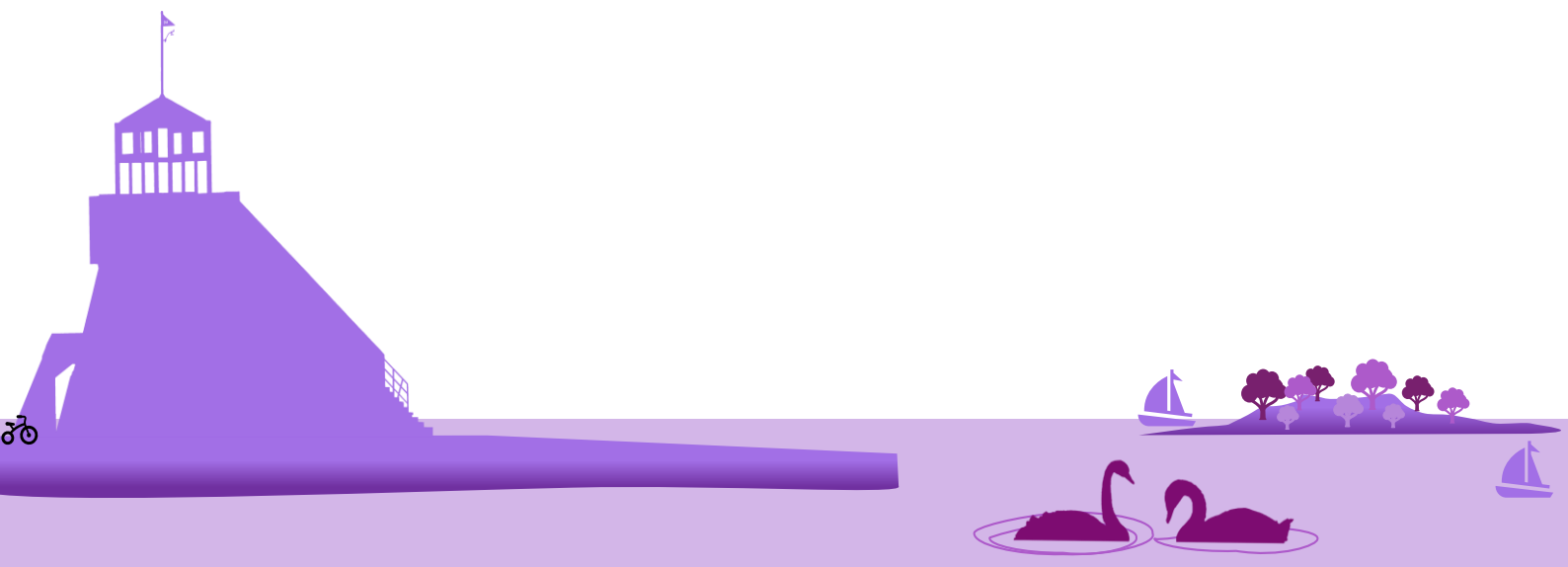
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NMR diffusion measurements provide versatile information about dynamics and structures of porous materials and reveal interactions of nuclei within their microscopic environment. The experimental data consist of decaying exponential signals and inverse Laplace-transform is used to extract decay-constant distribution; hence they fall into category of Laplace NMR (LNMR). In many cases diffusion might be restricted due to confinement of the liquid to porous medium, for example, a rock or wood. This lowers apparent diffusion coefficient as illustrated in Figure 1. The effect of restricted diffusion can be seen by repeating the diffusion measurement with varying diffusion time.[1]

Unfortunately, repeating several measurements with varying evolution delay or gradient strength is extremely time consuming. Some methods have been proposed to tackle the slowness, such as difftrain [2], or exploitation of spatial encoding [3] in ultrafast LNMR [4-7]. The price to pay is reduced sensitivity. However, the single-scan approach enables the use of hyperpolarization methods (e.g. PHIP, DNP [4] and SEOP [5]), which provide much higher sensitivity boost than the loss due to spatial encoding.

Here we introduce a method, in which spatial encoding is combined with a low angle reading pulses, utilizing partial magnetization with a loop structure. This enables measurement of full restricted diffusion data with different diffusion times in a single scan.

The method has great prospects especially for many biological applications, in which the sample concentrations may be small, system conformations can vary fast in time, system components decay biologically, or real time information is wanted. The method also has promising prospects in process control, for example in applications such as porous material characterization and emulsion analysis, where diffusion measurements can provide valuable insights into structural properties, stability, and composition while ensuring speed and efficiency.



Application of high temperature NMR for the study of structural properties in molten chloride fast reactor fuels

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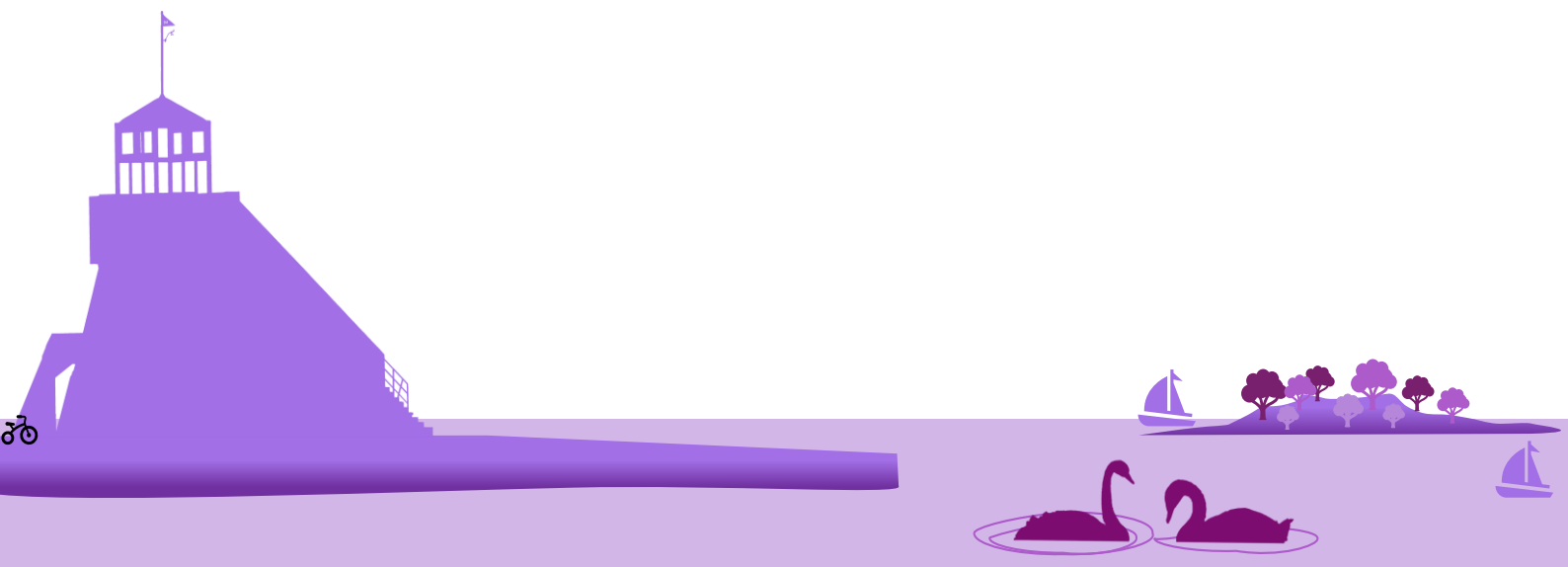
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Chloride salts have become the leading fuel choice for next-generation molten salt reactors, particularly molten chloride fast reactors (MCFRs). In France, the ARAMIS-A reactor design is investigating the use of $\text{NaCl-MgCl}_2\text{-PuCl}_3\text{-AmCl}_3$ as fuel for an actinide-burner reactor. However, research on the structural dynamics of molten chloride fuel salts remains scarce, limiting accurate modeling of fuel performance and behavior.

To address this gap, we conduct in-situ high-temperature nuclear magnetic resonance experiments on $\text{NaCl-MgCl}_2\text{-LaCl}_3$ as a surrogate for $(\text{Pu,Am})\text{Cl}_3$ fuel to explore the local structural chemistry of molten chloride salts. By integrating our experimental results with solid-state nuclear magnetic resonance measurements, classical molecular dynamics simulations, and density functional theory calculations, we reveal key structural interactions in molten chloride systems—such as lanthanide network formation and chlorine bridging—and demonstrate how these interactions vary with temperature and fuel composition. Our findings establish a direct correlation between ^{35}Cl and ^{139}La NMR chemical shifts and coordination numbers in molten salts, providing valuable insights into the local structural environments that govern the behavior of actinide-based fuels in molten salt reactors.



Aging of hydrated geopolymer samples followed by solid-state NMR spectroscopy

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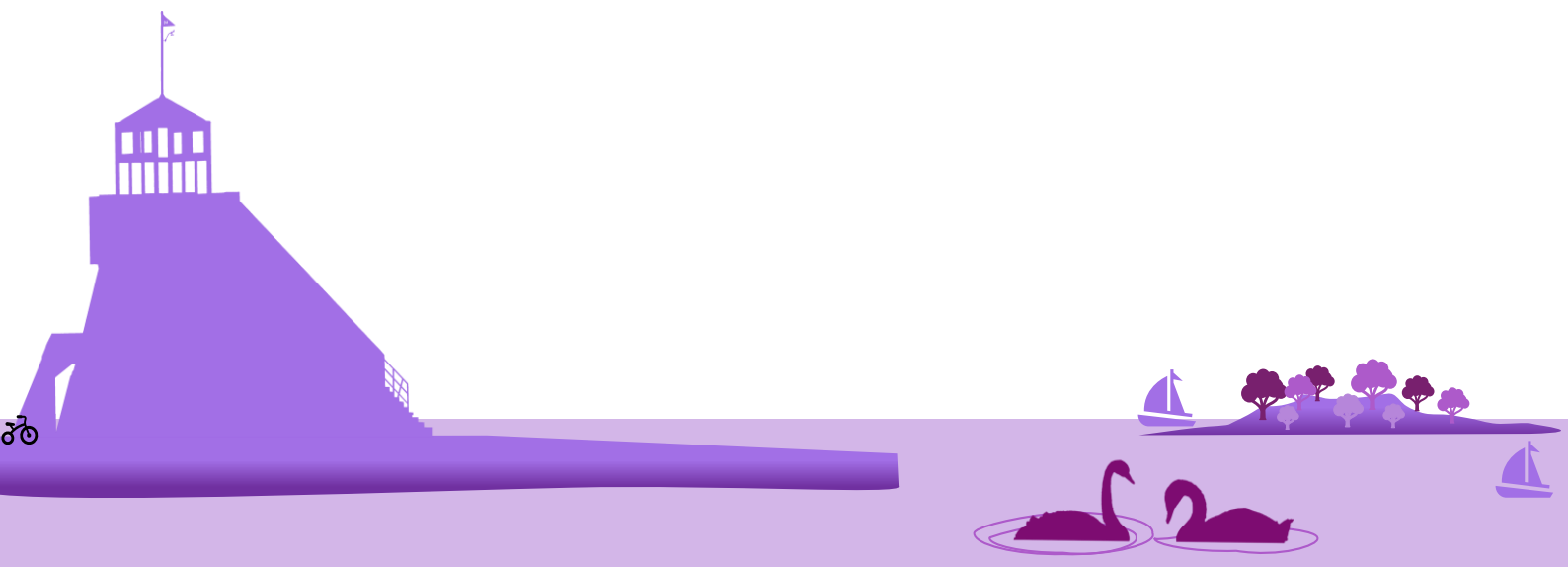
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This study investigates the potential of geopolymers as sustainable alternatives to traditional concrete as building material. Geopolymers are characterized by their amorphous three-dimensional structures formed from bridged aluminate and silicate units, activated through alkaline or acidic processes. Notably, this method eliminates the need for clinker sintering, resulting in significantly lower CO₂ emissions compared to conventional concrete production.

We focus on two aluminosilicate precursors: meta-kaolin and illite, exploring various ratios of alkaline activator solutions to precursor material. The compressive strength and structural integrity of the resulting geopolymers are analysed after hydration times of 2, 7, and 28 days. Solid-state NMR spectroscopy is employed to probe the coordination environments of aluminium (²⁷Al) and silicon (²⁹Si) within the geopolymer matrix. Our findings demonstrate that optimizing the activator-to-precursor ratio can enhance performance properties, highlighting the feasibility of geopolymers as a viable eco-friendly construction material.



Optimal Control Enables Efficient 5QMAS

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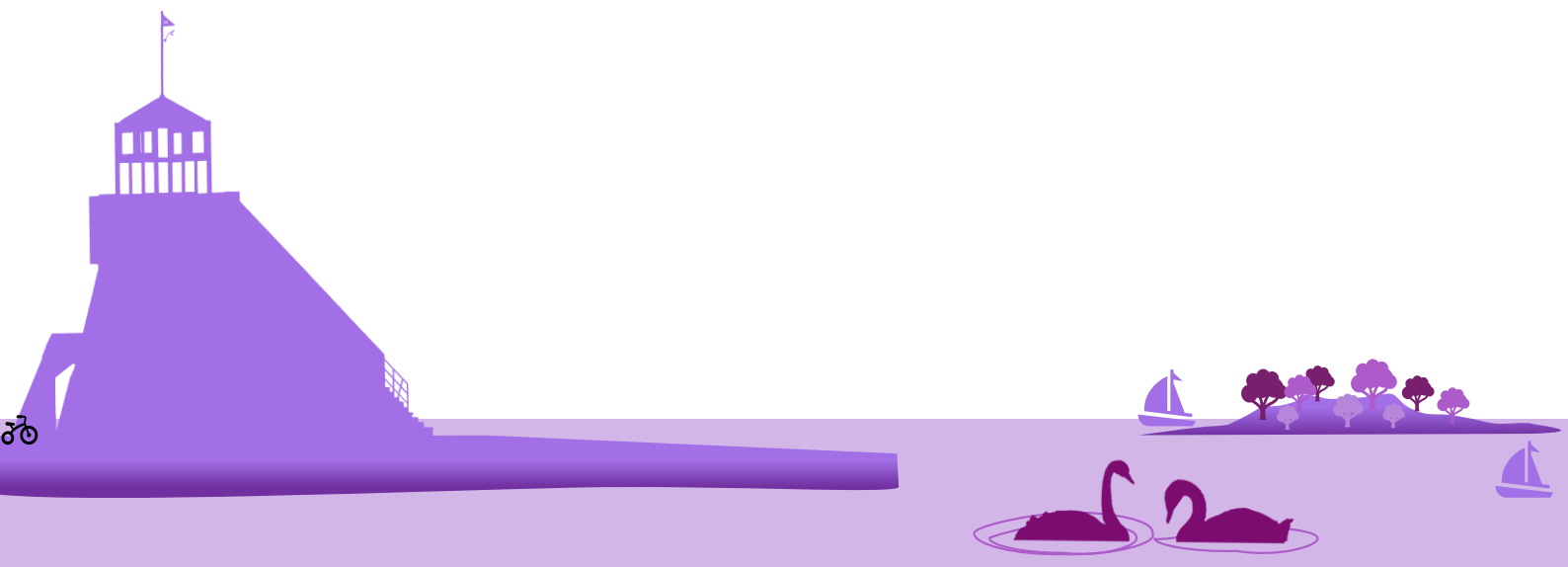
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Aluminum atoms ($I = 5/2$) play a crucial role in zeolite catalytic sites, which are typically studied using the Triple-Quantum Magic Angle Spinning (3QMAS) experiment. 3QMAS yields high-resolution spectra of half-integer spin nuclei by refocusing second-order quadrupolar broadening in the indirect dimension. However, in zeolites, 3QMAS may still fail to distinguish signals from distinct sites of the same coordination within tetrahedral or octahedral environments

A similar experiment, 5QMAS, provides potentially much higher resolution in chemical shifts due to the use of higher coherence order. Unfortunately, such experiments suffer from very low sensitivity and are not frequently used in practice.

We used Optimal Control Theory (OC) to replace excitation and reconversion hard pulses in the 5QMAS pulse sequence with numerically optimized shaped pulses. This approach significantly enhanced sensitivity across a range of quadrupolar coupling constants, asymmetries, and isotropic chemical shifts characteristic for tetrahedral aluminum in hydrated zeolites.

Our results demonstrate that OC-based pulse sequences can dramatically outperform traditional MQMAS methods in terms of sensitivity.



Phosphorus Oxidation Controls Epitaxial Shell Growth in InP/ZnSe Quantum Dots

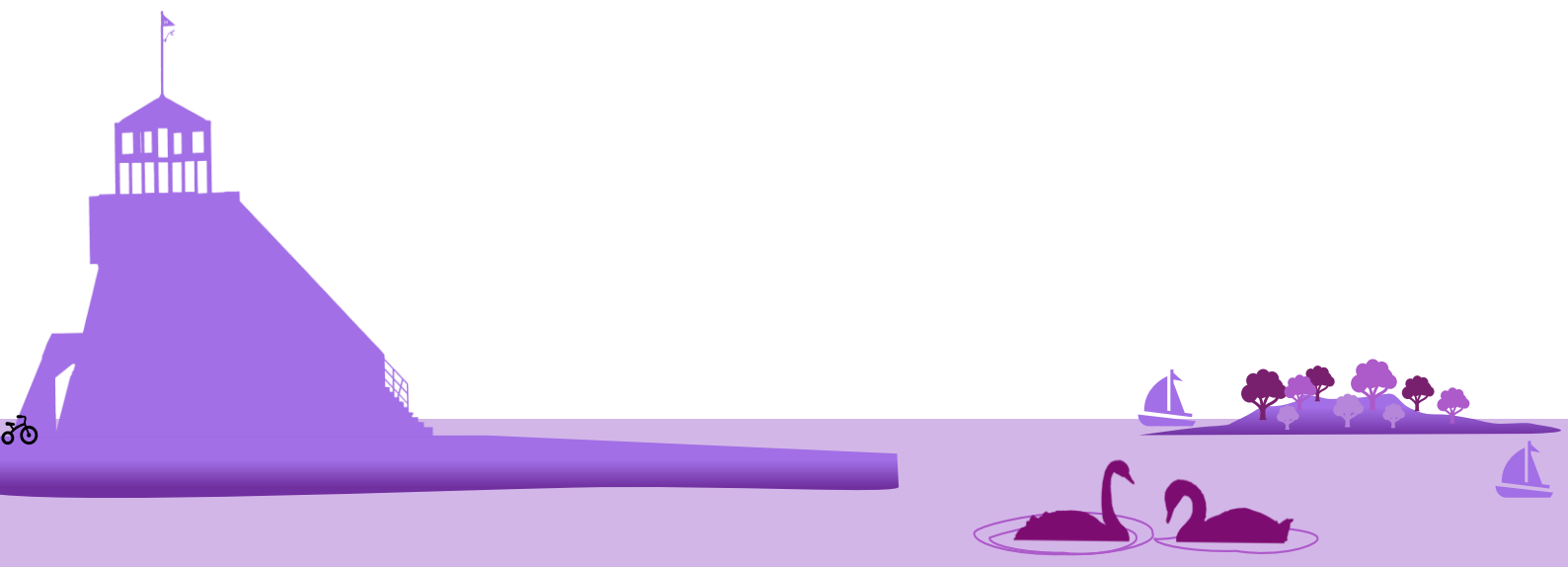
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InP/ZnSe/ZnS core/shell/shell quantum dots are the most investigated quantum dot material for commercial applications involving visible light emission. The inner InP/ZnSe interface is complex since it is not charge balanced, and the InP surface is prone to oxidation. The role of oxidative defects at this interface has remained a topic of debate, with conflicting reports of both detrimental and beneficial effects on the quantum dot properties. In this study we probe the structure of the InP/ZnSe interface at the atomic level using ^{31}P , ^{77}Se and ^{17}O ssNMR and HAADF-STEM. We observe clear differences in Se NMR spectra and crystal orientation of core and shell when the InP/ZnSe is oxidized on purpose. High levels of interface oxidation result in an amorphous phosphate layer at the interface, which inhibits epitaxial growth of the ZnSe shell.



19F NMR studies of highly conductive $K_xBi_{1-x}F_{3-2x}$ prepared by thermal plasma processing

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Introduction

Ion conduction in solids has been an important subject due, for example, to its application in all-solid-state batteries. NMR is a powerful tool to investigate ion dynamics because some NMR observables can be related to ionic conductivity. We report 19F-NMR studies of $K_{0.06}Bi_{0.94}F_{2.88}$, of which ionic conductivity at ca. 500 K is comparable to that of $PbSnF_4$ [1,2].

Aims

To deepen our understanding of the conduction mechanism of fluoride ions and contribute to the development of high-performance electrolytes.

Methods

The fine $K_{0.06}Bi_{0.94}F_{2.88}$ particles (30-150 nm) prepared by thermal plasma processing were annealed at 300, 400, and 500 °C. NMR measurements of linewidth and relaxation time in the rotating frame ($T_{1\rho}$) were done at 14 T. The correlation time of the fluoride ion estimated from the linewidth and the $T_{1\rho}$ value is used to elucidate the diffusion constant, which is compared with those measured by the static field gradient (SFG) method [3] at 4.7 T.

Results

The diffusion constants estimated from $T_{1\rho}$ and the linewidth differ appreciably from those determined by the SFG method, which are consistent with those elucidated from ionic conductivity using the Nernst-Einstein relation. The apparent differences among these diffusion constants are discussed with considering differences of measuring objects as well as different time-/distance-scales for different measurements.

Conclusions

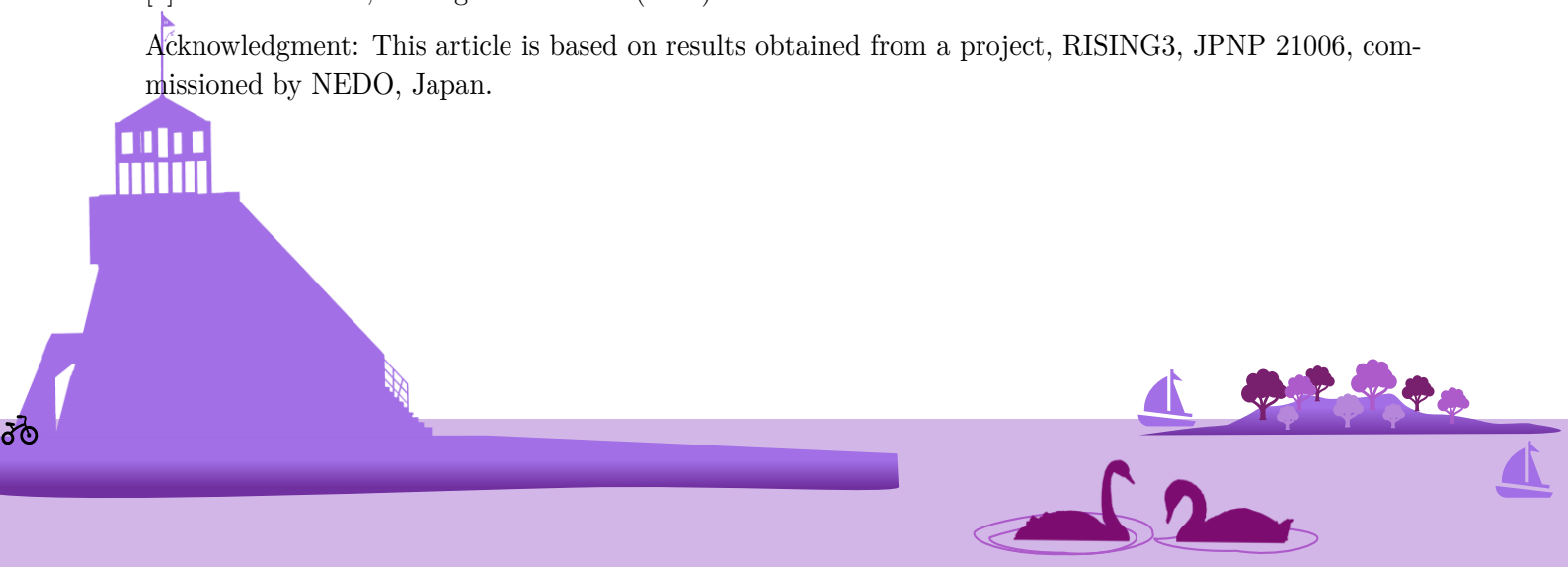
We believe that these multifaceted analyses can contribute to the development of high-performance electrolytes.

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Characterisation of lignocellulosic biomass residues by NMR spectroscopy

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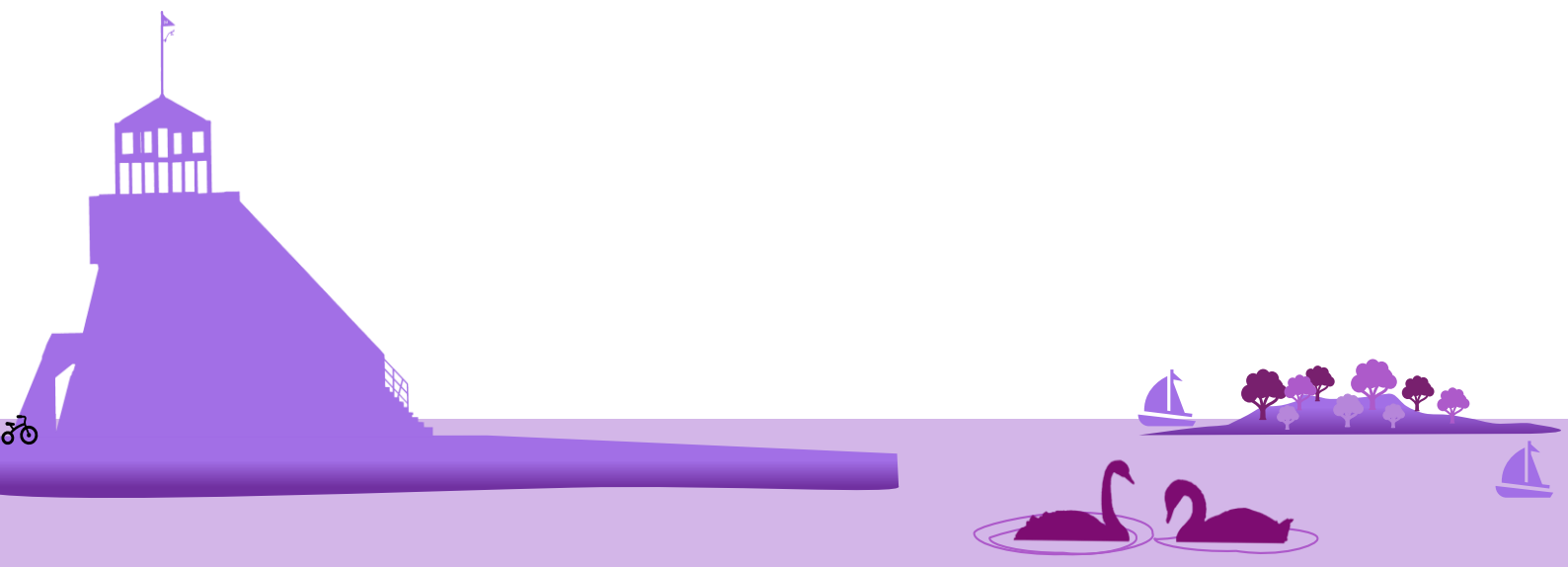
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Lignocellulosic biomass (LB) is promising feedstock for the production of various bio-based eco-friendly products including biochemicals and biofuels.[1] LB mainly consists of three types of biopolymers: cellulose, hemicellulose and lignin that are interconnected and form a very complex network structure. Due to its heterogenous character, complex chemical structure and recalcitrance, it is necessary to know its structural composition in order to optimize pretreatment process and further (bio) conversion into bio-based products.

Many different analytical methods and approaches were used to study properties and composition of lignocellulosic samples. In this work it will be demonstrated that nuclear magnetic resonance (NMR) techniques in solution and in the solid state are a valuable tool for investigating lignocellulosic residues from the vegetable oil industry: pumpkin oil cake (PC) and hemp pomace (HP). NMR spectroscopy is a fast and reliable method that can provide data on the molecular architecture and composition of lignocellulosic biomass.[2] Although NMR spectra of lignocellulosic samples are complex with overlapping signals, they still provide a plenty of useful information about the structural composition. This can further be exploited for industrial application of lignocellulose.

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Unveiling Molecular Interactions Between Cellulose and Isomeric Deep Eutectic Solvents Using NMR

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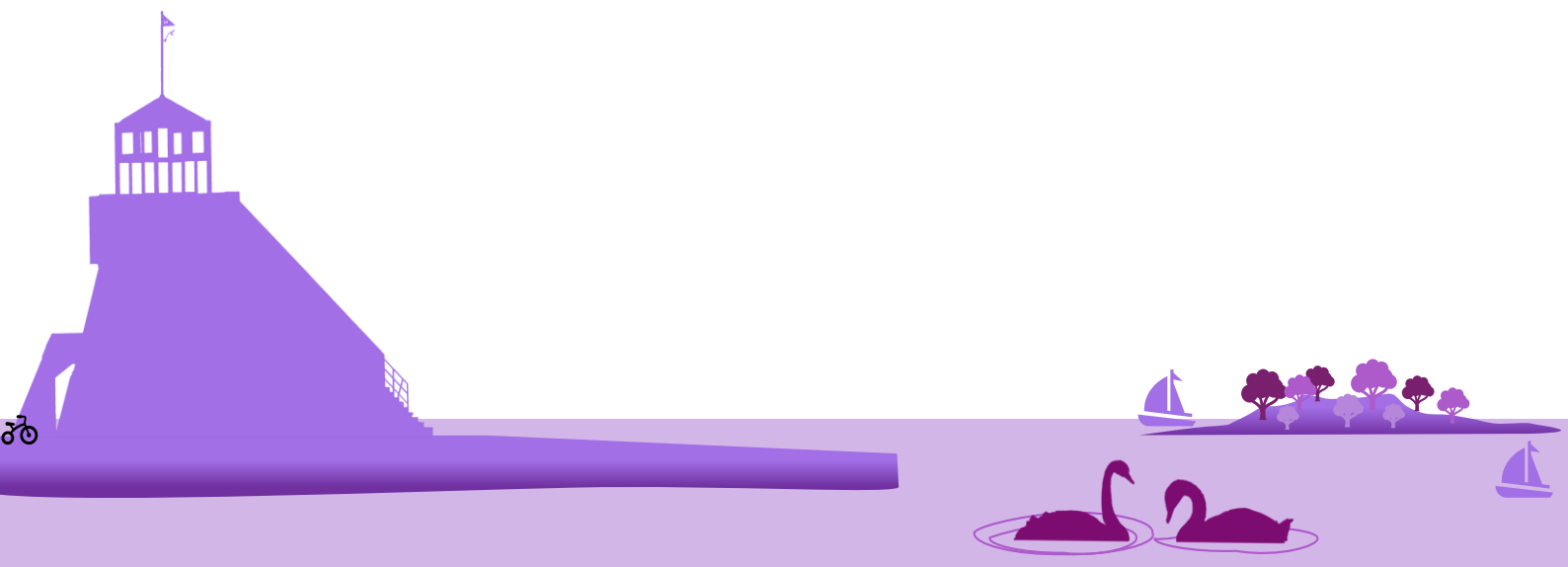
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Deep eutectic solvents (DESs) are known for their low toxicity, biodegradability, and versatile solvent properties, making them promising media for swelling, activation, dissolution, functionalization, and disintegration of cellulose fibers. These modifications depend on cellulose-DES interactions, which are governed by the chemical structure and characteristics of the DES components. In this study, the molecular interactions and dynamics between cellulose fibers and two non-derivatizing DESs, composed of triethylmethylammonium chloride (TEMACl) combined with either imidazole (Imi) or pyrazole (Pyra), isomeric five-membered aromatic heterocycles with two nitrogen atoms, were investigated using Nuclear Magnetic Resonance (NMR) spectroscopy. Specifically, the interactions between DESs and cellulose were analyzed using ^1H diffusion, T_1 and T_2 relaxation, and ^{13}C NMR spectroscopy across temperatures from 293–363 K. In the TEMACl-Imi system, cellulose fibers exhibited significant swelling, reflected by reduced T_1 and T_2 relaxation times of imidazole, while TEMACl signals remained largely unaffected. This suggests that imidazole plays a dominant role in interacting with cellulose. Additionally, the diffusion coefficients of both DES components decreased, likely due to increased viscosity from fiber swelling. The ^{13}C NMR spectra further confirmed cellulose-DES interactions by showing characteristic cellulose backbone signals, indicating molecular-level dispersion in the DES. In contrast, TEMACl-Pyra DES showed little change in relaxation times, diffusion behavior, or ^{13}C cellulose signals, suggesting minimal interaction. The stronger interaction in TEMACl-Imi is likely due to imidazole's higher basicity and superior hydrogen bonding capacity compared to pyrazole, enabling stronger interactions with cellulose hydroxyl groups. These results highlight how the molecular configuration of DES governs cellulose interactions and significantly impacts modification efficiency.



CO₂ locations and dynamics in a metal-organic framework revealed by solid-state ¹³C NMR and theoretical calculations

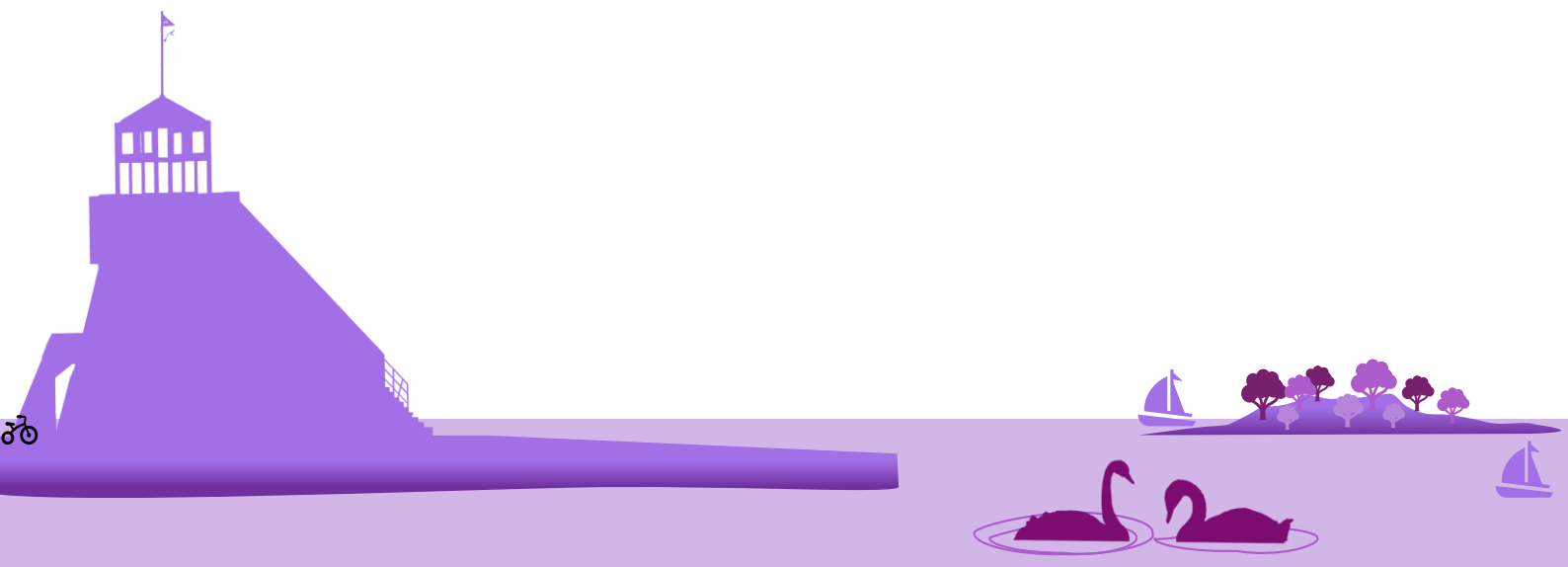
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CO₂ separation and storage using the gas adsorption abilities of porous materials has been intensively investigated. Understanding the CO₂ adsorption phenomena in porous materials is essential for designing new materials and developing applications for gas separation. In this work, we investigated the locations and dynamics of adsorbed CO₂ in a flexible metal-organic framework (MOF), MIL-53(Al), exhibiting a narrow pore (np)-large pore (lp) structural transition upon CO₂ sorption, by combining solid-state NMR analysis with theoretical calculations. We analyzed the local motions of adsorbed CO₂ by ¹³C chemical shift anisotropy (CSA) line shape simulations based on CO₂ locations in CO₂-loaded MIL-53(Al) structures predicted by density functional theory (DFT) calculations. The analysis revealed the coexistence of two types of CO₂-loaded pore structures in the np form and the presence of four CO₂ species captured by MOF-CO₂ and CO₂-CO₂ interactions in the lp form. Adsorbed CO₂ undergoes site-to-site random hopping and wobbling motions in the pores. The wobbling motion was also evaluated using ab initio molecular dynamics simulations.



Food, Health and Environment Research Infrastructures to tackle the impact of micro- and nanoparticles with a One Health approach

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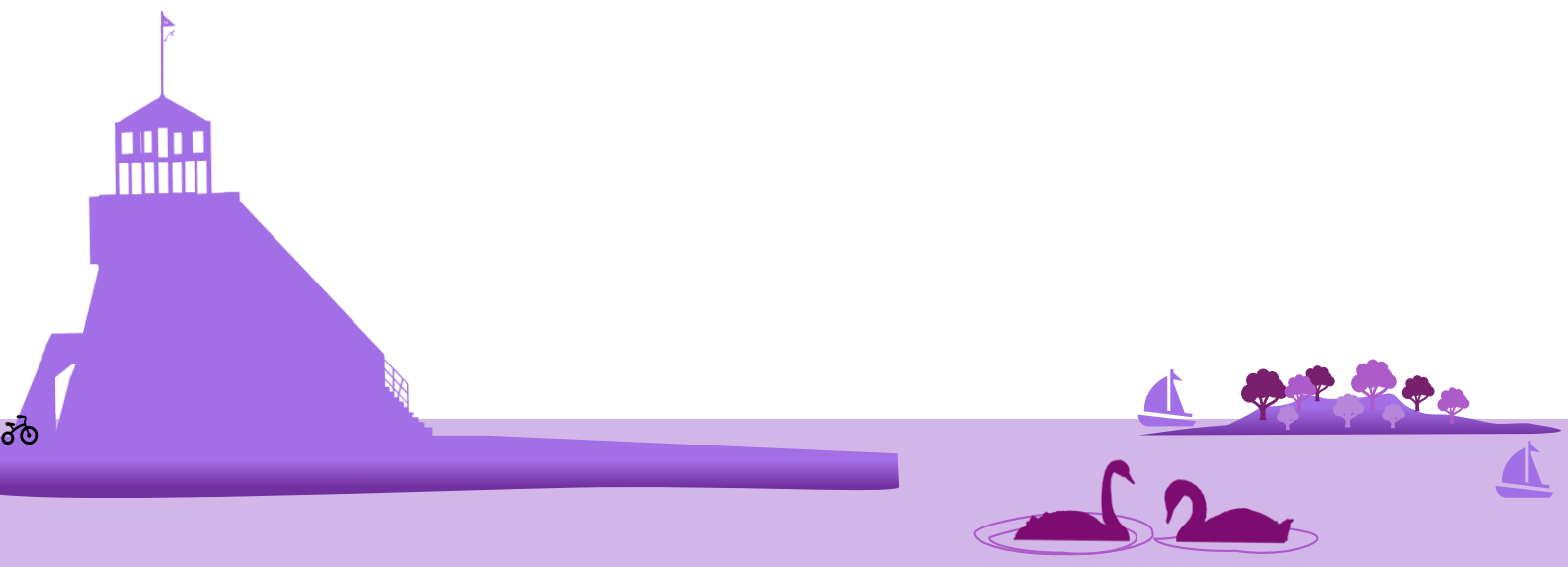
Environmental degradation, the widespread use of synthetic materials - particularly plastics - poses an escalating threat to human, animal, and environmental health. One of the most pressing concerns in this context is the increasing presence of micro- and nano-sized artificial particles in the environment. Due to their dimension, these particles can cross biological barriers, and become systematically distributed within organisms, posing serious risk to both health and ecological balance. To understand the extent of such environmental and health impacts, a "One Health" approach is critical. Despite a growth in research on these challenges, knowledge gaps persist.

The FHERITALE project (www.fheritale.eu) aims at establishing a centralized access point offering integrated services to the European scientist, in order to facilitate and widen the capacity of carrying on research on the impacts of synthetic materials at the micro- and nanoscale, with particular emphasis on four priority areas: micro- and nanoplastics, bioplastics, metals/metal oxides, and plastic additives.

To date, FHERITALE activities focused on an overview of the research currently addressing the impact on health of artificial micro- and nanoparticles and on performing a comprehensive cross-thematic landscape of European available services for a set of scientific priorities (doi.org/10.5281/zenodo.15211351). Results include a bibliometric study addressing the current research landscape and a first map of services offered by relevant European Research Infrastructures (<https://fheritale.eu/survey-outcomes>).

The work carried out revealed a significant imbalance in the application of analytical techniques, including a marked underutilization of Magnetic Resonance technologies. RIs play a pivotal role in addressing these limitations by enabling broader access to advanced instrumentation.

This contribution is presented on behalf of the whole FHERITALE project consortium (EC grant number: 101131588).



Towards the Structural Investigation of Bio-Based Blends from Functionalized Lignin for Additive Manufacturing by ^1H and ^{13}C variable temperature Solid-State NMR

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This study is conducted in the framework of ANR project MAGNELIN4D aiming to develop new generation of 3D printing filaments with a high load of lignin biomass. In this context, we focus on PA11 30GDA, a composite material of kraft lignin, functionalized with glycidyl allyl ether (GDA, 30 % weight ratio) and of polyamide (PA11) polymer.

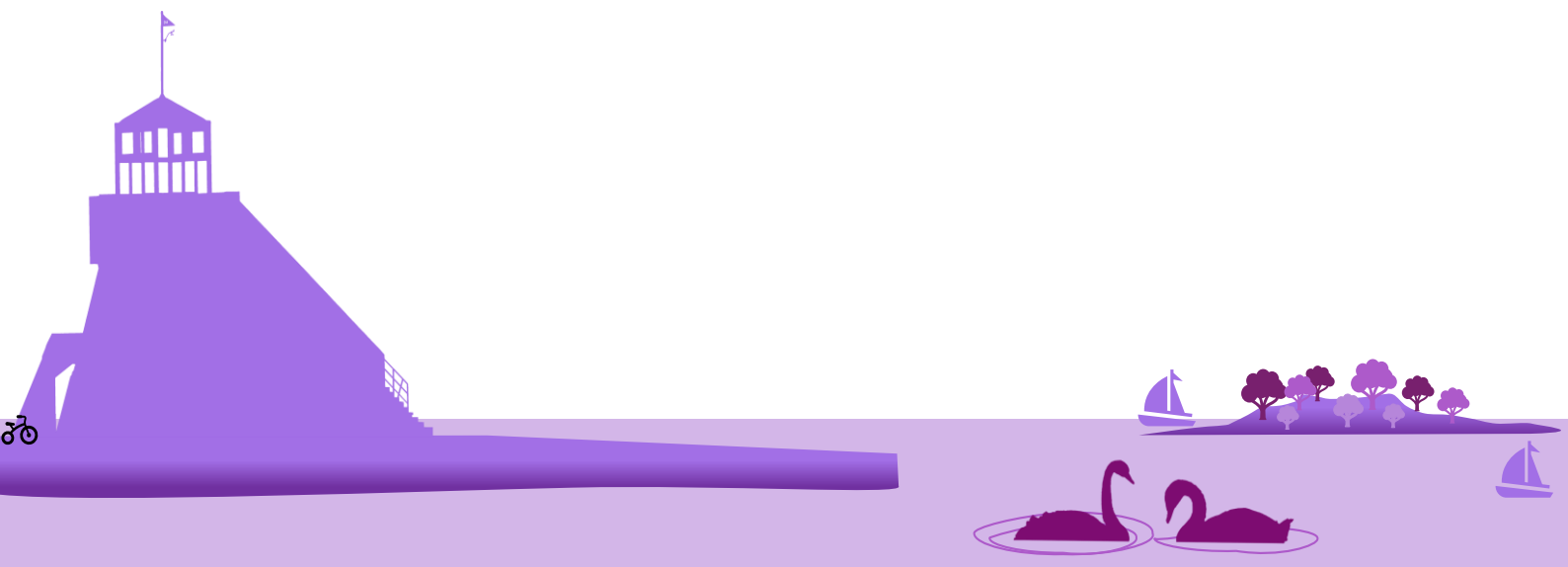
We use variable temperature solid-state NMR for the characterization of the native lignin and its blends to get atomic-scale information of PA11 30GDA, a polymer with functionalized lignin, at different temperatures from RT to 220 °C. It should be noted that the last temperature is the temperature in the 3D printer's extrusion nozzle.

The increasing temperature leads to narrowing of signals in the ^1H and ^{13}C spectra and allows us to assign the resonances corresponding to lignin-functionalized group (GDA) and the PA11 polymer chain. Obtained variable temperature data NMR compared with both the isolated lignin and the functionalized lignin spectra.

Besides structural details, we could obtain the spectroscopic fingerprint of extruded blends with functionalized lignin, setting the basis for further studies, which will involve other synthetic polymer, lignin functionalization group, and functionalized lignin ratios.

This work is financially supported by ANR (Project MAGNELIN4D ANR-22-CE10-0010).

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Understanding water adsorption in CALF-20 via ^1H , ^2H and ^{17}O NMR spectroscopy for carbon capture applications

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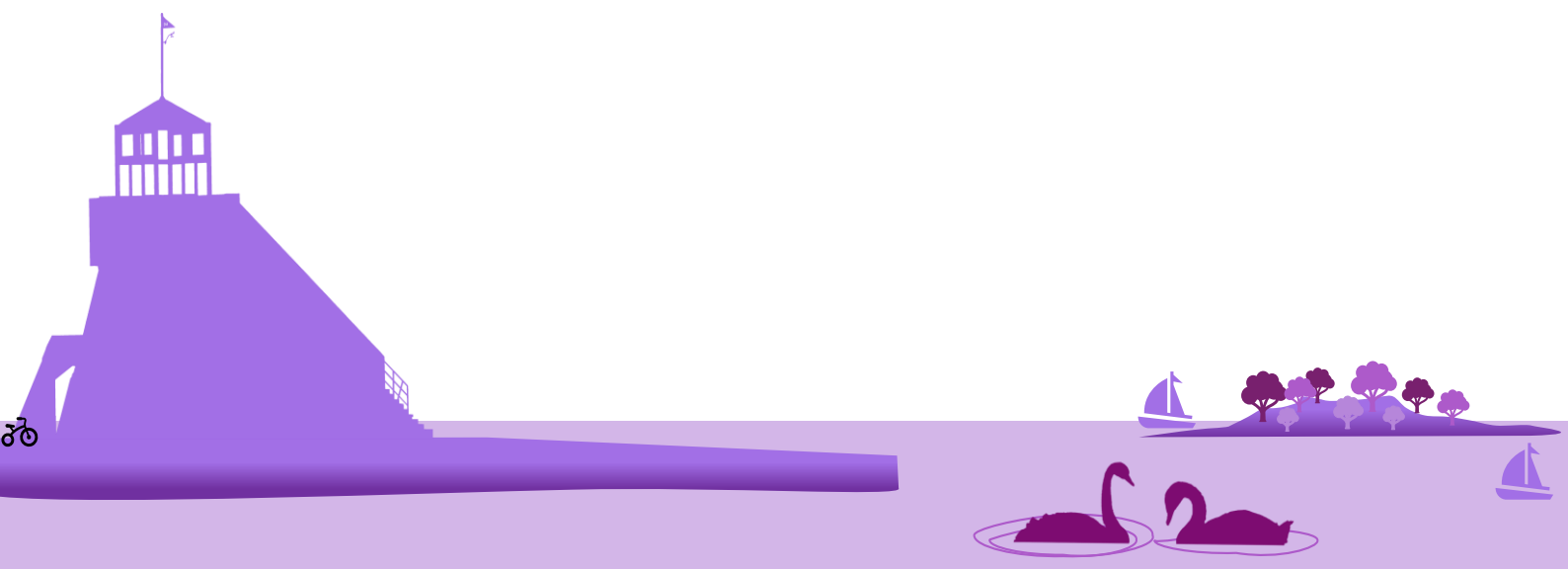
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The removal of carbon dioxide (CO_2) from our atmosphere is pivotal in the battle against climate change. CALF-20 is a metal-organic framework that is a highly promising material as a solid sorbent of CO_2 .¹ It is durable, scalable and has a relatively 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.

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New insights into Romanian archeological ceramics investigated by solid-state NMR spectroscopy

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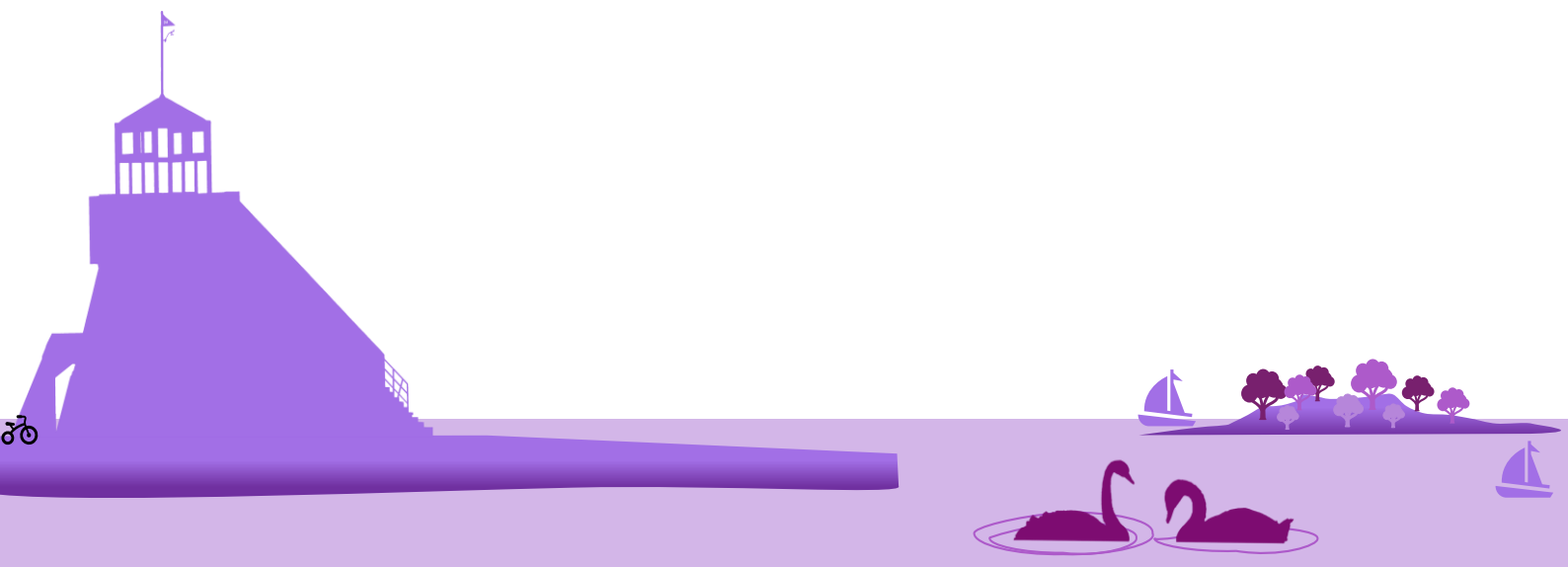
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Investigating archaeological ceramics is crucial for understanding past human cultures. Ceramics are often durable and abundant, providing valuable insights into daily life, trade, technology, and artistic expression. NMR spectroscopy is a valuable tool in this context because it provides detailed information about ceramic materials' molecular and structural composition.

In this work, we experimentally investigate three archeological ceramics from (1) Capidava, (2) the Dacian Fortress of Tilişca, and (3) Şura Mică, situated in Romania. These ceramics are from the Pre-Roman and Dacian period (9th century BC – 2nd century AD), and early medieval period (9th–11th century AD), respectively. For this purpose, samples were taken from the surface and from the depth of the ceramics, and analyzed by ^1H , ^{27}Al , ^{29}Si , and ^{31}P MAS NMR spectroscopy and electronic microscopy (SEM and TEM).

The ^1H MAS NMR spectra indicate a composition rich in oxides, with signal variations linked to ceramic porosity. The ^{29}Si MAS NMR spectra show silica mainly as Q3 and Q4 units, with varying ratios from surface to depth. Comparing with historical data, these results suggest that differing firing techniques were used. The ^{27}Al MAS NMR spectra reveal both AlO_4 and AlO_6 groups, with their ratio varying by sample; in Şura Mică ceramics, the equal presence of both suggests a more uniform forming process. Phosphorus is found throughout the samples, with its form and distribution—organic, inorganic, or metal-bound—depending on provenance.

As general conclusions, it was found that (1) the Romans had a sophisticated ceramic industry, with well-refined and treated materials, (2) the Dacians used traditional techniques and less refined materials, while (3) medieval ceramics show a method of production without concern for quality and durability.



Effect of nucleophilic delignification on the water behavior and porosity of hardwood

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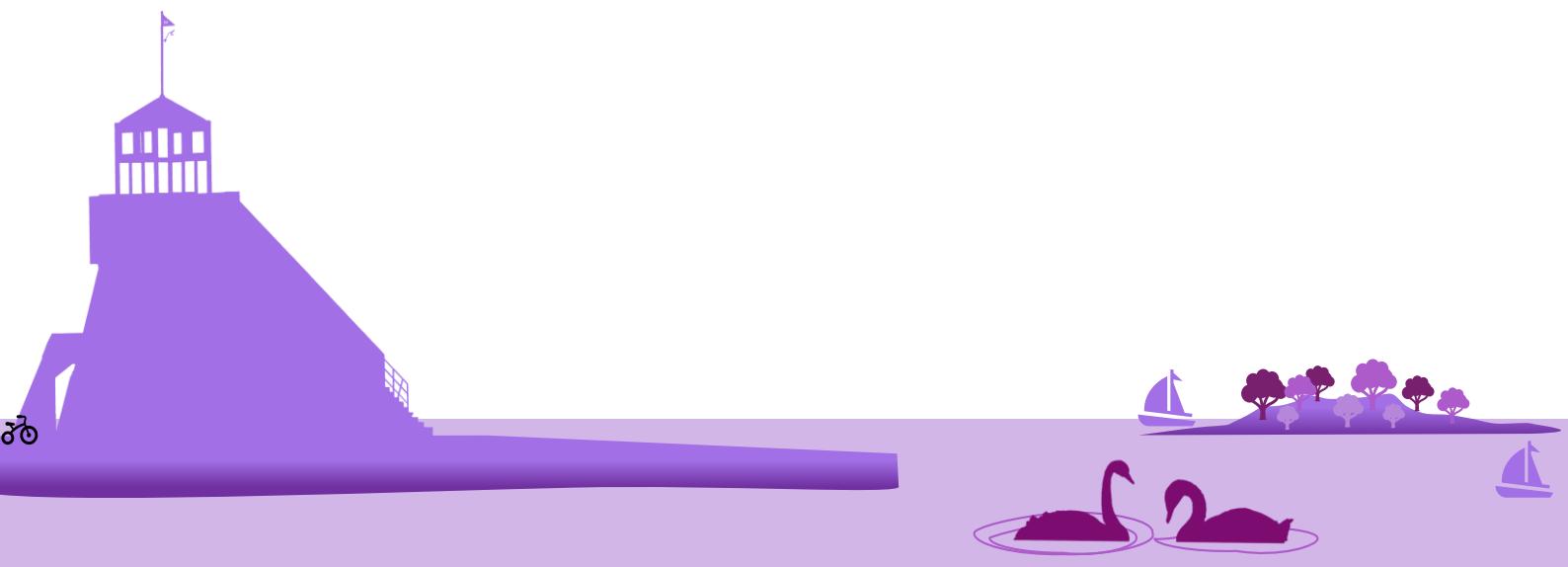
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The unique physico-chemical properties of wood and lignocellulosic materials facilitate their utilization across a diverse range of applications, including construction, papermaking, biofuels, bioplastics, biomedical applications, and advanced carbon materials. Lignocelluloses, which constitute the primary structural components of plant biomass, have sparked significant interest in process engineering due to their abundant availability and potential for sustainable applications. [1,2]

The aim in this research is to elucidate the structures and properties of fiber wall and middle lamella and study the behavior of bonds between relevant components and groups inside the wood samples, as well as observe the selectivity of chemicals used on the compounds of the wood. This is done using a novel nucleophilic delignification process to alter the lignin content of hardwood. Birch chips (*Betula pendula*), which are treated in mild conditions using acidic thiourea treatment. [3] Delignification is performed under minimal mechanical agitation to prevent the disintegration of wood during the delignification. Solid wood block is then analyzed using different NMR techniques to investigate water activity inside the wood as well as changes in porosity. [4,5] Chemical constitution of wood is also studied. Fiber liberation process of delignified wood is investigated by mechanical disintegration followed by fiber analysis and the results are compared to NMR data as well as some Magnetic Resonance Imaging (MRI) methods to acquire local information from the structure.

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Local structure and molecular motion analysis of imidazole-containing layered material and porous material by solid-state NMR

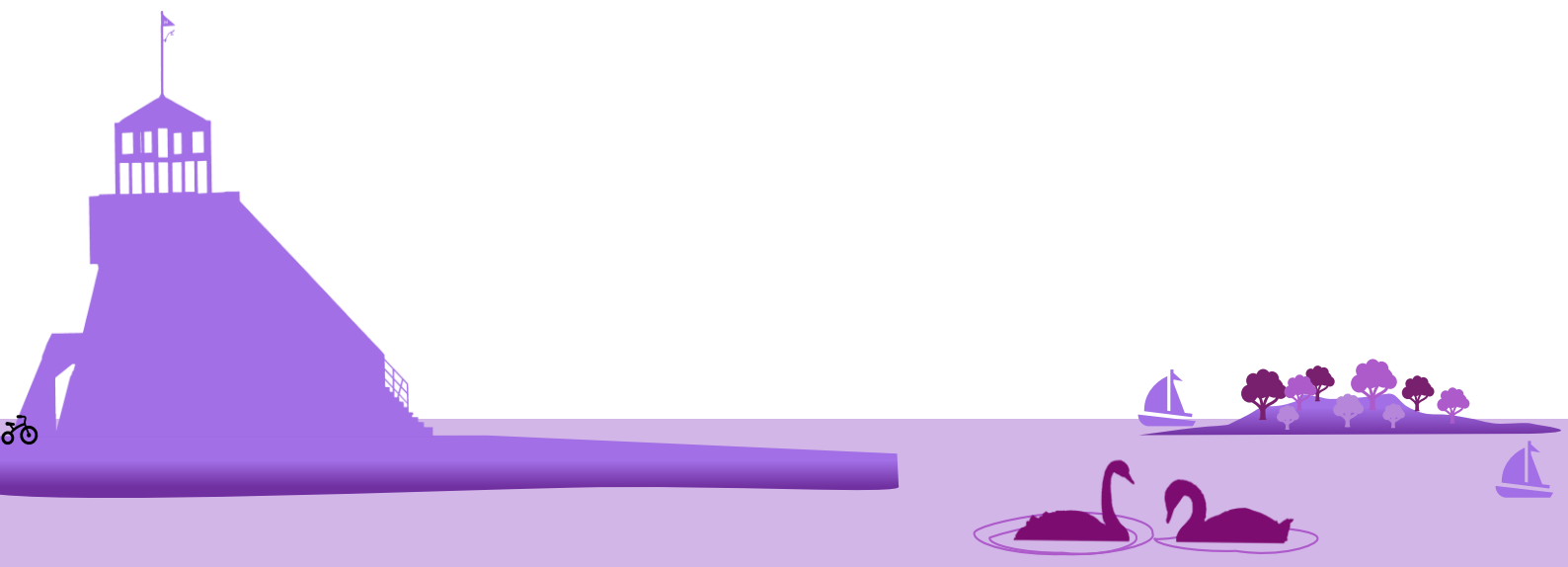
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Anhydrous proton-conducting materials available above 100 °C have been widely developed for their applications in solid electrolyte fuel cells. Several proton-conducting layered and porous materials including imidazole (Im) have been proposed. In these materials, Im is used as proton carriers and provide a proton conduction path. When proton conductivity of these materials is dominated by continuous proton transfer in the hydrogen bond network between Im molecules or between Im and the acidic group attached to the matrix (Grotthuss mechanism), the reorientational motion of Im molecules plays an important role in the proton conduction. In the present work, we analyzed the local structure and dynamics of α - zirconium phosphate/Im and phosphonic acid-modified mesoporous silica/Im composites using solid-state ^2H , ^{13}C , ^{31}P NMR to elucidate the proton conduction mechanism.



Evaluation of Lipid-Based GMO@DTPA-F127 Nanoassemblies for MRI: Gd^{3+} Loading Efficiency and Fast Field Cycling NMR Analysis

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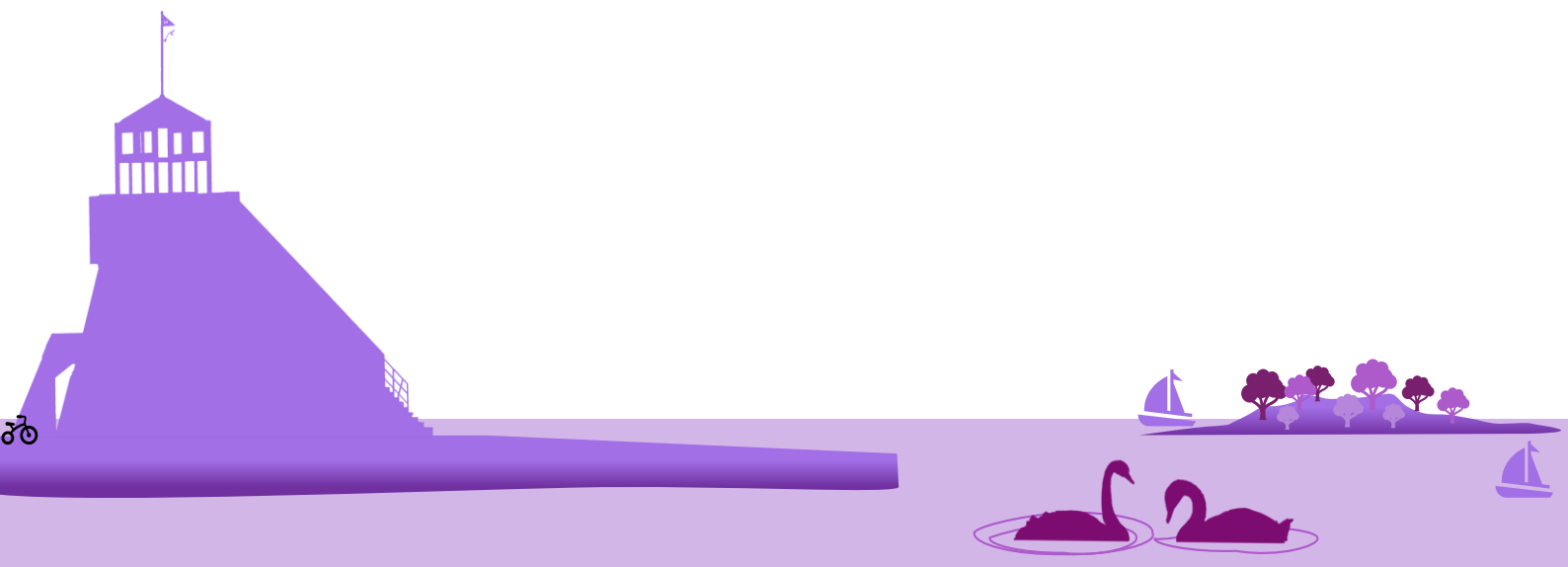
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Gadolinium-based contrast agents (GBCAs) are essential for enhancing MR imaging but raise toxicity concerns due to the release of Gd^{3+} ions, requiring safer alternatives with improved relaxivity and pharmacokinetics. Embedding high molecular weight Gd-chelating lipids within lipid nanocarriers presents a promising strategy to decrease Gd^{3+} dosage and enhance imaging efficiency. This study investigates GMO-based nanoassemblies loaded with DTPA-bis(stearylamide) gadolinium salt (DTPA-BSA-Gd), stabilized by Pluronic F127 in varying concentrations of DTPA-BSA-Gd (5-50% w/w) and F127 (10-25% w/w) to optimize contrast parameters. Characterization included particle size, stability, and structure. Lower DTPA-BSA-Gd favored internally ordered nanoparticles, while higher concentrations negatively affected self-assembly but maintained satisfactory relaxivity behavior. ICP–Mass Spectrometry analysis confirmed high Gd^{3+} loading efficiency and was corroborated by the NMR method. Relaxation time (T_1) and relaxivity (r_1) were investigated at 0.39 T (16.5 MHz). FFC-NMR was employed to analyze ^1H NMRD profiles, providing molecular insight into relaxivity across various magnetic field strengths. The optimal formulation, 20DTPA-25F127, yields maximal loading of Gd^{3+} to the system and achieves the highest r_1 value. At concentrations above the optimal, the incorporation of Gd was limited, likely due to disturbances in the system's structure caused by a phase transition, which leads to T_1 relaxation lengthening. Fixed-field NMR on HeLa cells showed reduced T_1 vs. commercial Gadovist at equivalent concentration of Gd^{3+} , confirming high cellular uptake and retained relaxivity. In conclusion, these GMO@DTPA-F127 nanoassemblies exhibit properties that qualify them as high molecular weight MRI agents superior to commercial GBCAs in longitudinal relaxivity, offering a potentially safer alternative with enhanced MRI performance compared to currently used agents burdened with toxicity concerns.



Influence of Aliovalent Substitution in Na_2BCl_6 ($\text{B} = \text{Y}/\text{Nb}$) Metal Halide Electrolytes Using Solid-state NMR Spectroscopy

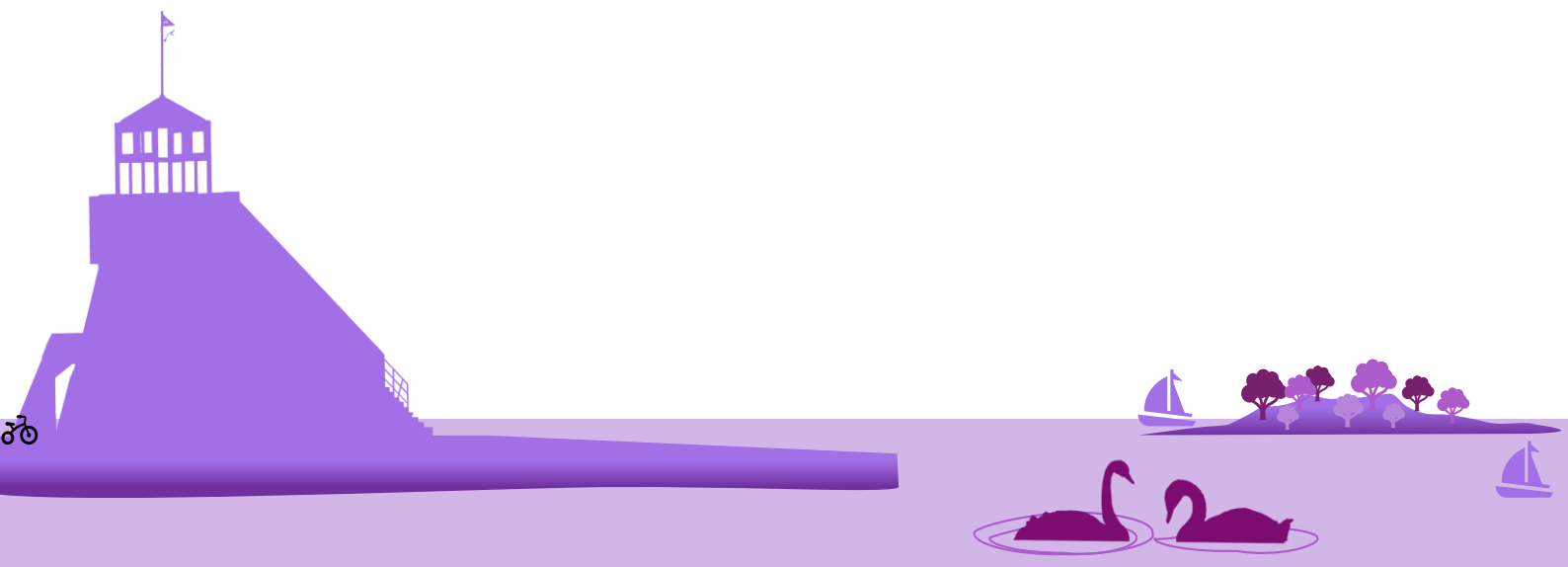
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Lithium-ion batteries (LIBs) are essential energy storage devices that power technologies ranging from portable electronics to electric vehicles to grid-scale energy storage. However, current lithium-based energy storage solutions are unsustainable and continue to raise ethical questions regarding mining practices. Compared to lithium, sodium is approximately 1100 times more abundant globally and more affordable to extract. An analysis of the environmental impact between sodium-ion battery (SIB) and LIB production indicates that SIBs have a lower potential for human toxicity and minimise conflicts over mineral and metal resources.

This presentation will discuss our recent efforts to understand the dynamics and structure of a series of Na_2BX_6 compositions, where $\text{B} = \text{Y}/\text{Nb}$ and $\text{X} = \text{Cl}$. Mechanochemistry is employed to synthesise candidate materials. A suite of characterisation techniques (XRD, SEM, EIS, etc.) is utilised to analyse the bulk structure and electronic properties. Solid-state nuclear magnetic resonance (NMR) spectroscopy is used to probe the local atomic structure, identify phase changes, and understand how sodium dynamics within these materials are influenced as a function of extreme temperatures ranging from -40 to $+80^\circ\text{C}$. Preliminary findings suggest that vacancies in the structure arise from aliovalent metal substitution ($\text{Y}^{3+}/\text{Nb}^{5+}$) in the B site. At lower Nb loading, at least two distinct peaks are present, representing different sodium chemical environments. Using 1D and 2D ^{23}Na MAS NMR and variable temperature methods, we will discuss the unique structure and jump rates exhibited within these materials. The presence of vacancies may enhance ionic conductivity; however, further analysis is ongoing to distinguish between local and bulk diffusion. Lastly, ultrahigh-field ^{93}Nb NMR reveals the BX_6 octahedra appear to be influenced by Y substitution.



A Combined Experimental/Theoretical Study: Structural Flexibility and Ion Dynamics in Solid Electrolytes for Chloride-Ion Batteries

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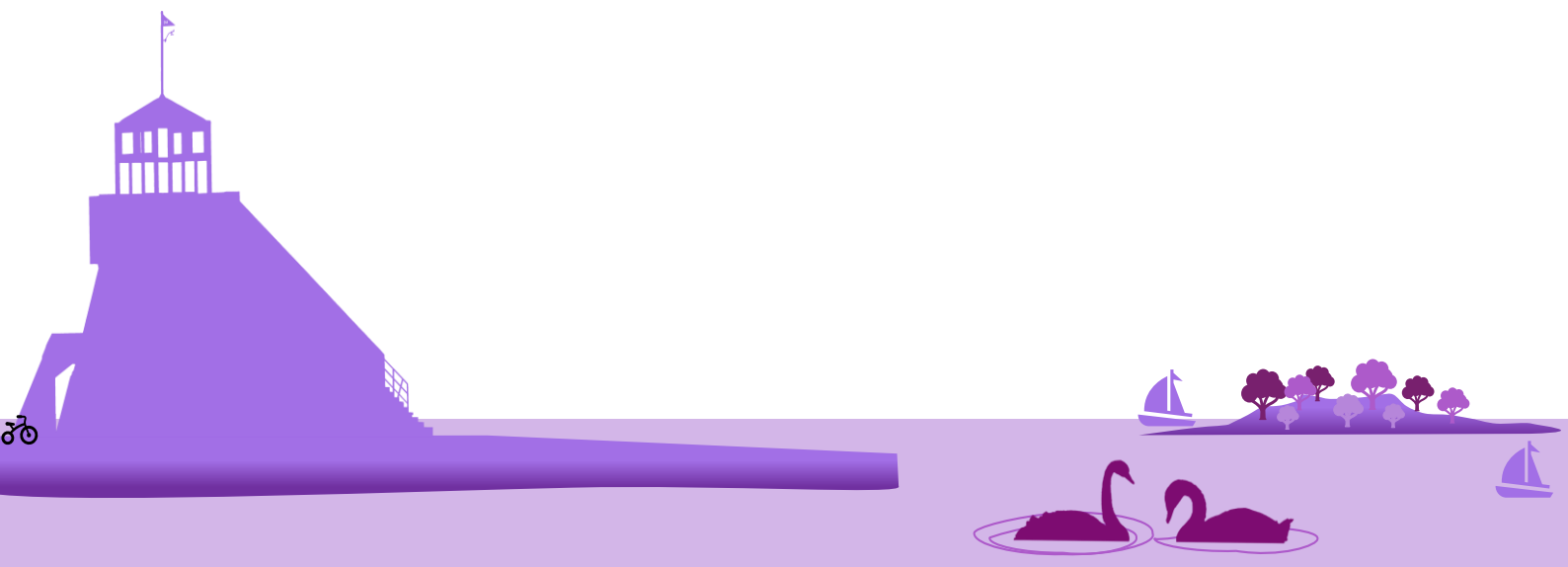
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CsSnCl₃-based solid electrolytes doped with trivalent ions (In³⁺, La³⁺) demonstrate exceptional potential for chloride-ion batteries. Using solid-state ³⁵Cl NMR, DFT modelling, and COSMOS-based tensor simulations, we elucidate the correlation between structural flexibility and enhanced ionic conductivity. Doped CsSnCl₃ exhibits significantly narrowed NMR linewidths (~3-4 ppm), indicative of rapid chloride-ion mobility averaging quadrupolar interactions to below 0.5 MHz. Spin-lattice relaxation times (T₁) shorten substantially with doping, confirming accelerated ionic motion. DFT calculations highlight that doping-induced lattice distortions significantly reduce chloride migration barriers (~0.2-0.3 eV), linked directly to the off-centring of Sn/In ions within flexible Sn/InCl₆ octahedra. Incorporating lattice dynamics and Sternheimer corrections into COSMOS molecular dynamics simulations accurately reproduces experimental NMR results, providing predictive insights into the interplay between lattice vibrations, structural disorder, and ionic diffusion. These comprehensive analyses connect microscopic lattice flexibility with macroscopic electrolyte performance, advancing the development of high-conductivity, stable chloride-ion solid electrolytes for next-generation batteries.

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Crystal Structure, Particle Size, and Ionic Conductivity of Solid-State Electrolytes Studied By Diffusion and Relaxation NMR

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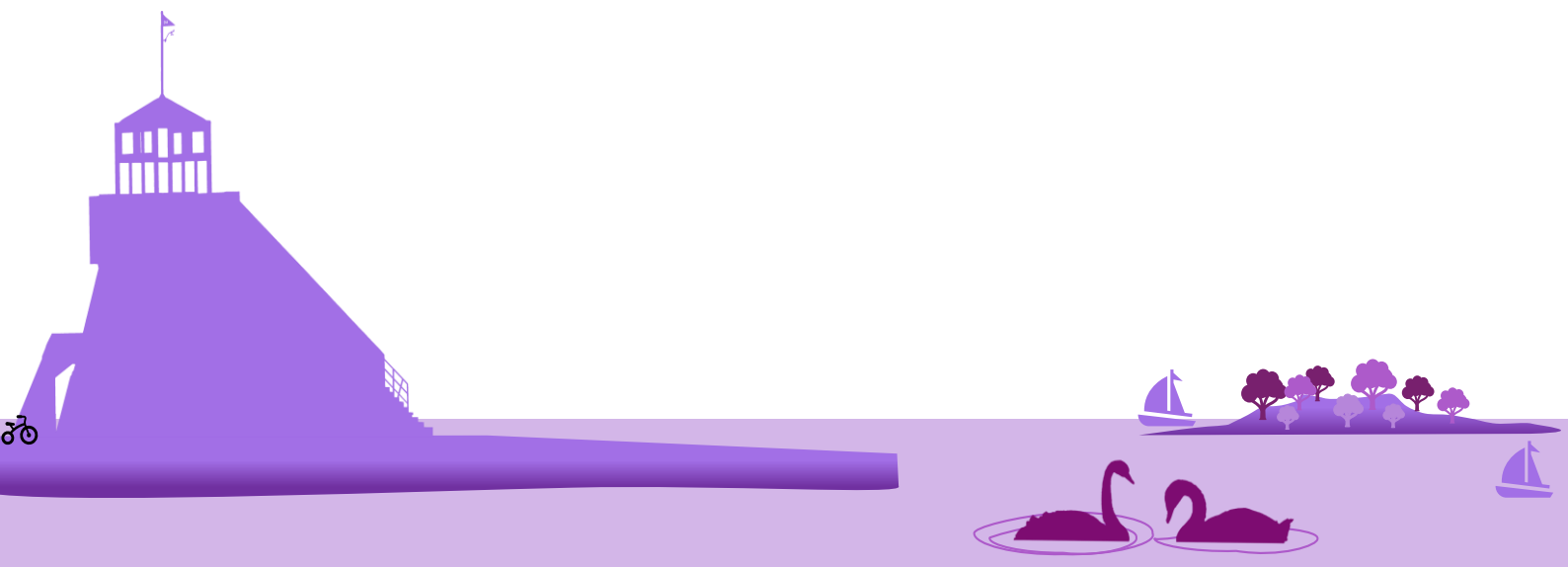
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Introduction: Development of new sustainable technologies relies on the development of safer, low cost, and higher energy density batteries, such as all solid-state batteries. In all solid-state batteries, ions migrate from anode to cathode through defects in the crystal structure of the solid-state electrolyte. Diffusion based NMR measures this migration in the crystal structure. Diffusion is correlated with electrical conductivity while diffusion measurements as a function of temperature and observation time allow for the measurement of activation energy. Relaxation measurements give insight into motion on the atomic scale. In this work, migration of Li in two solid-state electrolyte materials, cubic $\text{Li}_6\text{PS}_5\text{Cl}$ and monoclinic Li_3InCl_6 , is characterized.

Methods: The powder samples of $\text{Li}_6\text{PS}_5\text{Cl}$ and Li_3InCl_6 in an Argon atmosphere were sealed in 5 mm glass NMR tubes. NMR Measurements, measuring D, T2, T1, D-T2, and T2-T2, were acquired using 9.39 and 14.1 T Bruker spectrometers equipped with 5 mm Diffusion Broadband Probes with a maximum gradient strength of 17.4 Tm⁻¹ and 28 Tm⁻¹. Data was acquired from -60 to 180°C.

Results and Discussion: To test the effect of defects in the crystal structure on ion migration, which includes ion hopping and long-range motion, monoclinic Li_3InCl_6 was studied before and after annealing, which introduces impurities. R2 relaxation rates and D were faster in the annealed sample, indicating an increased mobility between atomic sites in the crystal lattice and faster long-range motion. To compare the effect of crystal structure, the diffusion in cubic $\text{Li}_6\text{PS}_5\text{Cl}$ was studied. D(T) was faster and had a smaller activation energy in cubic $\text{Li}_6\text{PS}_5\text{Cl}$ than in monoclinic Li_3InCl_6 .

Conclusion: Relaxation and diffusion NMR are powerful tools for measuring the ion motion in solid-state electrolytes that give insight into short range motion and long-range motion.



Micellar growth upon addition of fatty alcohol investigated by NMR diffusometry

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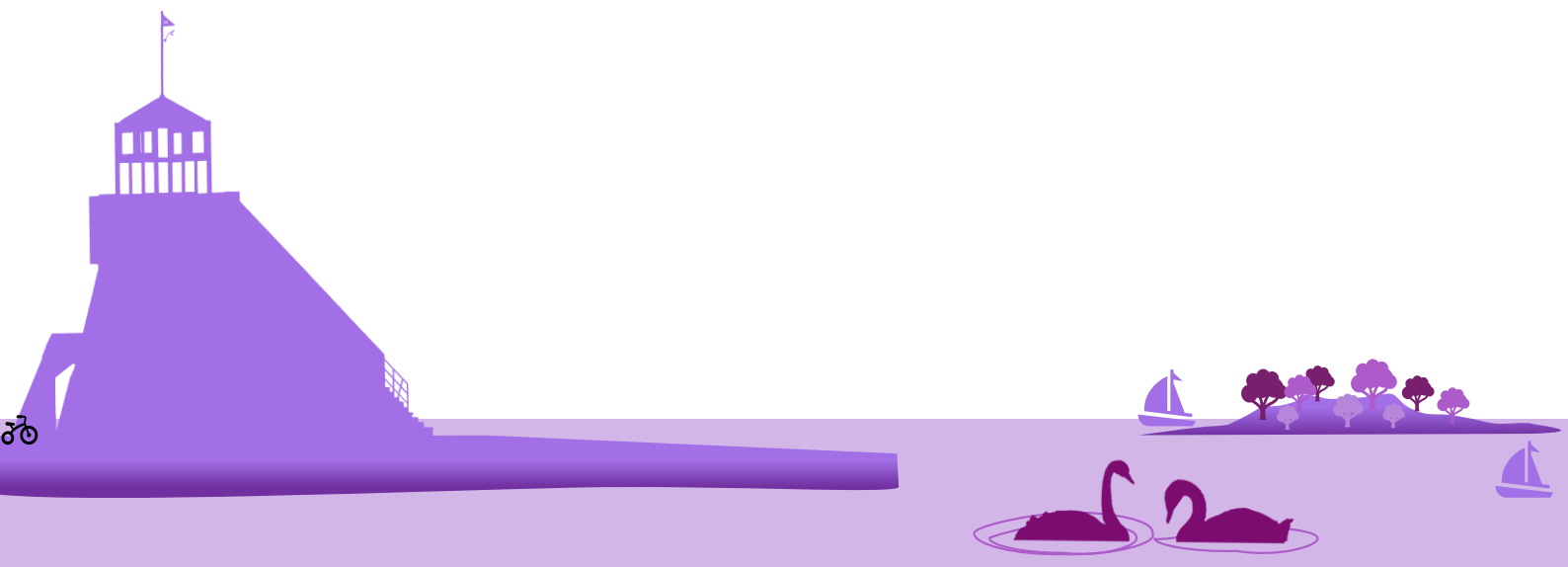
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NMR diffusometry is a powerful tool for studying the aggregation of molecules, for example the formation of micelles in aqueous solutions [1]. The shape of the micelles depends on the curvature of the interface and can be adjusted by concentration or temperature. Another way to change the interfacial curvature is by adding a co-surfactant with a different packing parameter [2]. If the co-surfactant itself is insoluble in water, its diffusion coefficient corresponds to the diffusion coefficient of the micelles. In this case, information about the micelle size can be obtained directly from the diffusion coefficient of the co-surfactant via the Stokes-Einstein equation. The measured diffusion coefficient of the water-soluble surfactant, on the other hand, is a weighted average of the diffusion coefficients of free monomeric surfactant and surfactant in micelles due to the rapid exchange between the two environments.

According to this concept, we have investigated ternary systems containing sodium dodecyl sulphate (SDS), water and a fatty alcohol as co-surfactant. We found that the diffusion of the micelles slows down when more and more SDS is replaced by the co-surfactant until phase separation occurs. The decrease in the micellar diffusion coefficient indicates a growth of the micelles, which is to be expected as the small head group of the co-surfactant (large packing parameter) lowers the interfacial curvature and leads to a cylindrical growth of the micelles. Furthermore, the observed diffusion behavior of the surfactant is discussed, taking into account additional effects such as a dependence of the critical micelle concentration on the composition of the system and obstructed diffusion.

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Modified lipid-based multifunctional nanoparticles as MRI contrast agents

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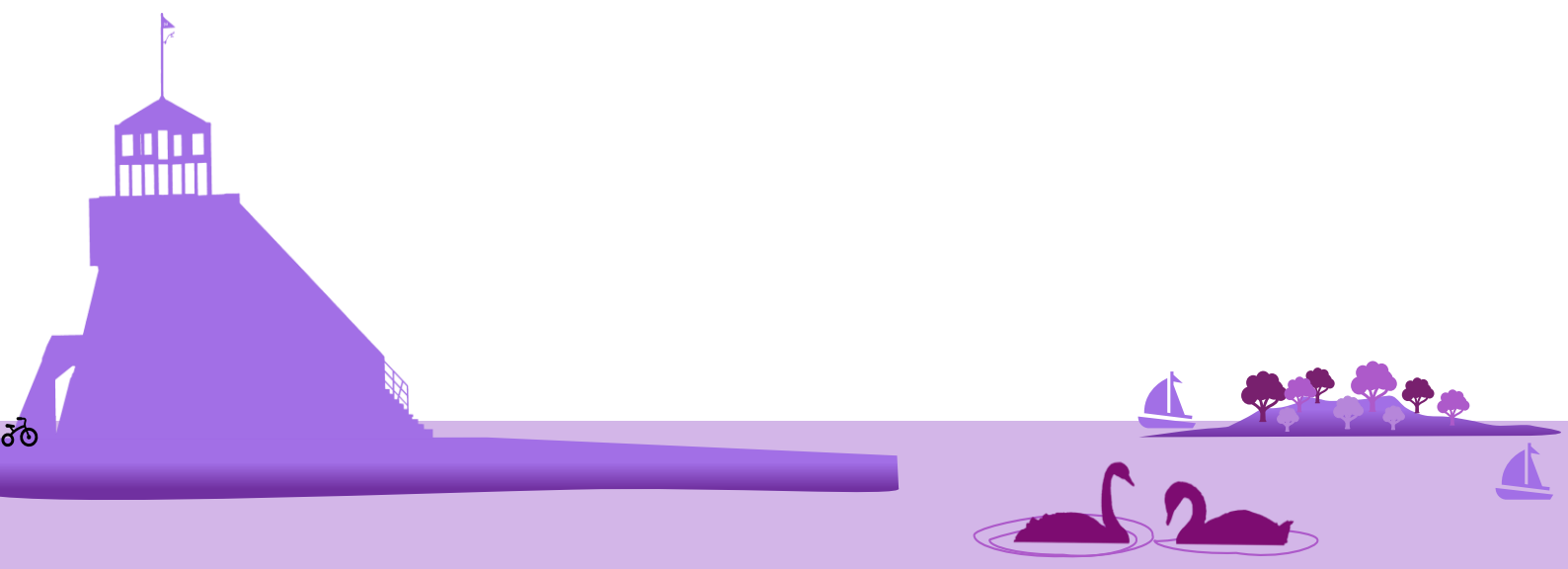
Modified lipids could be used to create many types of nanoparticles with different geometries. The hydrophilic and hydrophobic properties allow it to be used as a carrier for many biologically active materials. The aim of this study was to create two types of multifunctional hybrids containing gadolinium chelate-based MRI contrast agents (Gd-DTPA) and the first with a non-metal hydrophobic photosensitizer (Verteporfin), and the second type with a metal hydrophobic photosensitizer (ZnPc) which is a gold standard photosensitising drug, both using for photodynamic therapy. These systems were prepared by the microfluidic method, which allows double the loading capacity of active components compared to the system made by traditional extrusion methods.

The NMR spin-lattice relaxation methods were used to assess the MRI contrast properties of the nanocarriers and the sensitivity of the contrast agent (relaxivity) for both systems. Moreover, the T1 relaxation study in a wide range of magnetic fields gives a proper tool for investigating the influence of the VER component on the nanoparticle behaviour, which should correspond to the increase in contrast properties. To confirm the desired structure and size of nanocarriers, dynamic light scattering and transmission electron microscopy were used.

The results of the relaxivity parameters obtained for liposomal contrast agents are higher than results known for standard clinically used contrast agents. Moreover, the TEM results confirm liposomal geometry with well adjusted size of nanoparticles. At the last step, MRI studies were performed for the set of samples with dispersion of liposomes in different Gd concentrations, and finally, MRI in vivo experiments on rodents.

All experimental data confirm the potential to use the lipid-based nanocarriers containing photosensitiser as modern contrast agents in a newly designed cancer therapy.

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The influence of cation change in ammonium-based ionic liquids on its structure and molecular dynamics in bulk and at interfaces

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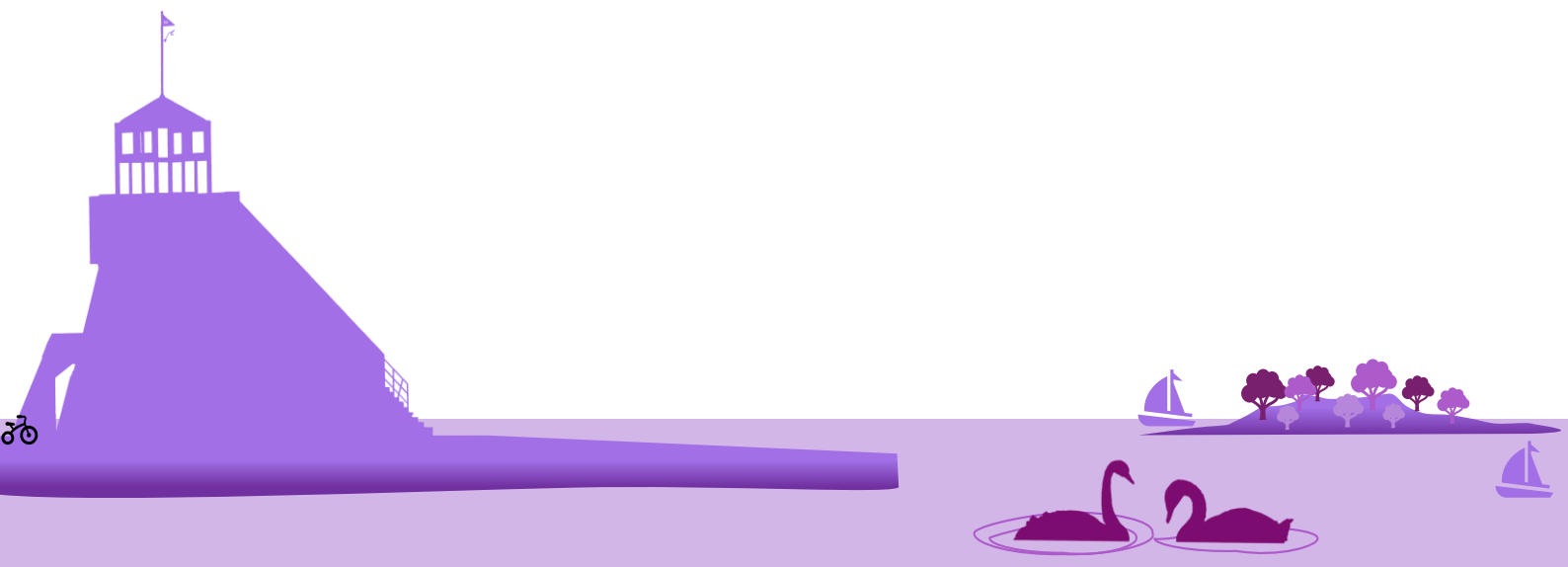
Numerous studies have documented the use of ionic liquids to remove various organic and inorganic contaminants from residential and industrial wastewater. Moreover, the hydrophobic ionic liquids are suggested as extracting solvents for the removal of plastic particles from aqueous systems, to reduce the concentration of these potentially hazardous materials.

The main objective therefore was the evaluation of potential use of ionic liquids as extraction media for plastic nano- and microparticles. Apart from the preparation and characterization of three families of ionic liquids with varied alkyl chain length (with aromatic – alkylbenzyltrimethylammonium, cyclic – alkylcyclohexyltrimethyl-ammonium and linear – alkyltriethylammonium cations, with alkyl chains varied from 2 to 18 carbon atoms, with bis(trifluoromethanesulfonyl)imide anion), the results from NMR relaxometry. In this way, dynamical processes occurring on timescales from milliseconds to nanoseconds can be studied, including translational and rotational dynamics of ions. Such variety of proposed structures enables different spatial heterogeneity (creation of ionic networks, nanostructure, formation of clusters, aggregates etc.) which has enormous effect on their behaviour at the solid-liquid interface, their physicochemical properties and their application performance.

In our case, the molecular interactions at the solid-liquid interface (between the ionic liquid and plastic particles), will be presented. Explanation of the behaviour of such mixtures is crucial for the understanding of the potential of ionic liquids as plastic particles' extraction media.

Acknowledgements:

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Tailoring the Room Temperature Spin Dynamics in Molecular Quantum Materials via Host–Guest Crystal Engineering

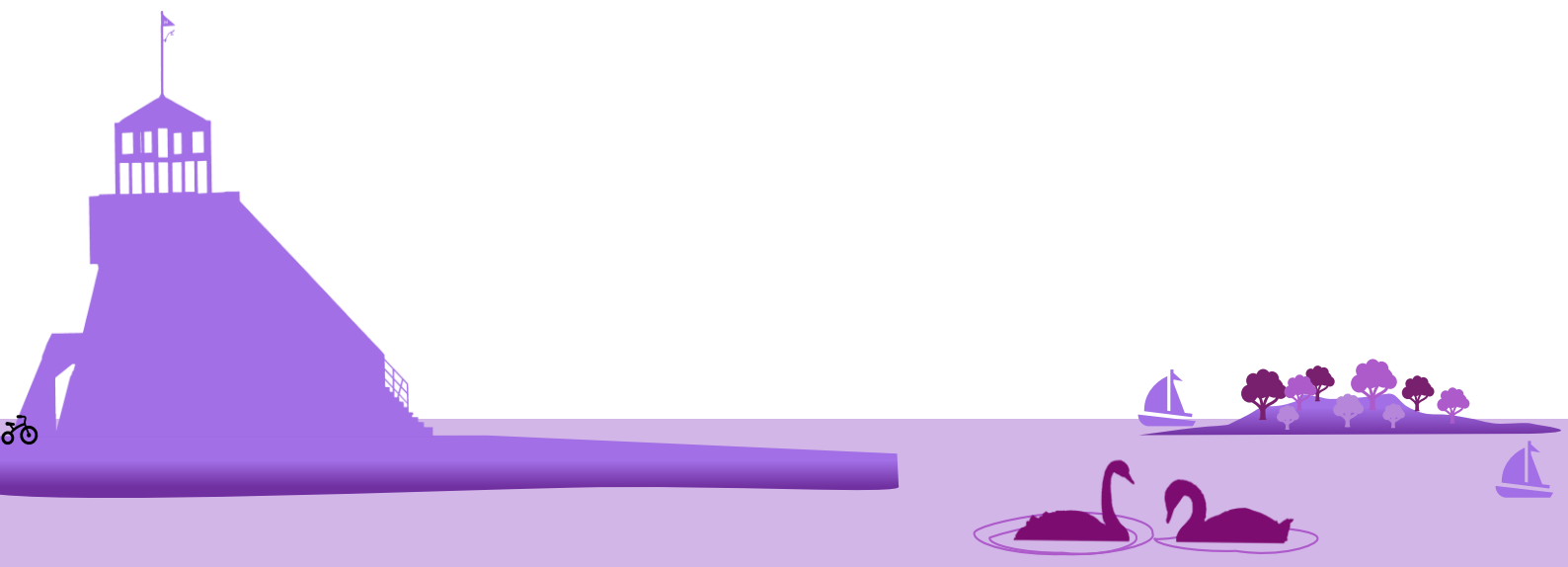
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The search for better molecular quantum materials is driven by the demand for long-lived electron – spin states, and organic triplet, particularly those of pentacene offer a promising platform for technologies such as room-temperature masers [1] and quantum sensors [2], but their spin properties are sensitive to their surrounding host environment. To investigate the influence of host environment on spin dynamics and quantum properties, we synthesised a series of host-guest systems consisting of pentacene doped into: p-terphenyl (PTA:PTP), naphthalene (PTA:NAP), picene (PTA:PIC) and perdeuterated p-terphenyl (PTA-d14:PTP-d14) and studied their triplet states using photoexcited time-resolved electron paramagnetic resonance (trEPR) and theoretical calculations.

Our findings reveal that host properties such as π – π stacking, and nuclear spin environment dramatically affect the spin–lattice relaxation time (T_1), and spin decoherence time (T_2), leading to significant differences in the maser threshold and output power. As a result, a quantitative framework was established to link the differences in host structure and nuclear environment to the potential of these materials in quantum devices, offering new insight into the rational design of organic quantum materials.



Structural and Dynamic Insights into the Loss of Activity of Tolaasin I, an antimicrobial cyclic lipopeptide.

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Cyclic lipopeptides (CLiPs) are secondary metabolites characterized by a peptide moiety with an N-terminal lipid tail and a macrocycle formed via a depsi bond. Produced by various bacteria like *Pseudomonas* and *Bacillus*, CLiPs have diverse biological functions, including enhancing bacterial motility and exhibiting antibacterial and antifungal activities.[1] Tolaasin, a CLiP from *Pseudomonas tolaasii*, is responsible for brown blotch disease in mushrooms and shows inhibitory effects against fungi and Gram-positive bacteria. A natural defense mechanism, where tolaasin's antagonistic properties are neutralized by hydrolysis of the depsi bond, involves an enzyme from a cohabiting bacterium.[2] Understanding how structural changes impact membrane interactions is vital for developing new therapeutics and biocontrol agents.

In this study, we use NMR spectroscopy to investigate conformational changes and membrane interactions of both native and hydrolyzed tolaasin in SDS micelles. To enable multidimensional NMR analysis, ^{13}C - and ^{15}N -labeled tolaasin and its hydrolyzed form are produced by growing the bacterium on minimal medium. Followed by full resonance assignments and ^{15}N relaxation experiments provide insights into peptide backbone dynamics, with order parameters (S^2) shedding light on molecular motions on the ns-ps timescale. 3D-HNHA experiments were conducted to access backbone conformation dependant $^3\text{J}_{\text{HNHa}}$ scalar couplings. Long-range HNCOC measurements identify long-lived hydrogen bonds through $^3\text{hJ}_{\text{NC'}}$ scalar couplings. Additionally, NH R1 rates, recorded with and without a paramagnetic agent, map the paramagnetic relaxation enhancement (PRE) wave of tolaasin, allowing to determine helix orientation.

Our findings suggest that opening the macrocycle leads to partial loss of peptide backbone rigidity, resulting in conformational changes in exocyclic residues. These insights are crucial for the design of biocontrol agents and novel therapeutics.



Lithium-ion motional behavior under confinement: An NMR study of Carbon aerogel

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In recent time, structural characterization and quantification of ion dynamics in carbon aerogel (CA) materials have become topically relevant. NMR spectroscopy offers exciting avenues to investigate CA, particularly in understanding ion adsorption and dynamics at the solid-water interface. The present study has employed ^1H restricted diffusion and relaxation measurement to evaluate pore parameters of a synthesized CA that reveal the presence of mesopores, a high surface-to-volume ratio, low tortuosity, high porosity, and fluid-free index. Inverse Laplace transformation (ILT) of ^1H spin-spin relaxation data indicates the existence of four regions of CA where water can exist. Advanced ^1H diffusion-relaxation correlation spectroscopy (DRCOSY) recorded with specific diffusion time (Δ) corroborates the same. Further, to understand ion dynamics in these pore spaces of CA, temperature-dependent analysis of ^7Li linewidth and relaxation rates are carried out for lithium-based electrolytes with the specific goal of elucidating how pore parameters of the aerogel alter and further influence the mobility and solvation of the lithium ions in the solution. ILT of ^7Li spin-spin relaxation data confirms the presence of Li ions in all the detected regions exhibiting complex relaxation curves. Multi-exponential fitting of the spin-spin relaxation data allows evaluation of fluid-free index, quantifying movable and immovable fluid near the surface. Relaxation analysis further confirms that incorporation of these electrolytes in the CA pore spaces considerably alters the pore parameters, especially the tortuosity and surface relaxivity. The Li ion hopping process amongst various pore spaces are investigated by analyzing the temperature-dependent ^7Li linewidth and spin-lattice relaxation rates. It is observed that Li ion migration in CA-based systems is largely affected by the interplay between the confinement effect and the interaction of the electrolyte with the pore walls.



Water-detected NMR reveals novel insights into disease-associated RNA condensates

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RNA condensation is implicated in the formation of toxic RNA foci in cells affected by genomic expansions of trinucleotide repeats. Given the critical role of this process in neurological diseases, it is important to understand the physical properties of such condensates, which are poorly understood due the limited number of available methods for their characterization. Using NMR, we show that CAG repeat-expansion RNA condensates are broadened beyond detection and in addition not amenable for indirect characterization via DEST due to slow exchange of RNA molecules between condensed and dilute phase. However, selective saturation of condensate resonances can be relayed to H₂O via fast through-space dipolar transfer in rigid materials, allowing information about condensed RNA to be encoded in solvent water. Based on this, we developed a method, termed Semi-solid Condensate Observation by Proton Exchange (SCOPE), for characterization of NMR-invisible biological condensates. SCOPE is based on semi-solid magnetization transfer and exploits multiple exchange processes to water during prolonged saturation. The resulting 150-fold signal amplification enabled detection of condensates at RNA levels as low as 10uM. SCOPE revealed a 1000-fold decrease in molecular tumbling of RNA upon condensation, leading to T₂ relaxation constants below 8us and suggesting semi-solid material properties of these condensates. We show that, upon condensation, RNA partitioning depends on the MgCl₂ concentration, implying that this process is driven by heterotypic interactions between RNA and Mg²⁺. Further, we detected a dramatic reduction of condensate-bound water when increasing the number of CAG repeats per RNA oligomer. Given the crucial role of the number of CAG repeats for disease progression, our work provides new mechanistic insights in RNA foci formation in the context of disease and yielded a broadly applicable NMR method for characterization of disease-associated biological semi-solids.



A new method to extract couplings between prochiral methylene protons with very close chemical shifts

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Homonuclear couplings provide a wealth of information for constitutional, configurational and conformational analysis of organic compounds.[1,2] In particular, within prochiral methylene (CH_2) moieties, the geminal ^2JHH scalar coupling constants display interesting dependencies on local conformation.[1] Also, two-bond ^1H - ^1H residual dipolar couplings (RDCs) are attractive for structural analysis given the fixed interproton distance.[2] Several experiments have been described that measure geminal ^1H - ^1H couplings individually, but these only work well when the two protons are well-separated in the spectrum.[3-5] When the protons have similar chemical shifts and are strongly coupled—i.e., when their multiplets appear ‘merged’—their extraction is challenging, especially when further overlapped with other multiplets. Recent selective J-resolved experiments for individual coupling measurement in cases of chemical shift degeneracy equally cannot work for the particular case of geminal ^1H - ^1H couplings.[6]

Here we present the ‘Inverse’ TSE-PSYCHEDELIC experiment that yields individual geminal ^1H - ^1H couplings at pure shift resolution. This new experiment – a variation on the original TSE-PSYCHEDELIC[5] – works well as long as the parent TSE-PSYCHE experiment[7] is able to resolve pure shift responses of the two strongly coupled protons. This includes many cases that until now were considered challenging. This method will be very useful for configurational and conformational analysis based on J-couplings and RDCs.

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Shaping of the selectivity profile of single-scan ultraselective NMR experiments

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Extracting the spectrum of a targeted molecule from a mixture is an efficient way of quickly accessing information from an otherwise complex spectrum. Classical selective methods are based on the excitation of a region of the spectrum containing a signal from the system of interest. This requires a well-resolved multiplet, which can be difficult to find in ^1H 1D spectra due to multiplicity induced signal overlap. “Ultraselective” methods such as Chemical Shift Selective Filters (CSSF) have been developed to overcome the problem of overlap in selective excitation. CSSF allow selection based on chemical shift alone, but they require the acquisition of a large number of time steps, resulting in long experimental times.

GEMSTONE(1) allows CSSF-type selection to be performed in a single scan. This fast “ultraselective” method uses spatial parallelisation to obtain the time increments of the CSSF in a single scan. By dividing the spatial parallelisation process into smaller blocks, sensitivity losses caused by translational diffusion can be mitigated(2). However, GEMSTONE has a sinc-shaped selectivity profile, which can lead to incomplete selection depending on the distance between the signal of interest and the neighbours (which may coincide with a side lobe or a zero of the sinc).

Here we show how to control the selectivity profile of GEMSTONE, and in particular how to change it from a sinc to a Gaussian shape. We achieve such a change by “spatial sculpting”, using a Gaussian selective pulse combined with a magnetic field gradient that allows the distribution of the magnetisation in the sensitive volume to be modified, resulting in a Gaussian selectivity profile. These changes lead to an overall improvement in the selectivity of the GEMSTONE which, when used together with a polarisation transfer block, allows the analysis of more complex samples.

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What is my reaction intermediate? Resolving individual spectra in real-time NMR reaction monitoring

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NMR spectroscopy is widely used in live reaction monitoring as it provides rich structural information and quantitative concentration profiles; yet, extracting detailed insight from reaction mixtures remains a significant challenge. Combining NMR methods with chemometrics has shown great success in resolving pure component spectra of mixtures without requiring physical separation.¹⁻³ Here, we demonstrate the power of a simple multivariate curve resolution (MCR) method for continuous reaction monitoring using NMR. This approach facilitates the analysis of reaction pathways and mechanisms by providing individual spectra of all reaction mixture components.

We applied the method to a photodegradation study of betamethasone, a well-known anti-inflammatory drug, using the NMR Torch4 for in situ, real-time reaction monitoring by NMR. Interleaved 1D ^1H and $^{19}\text{F}\{^1\text{H}\}$ NMR spectra were recorded, followed by MCR analysis. This decomposes the NMR dataset (X) into component spectra (S) and concentration profiles (C) according to the model $X = CST + E$, where E represents the experimental noise and T denotes the transpose. Using concentration profiles C from resolved ^1H or ^{19}F signals, the dataset X can be decomposed by solving the system of linear equations in a single step to yield pure component spectra S . In the case of betamethasone photodegradation, we successfully separated the spectra of the substrate, two intermediates, and major products.

MCR processing of reaction monitoring NMR data is a versatile tool for complex reaction mixture analysis. It facilitates the understanding of chemical reactions by enabling computationally efficient multicomponent analysis through “matrix division”, while eliminating the need for physical mixture separation. This approach is broadly applicable to studies of reaction kinetics and catalysis.

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Real-time NMR Investigation of Electrolyte Degradation in Aqueous Organic Redox Flow Batteries

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Introduction: Large-scale energy storage is essential for balancing renewable energy production and consumption. Aqueous organic redox flow batteries (ARFBs) offer a cost-effective and environmentally sustainable alternative to vanadium-based systems. However, their commercial viability is hindered by shorter lifetimes and capacity fade, primarily due to molecular degradation [1].

Aim: To investigate, in real time, the underlying chemical and electrochemical processes occurring under real environmental conditions—such as temperature variations, prolonged cycling, and ageing—to optimize redox flow batteries (RFBs) more effectively.

Methods: Real-time pseudo-2D ^1H NMR spectra were acquired with high temporal and spectral resolution. Additionally, various 2D NMR methods, including NOESY, COSY and TOCSY were employed to validate the identified degradation products and their chemical interactions. These analyses focused on understanding the degradation pathways of 2,6-dihydroxyanthraquinone (DHAQ)/ $\text{Fe}(\text{CN})_6$ RFBs, a promising candidate for the future of RFB technology.

Results: Key degradation products, including 2,6-dihydroxy-anthrone (DHA) and 2,6-dihydroxy-anthranol (DHAL), along with their stereoisomeric dimers, were identified and quantified [2]. A two-step electrochemical regeneration process was demonstrated to restore DHAQ, effectively mitigating capacity loss. Furthermore, the underlying mechanism of capacity loss and the processes preventing complete electrolyte regeneration were identified.

Conclusion: Real-time NMR monitoring directly observes electrolyte decomposition and charge transfer dynamics under operating conditions. Real-time Variable-temperature NMR reveals degradation mechanisms, capacity loss, and the structural characteristics of degradation species.

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Robust bilinear rotations and HUGE-BIRD

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Bilinear rotations form an essential tool in modern pulse sequence design as it allows the manipulation of spins based on their specific bilinear interactions. The original Bilinear Rotation Decoupling (BIRD) element [1] effectively applies a universal π -rotation when heteronuclear one-bond coupling is allowed to evolve and leave spins untouched for couplings close to zero. The main drawback of the BIRD element is that delay mismatch, and – more importantly – coupling variation within the sample, leads to dissipation of magnetization and this may make signals become uninterpretable and unphaseable. Using the COB approach [2], we optimized the BIRD element to be robust over Coupling, Offset and B1-variations into the new COB-BIRD. We apply this new sequence in a $\{^1\text{H}\}^{13}\text{C}$ 2D-INEPT type experiment on partially aligned (–)-nicotine with total coupling values ranging from 71 Hz to 253 Hz. We then design a new homonuclear BIRD into the Homonuclear Universal Gyration or Excitation Bilinear Rotation Differentiation (HUGE-BIRD) and apply this to an S/N optimized INADEQUATE sequence for effective ^{13}C - ^{13}C spin system selection.

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High Throughput in NMR: Rapid Sample Analysis using Localised Spectroscopy

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Screening in NMR spectroscopy is often limited by the time-consuming data acquisition even when the sensitivity of the experiments is high enough to allow the measurement of multiple experiments at once. Localised spectroscopy can address this challenge, by measuring several samples in parallel and thus enhancing the throughput of experiments.

In this work, we discuss the experimental protocols for 2D-MRSI experiments, including water suppression to acquire ^1H NMR spectra at 950 MHz (22.3 T) for a bundle of capillaries in a Bruker micro5 probe equipped with a 5 mm birdcage coil. Each sample holder contains up to 25 capillaries containing 1 μL of sample. In under one minute per sample, spectra for samples in the 5 mM range can be obtained with a resolution of better than 0.03 ppm.

Using this setup we investigate the efficacy of various thymidine phosphorylase (TP) inhibitors. TP is an enzyme which plays a key role in recycling DNA building blocks and is upregulated in many cancers. TP inhibition could disrupt the rapid replication of cancer cells, making it a target for therapeutic development. With this method, all considered TP inhibitors can be measured at once. Spectra for each sample are obtained making it possible to assess inhibitor potency based on chemical shift differences as small as 0.03 ppm. This small separation occurs between the substrate and product methyl peak, which serves as probe for the enzymatic activity. Furthermore, the half maximal inhibitory concentration – IC₅₀ values – for all inhibitors could be determined through a single experiment.

The presented work enables parallelised NMR measurements and rapid acquisition of NMR spectra, thus providing a powerful tool for large-scale screening of therapeutic candidates. The ability to measure a high number of samples in a short period, opens new possibilities for accelerating NMR-based assays in drug discovery if the sensitivity of individual experiments is high enough.



Optimal Control Pulses for Ultrawide Bandwidth NMR Using Seedless

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In high resolution NMR the inability to produce uniform excitation across a broad bandwidth hinders data acquisition. Whilst hard pulses can readily excite the entire ^1H spectrum, they can only cover a fraction of ^{19}F and ^{59}Co frequency space. Both CHORUS (Foroozandeh et al, 2019, JMagRes) and Seedless (Buchanan et al, 2025, accepted NatComms) were developed to improve bandwidth for ^{19}F excitation; however, since ^{59}Co has a 20000ppm chemical shift range, locating the peak of an unknown compound requires rastering across the entire spectrum. This process is both time and labour intensive, and so a method able to excite the whole frequency space aids rapid identification of unknown catalytic intermediates and counters broad linewidths not observable with high resolution spectroscopy.

In Seedless simulations, we worked to improve the fidelity of 5000ppm wide pulses, varying finite length, pulse duration, pulse power, and spin density. We tested the pulses on the ^{59}Co reference to ensure the simulations matched the data. Having determined that the main factors governing pulse fidelity were spin density and number of points, we expanded the spectral width to 15000ppm using a 10kHz power at 10ms duration on a 400MHz Bruker spectrometer with a room temperature BBFO probe. This pulse was verified by repeating pulse-acquire experiments, varying the carrier, and further validated by the simultaneous excitation of $[\text{Co}(\text{CO}_3)_3]^{3-}$ (14140ppm) and $[\text{Co}(\text{CN})_6]^{3-}$ (0ppm) in a single coaxial sample.

Our results demonstrate uniform excitation of a 1.5MHz bandwidth, corresponding to 75% of the ^{59}Co bandwidth. These pulses will aid in wide bandwidth excitation in both solution and solid-state NMR, with potential applications including mechanistic insights involving transition metals. Furthermore, our work here, exciting nuclei beyond the range of tuning and matching, has laid the groundwork for simultaneous single-channel multinuclear detection, and the targeting of hard-to-reach nuclei.



Phosphonate-coated Superparamagnetic Iron Oxide Nanoparticles: A promising Contrast Agent for Magnetic Resonance Imaging

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Contrast agents are often useful in magnetic resonance imaging MRI. Among them, those based on superparamagnetic iron oxide nanoparticles were previously used but were later withdrawn from the market due to both scientific-related and commercial concerns. These particles are primarily known to shorten the transverse relaxation time of water protons, resulting in a hypointense signal in MRI. This work presents ultra-small superparamagnetic iron oxide nanoparticles coated with a novel low-cost commercially available coating agent—methylene phosphonic acid. This coating agent significantly enhances particles' stability at physiological pH. We found that these particles also shorten the longitudinal relaxation time of water protons. Hence, hypointense or hyperintense signals can be achieved with these particles. Their efficiency (relaxivity) varies with magnetic field strength as described by nuclear magnetic resonance dispersion (NMRD) profiles. Here, this profile was assessed across a wide range of magnetic field strengths from 0.24 mT to 14.1 T to encompass all medically relevant magnetic fields. Furthermore, we analyzed the measured dispersion profile using quantum mechanical formulations and showed it can be used as a universal physicochemical characterization tool for such systems. A biocompatibility assessment through cytotoxicity assays, including LDH and MTT tests, revealed that these new phosphonate-coated superparamagnetic iron oxide nanoparticles are non-toxic.



Double-Quantum Excitation in Systems at Near-Equivalence

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Double-quantum coherence excitation via standard methods such as INADEQUATE [1] is inefficient in the near-equivalence limit, where the chemical shift difference between two spins is much smaller compared to the respective scalar coupling. This results in extensive signal losses due to T2 relaxation.

A recently devised [2] double-quantum excitation method, GEODQ, exploits the Aharonov-Anandan geometric phase, and has shown to be more efficient than conventional INADEQUATE, leading to stronger signals.

Pulse sequences such as SLIC [3] and PulsePol [4] have been used to access nuclear singlet order in the past. We adapt both methods, now SLIC DQ, and PulsePol DQ, to excite double-quantum coherences within an AB type chemically inequivalent two-spin system. In addition, we plan to explore the case of an AA'MM'XX' type six-spin system where the spins are chemically equivalent, but magnetically inequivalent.

We show that the GEODQ, SLIC DQ, and PulsePol DQ pulse sequences can be employed for efficient double-quantum excitation in the near-equivalence limit. Possible applications [5,6] include selectively observing double-quantum filtered signals from metabolites, providing scope for in vivo experiments, as well as for new contrast agents.

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Mixture analysis of synthetic (-)-cannabidiol derivative atropisomers using Viscosity-enhanced spectroscopy (ViscY) combined with dynamic NMR

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Complex mixture analysis by liquid-state NMR has been extensively studied for over 20 years. However, only a few ways have been explored, and the field still faces many challenges. The recent use of viscous solvents has led to the development of a promising Viscosity-enhanced-spectroscopy (ViscY) approach. This approach exploits the combination of viscous solvents and temperature control to reduce the molecular tumbling rate in solution. As a result, molecules exhibit a negative nOe regime, enabling their resonances to exchange magnetization through intramolecular spin diffusion. The 2D ^1H - ^1H NOESY spectrum under spin diffusion conditions reveals correlations between all ^1H resonances of each compound within the mixture, allowing the individualization of their individual 1D ^1H NMR spectra.

Dynamic NMR is a suitable technique for characterizing atropisomerism, which predominantly occurs in single bonds that link hindered planar groups. The kinetic and activation parameters associated with the chemical exchange process influenced by rotational barriers can be determined. The exploration of conformational control concerning $\text{C}(\text{sp}^2)$ - $\text{C}(\text{sp}^3)$ single bonds has been limited, primarily due to the infrequency of restricted rotation around these bonds.

We first combined spin diffusion phenomena and dynamic NMR to achieve alongside structural elucidation and the determination of kinetic and activation parameters for the restricted rotation around the Aryl- $\text{C}(\text{sp}^3)$ bond in synthesized (-)-cannabidiol derivatives, such as phenyl, naphthyl, and fluorinated derivatives. This procedure was conducted using the viscous binary solvent DMSO- d_6 /water.

This viscous blend solvent offered an extensive temperature range (213 K to 423 K) for dynamic NMR investigations. We conducted 2D ^1H - ^1H and 2D ^1H - ^{19}F EXSY experiments to ascertain the thermodynamic and kinetic parameters. Our findings indicated that phenyl and fluorine derivatives induced atropisomerism in contrast to the naphthyl derivative



Cost-Efficient Stable Isotope Labeling of Proteins Overexpressed in Mammalian Cells

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NMR for investigating protein structure and dynamics requires uniform ^{13}C and ^{15}N isotopic labeling. Preferably, the protein of interest is expressed in its native structure in eukaryotic cells. However, the production of proteins with high label incorporation in eukaryotic cells is challenging and costly. We have developed a cost-efficient media for the culture of mammalian cells using a bacterial protein hydrolysate and yeast autolysate. The media are based on stable isotope amino acids generated by lysing the protein fractions of *Cupriavidus necator* and *Saccharomyces cerevisiae*. Multiple passage and starvation protocols were used to optimize the isotopic purity and yield of overexpressed green fluorescent protein (eGFP) in HEK293 cells.

HEK293 cells cultured for several passages in ^{15}N SI Medium and standard DMEM/F12 medium resulted in similar growth and average doubling time. Furthermore, cell morphology and differentiation ability in ^{15}N SI Medium was comparable to DMEM/F12 medium. An average protein yield of 0.2565 ± 0.034 mg/ml was obtained after 5 passages in ^{15}N SI Medium, and 0.2841 ± 0.022 mg/ml in DMEM/F12 medium.

eGFP overexpressed in HEK293T cells cultured in ^{15}N SI Medium supplemented with ^{15}N -labeled glutamine resulted in $>90\%$ eGFP ^{15}N incorporation after one passage. In addition, we have developed an alternative, innovative protocol to enhance the isotopic enrichment of overexpressed proteins. Laboratory testing has explored strategies such as an 8-hour starvation period and a temperature shift to 33°C , both of which improved isotopic purity and protein yield.

'Silantes Isotope-labeled Growth Medium for Mammalian Cells' offers several advantages compared to commercially available eukaryotic cell media. It has a lower carbon footprint since only biological matter is used. Additionally, protein yields are comparable to those achieved with standard DMEM/F12 media, while high label incorporation rates of over 90% can be achieved.





Ultrafast T1-D NMR experiment

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Nuclear magnetic resonance (NMR) is capable of studying molecular motions through relaxation and diffusion experiments. These provide information on chemical and physical environments of molecules and their interactions. Relaxation and diffusion coefficient distributions can be extracted from measurement data with an inverse Laplace transform and thus these methods are collectively known as Laplace NMR (LNMR).

Multidimensional approaches increase the chemical resolution of NMR experiments. The downside of this approach is increased measurement times since the experiment needs to be repeated multiple times. This reduces the applicability of the approach for example with samples which deteriorate fast, giving only a small window for data collection. A current field of research in Laplace NMR is the development of faster measurement methods. A powerful solution to the problem is to introduce a spatial encoding of two-dimensional data. Gradients and frequency-swept pulses are used to encode the indirect dimension into different layers of the sample. This has led to ultrafast NMR spectroscopy [1] and ultrafast LNMR [2,3,4].

In this presentation we introduce an ultrafast spin-lattice relaxation - diffusion (T1-D) correlation experiment. We demonstrate that this experiment is capable of distinguishing molecules by their relaxation T_1 and diffusion D properties, especially in situations where conventional NMR spectroscopy is unable to do so due to for example overlapping spectra. This new experiment shortens the measurement time significantly while maintaining comparable data quality with conventional and slower 2D-experiments.

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Open-air green light ATRP in-situ monitoring and dispersity determination by Diffusion NMR

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Introduction

Atom Transfer Radical Polymerization (ATRP) with dual photoredox/copper catalysis is a powerful tool for obtaining good-quality polymers. The advantages of this method are high oxygen tolerance and the fact that the reaction runs under green light irradiation instead of UV light. The Time-Resolved Diffusion NMR is a perfect tool for studying this process.

One of the most crucial parameters to describe a polymer is Dispersity ($\bar{M}_w/\bar{M}_n$). It can be measured using NMR diffusion experiments, but for low $\bar{M}_w/\bar{M}_n$ samples, the high SNR is needed to calculate $\bar{M}_w/\bar{M}_n$ precisely. In this work, we show the simulation results of various acquisition strategies to circumvent this problem.

Aims

We want to monitor in-situ ATRP with dual photoredox/copper catalysis reaction using NMR spectroscopy and then measure the dispersity of the sample.

Methods

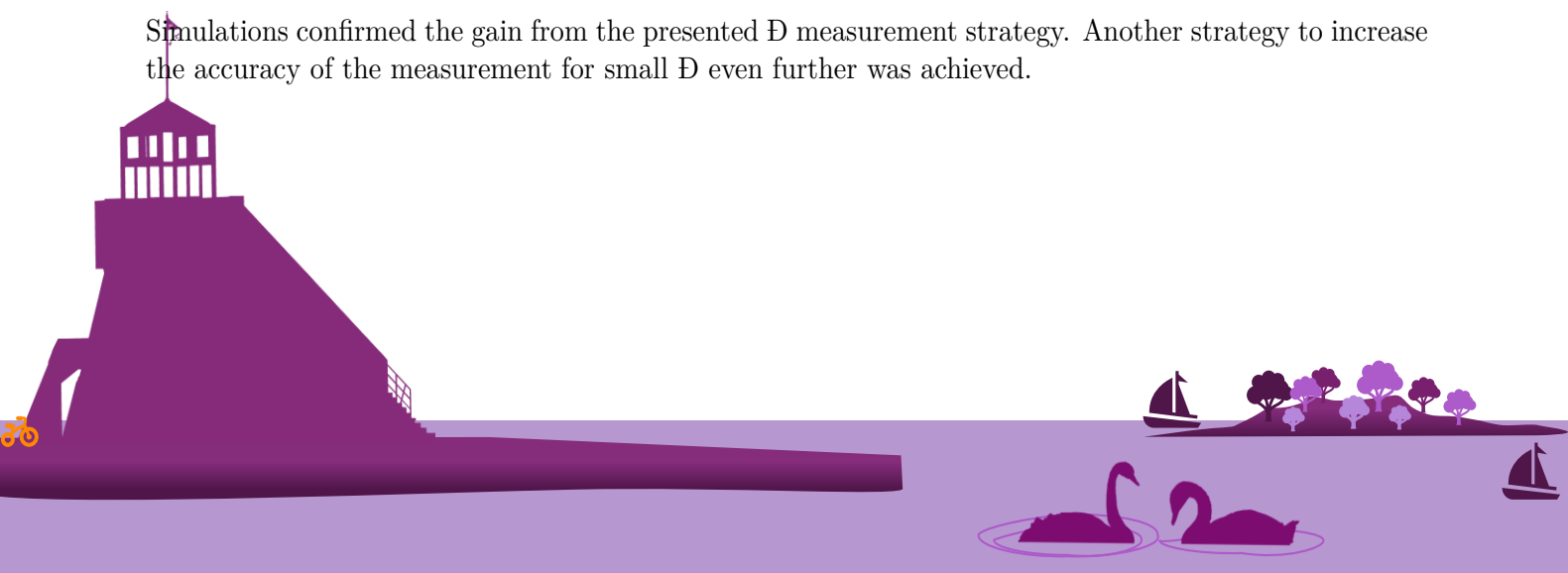
We performed the TR-DOSY interleaved with proton experiments on the sample illuminated in situ. The illumination was turned off automatically by an Arduino module after the reaction reached a given stage. When the reaction was finished, the dispersity of the sample was measured using a DOSY experiment in which the number of scans was adjusted to keep the noise in the spectra at the same level. The results were validated using GPC/SEC measurement.

The simulations of DOSY experiments were carried out to approximate the gain from the $\bar{M}_w/\bar{M}_n$ measurement method. A few strategies for measurements were tested in 100 000 simulations each.

Results and conclusions

During reaction monitoring, we obtained a series of spectra for samples of oligo(ethylene glycol) methyl ether methacrylate with final molar masses in the 25-400 kg/mol range. We demonstrated that the polymerization reaction can be monitored using the TR-DOSY. We obtained the molar mass evolution with conversion and time.

Simulations confirmed the gain from the presented $\bar{M}_w/\bar{M}_n$ measurement strategy. Another strategy to increase the accuracy of the measurement for small $\bar{M}_w/\bar{M}_n$ even further was achieved.



Long-Lived States Involving a Manifold of Fluorine-19 Spins in Fluorinated Aliphatic Chains

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We show here that long-lived states (LLS) of ^{19}F spins can be excited in achiral fluorinated aliphatic chains in solution. LLS lifetimes are measured in three per- and polyfluoroalkyl (PFAS) containing 2 or 3 consecutive CF_2 groups. The spin system can be denoted as $\text{AA}'\text{MM}'$ or $\text{AA}'\text{MM}'\text{XX}'$ in Pople's notation. Indeed, the vicinal scalar couplings are not identical ($\text{JAM} \neq \text{JAM}'$, $\text{JMX} \neq \text{JMX}'$, etc.) because the potential wells of the different rotamers that result from rotations about the C-C bonds are unequally populated [1]. LLS excitation is performed using polychromatic Spin-Lock Induced Crossing (poly-SLIC) by applying 1, 2 or 3 radio-frequency (RF) fields on resonance with chemical shifts of the CF_2 groups simultaneously to create delocalized long-lived populations [2]. Importantly, the amplitude of a SLIC pulse must match the sum of geminal couplings. These couplings manifest themselves as weak combination lines around the multiplet with the intensity of ca. 10^4 times weaker than those of the central transitions. We were able to measure geminal 2JFF-couplings accurately using an approach that enhances the combination lines intensities by up to two orders of magnitude in the studied cases [3]. The measured couplings fall in the range of 560 to 590 Hz. In a static magnetic field of 11.6 T, the lifetimes $\text{TLLS}(^{19}\text{F})$ of the long-lived states exceed the longitudinal relaxation times $\text{T}_1(^{19}\text{F})$ by about a factor 3. The lifetimes $\text{TLLS}(^{19}\text{F})$ are believed to be strongly affected by binding to macromolecules, a feature that can be exploited for screening of fluorinated drugs.

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Active Pharmaceutical Ingredients Analysis by ^{19}F - $\{^1\text{H}\}$ NMR using PBLG-based Liquid Crystals as Enantiodiscriminating Weakly Orienting Systems

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Molecular chirality is a concern in the pharmaceutical chemistry as enantiomers may have different bioactivities with potential toxic side-effects. There is, therefore, a need for analytical methods to determine the enantiomeric purity of a bioactive, chiral ingredient

In isotropic solution NMR, the most widespread and used method for enantiomeric discrimination relies on the use of chiral auxiliaries in interaction with the enantiomers of the chiral analyte, thus forming temporary or permanent diastereomeric clusters (1)

An alternative approach based on lyotropic chiral liquid-crystals (CLC) can be used for this purpose. In such CLC, enantiomers exhibit a difference of averaged molecular orientation with the magnetic field. This may induces spectral enantiodiscriminations through variations of residual anisotropic NMR interactions, such as chemical shift anisotropy (RCSA), dipolar coupling (RDC) and quadrupolar coupling (RQC) (2)

The analytical potential of such CLC has recently been highlighted by anisotropic ^{19}F - $\{^1\text{H}\}$ NMR to discriminate fluorinated drugs (e.g., Efavirenz and Flurbiprofen) with a routine NMR equipment. ^{19}F -RCSAs and (^{19}F - ^{19}F)-RDCs have enabled efficient enantiomeric discriminations and determination of enantiomeric excess (3)

We extend this methodology to more challenging drugs (hydrochloride or not) such

as Fluoxetine or Ofloxacin dissolved in a lyotropic CLC made of poly- γ -benzyl-L-glutamate (PBLG) with-weakly polar organic solvents. The importance of the experimental conditions (mesophase composition, sample temperature, ...) onto the resulting enantioresolution of the anisotropic ^{19}F NMR is explored and discussed in details (4), along with the influence of B_0 strength on the suitability of this approach

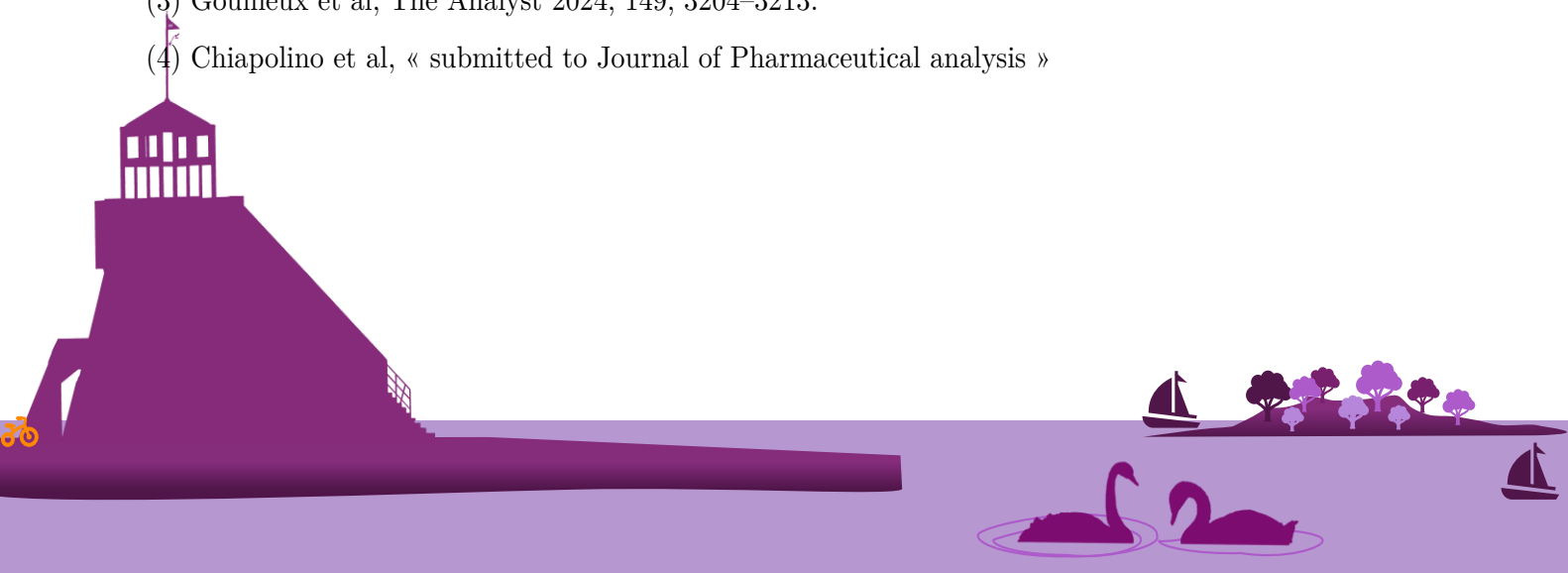
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The synergy of multiple receivers and heteronuclear detection to explore interactions involving intrinsically disordered proteins

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Intrinsically disordered proteins and intrinsically disordered regions of complex multidomain proteins are central to structural biology due to their crucial biological functions and involvement in diseases, making their characterization essential for biomedical research. However, conventional NMR experiments often fail to fully elucidate their interaction mechanisms.

Experiments focusing on protein backbones provide limited details on the actual interaction regions, whereas side chain nuclei are likely more informative, but more challenging to investigate with standard ^1H -based methods.

To overcome these limitations, we opt for the combination of heteronuclear direct detection with multiple receivers (mr_NMR).[1] The former ensures high resolution, crucial for the characterization of flexible regions. The latter allows to acquire one experiment during the recycle delay of a second one. Combining ^{13}C -detection with mr_NMR permits saving time and obtaining complementary information from different types of detected correlations.

Here I will focus on three key experiments providing different kinds of correlations through appropriate combinations of selected 2D experiments. The synergy of multiple receivers and heteronuclear direct detection will be highlighted through its application to biologically relevant examples.

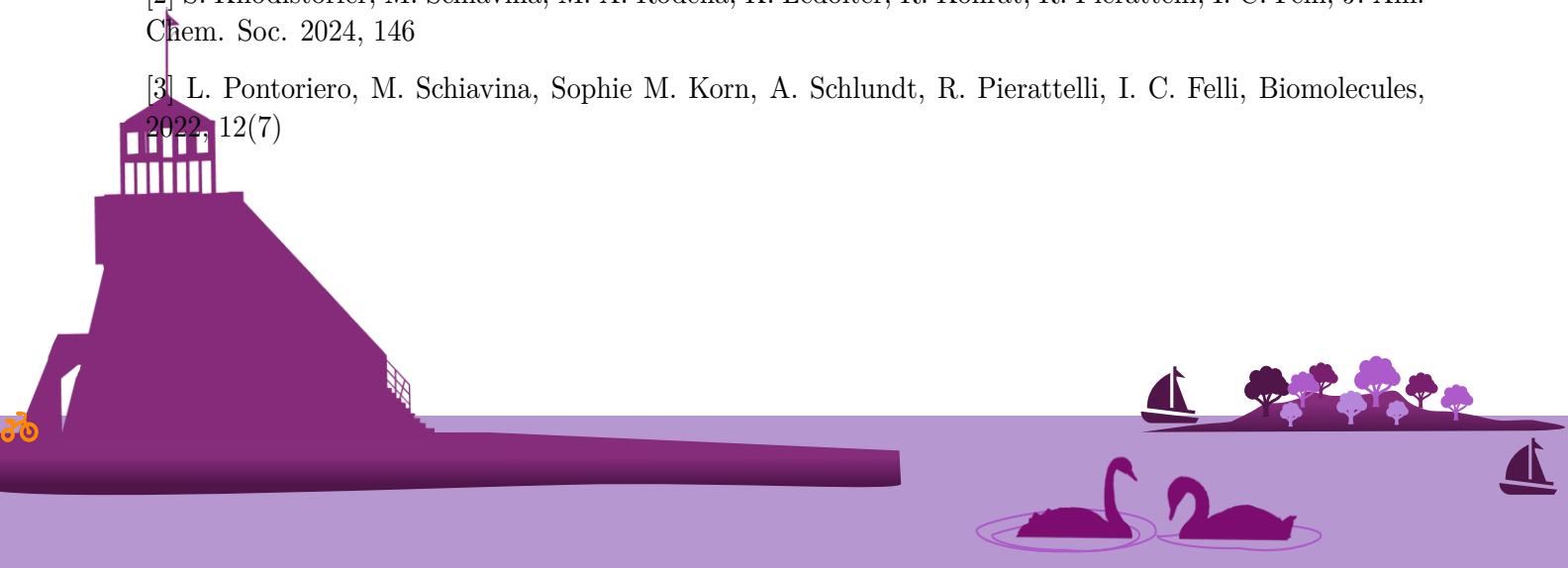
In particular I will present three examples that, starting from the investigation of backbone nuclear spins (mr_CON//HN[3]) gradually shift the focus to key side chain resonances to monitor the role of negatively and positively charged residues (mr_CACO//btHN[2], mr_HeN ϵ //C ζ N η).

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Unlocking New Capabilities of Electrophoretic NMR

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In view of the increasing importance and demand for electrochemical energy storage devices, research on electrolyte materials is essential. At that, electrophoretic NMR (eNMR) is an established technique to study migration velocities of individual species. In contrast to the related pulsed field gradient (PFG)-NMR, it directly reflects the situation in a battery where electric fields drive the migration of ions between the electrodes. This is achieved by the application of up to several hundred Volts through palladium electrodes directly above and below the NMR active volume. However, upon applying such high fields, problems can arise which include thermal convection by resistive heating, gas bubble formation, electrolyte decomposition and electroosmosis. Although certain precautions like the use of convection compensating pulse sequences enabled the successful application even in concentrated ionic liquids, especially the analysis of sample compositions combining high salt concentration and low viscosity remains unfeasible so far.

Herein, we present an innovative experimental setup which uses reference electrodes to not only enhance the precision of applied voltages but to also probe electrochemical processes at the electrode interfaces. As an example, we show by an analysis of the electrode capacitances taken from the voltage profiles, that electrochemical double layer break-down by electrolyte decomposition is crucial for measuring reliable electrophoretic mobilities. Furthermore, we directly connect different flux patterns - arising from the above-mentioned experimental artefacts - with their resulting NMR phase shifts and show how to possibly separate and compensate the individual effects. Our findings can therefore serve as a guideline to perform eNMR experiments on challenging samples and show how to spot and circumvent undesired artefacts. In the end, this might even unlock the so far unfeasible examination of highly concentrated water-in-salt electrolytes.



Single-Channel Multi-Nuclear NMR

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High-resolution NMR spectroscopy typically excites only the nuclei of a single isotope per channel. This is due to the narrow tuning and matching range as well as restrictions from the bandwidth of rectangular pulses. To bypass these limitations, we used Seedless (Buchanan et al, 2025) to design phase modulated optimal control pulses that simultaneously excite different regions of frequency space from a single channel and carrier frequency.

Initially, we used Seedless to design pulses that can excite multiple narrow bandwidths at a megahertz distance from the carrier frequency. We validated these pulses on a 400 MHz Bruker spectrometer with a BBFO probe. Here, we simultaneously excited discontinuous regions of frequency space corresponding to ^{13}C (100.6 MHz), ^{23}Na (105.8 MHz), and ^{59}Co (94.9 MHz) to produce a 1D spectrum with resonances almost 10 MHz apart. This multi-nuclear methodology enabled acquisition of different combinations of nuclei, including ^{25}Mg - ^{43}Ca - ^{37}Cl (24.5, 26.9, and 32.6 MHz) and ^7Li - ^{31}P (155.5 and 161.9 MHz). At this stage, the 1D spectral bandwidth was constrained by our detector, which could not acquire spectra wider than 10MHz.

We applied the multi-nuclear method to 2D data collection, including a ^1H - ^{19}F HSQC. Each ^{19}F pulse was designed to excite from the proton channel. We replaced the pulses in the first inept of the HSQC with only pulses from the proton channel and acquired an HSQC. This demonstrates excitation 24 MHz from the carrier frequency.

In the future, we envisage this development will enable multi-dimensional heteronuclear NMR data to be collected from a single channel. This will expand the scope of experiments that can be performed on a standard two channel BBO probe, including correlation experiments without proton. Furthermore, this has the potential to expand to other magnetic resonance methods, such as MRI, where Seedless methodologies could enable proton MRI machines to detect ^{19}F labelled probes.



Advancing GHz-class NMR: High sensitivity through large volume cryoprobe and optimal control sequences

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Improving the sensitivity of nuclear magnetic resonance (NMR) spectroscopy requires advancements in both instrument technology and experimental methodology. In this study, we introduce the first proton-detected large volume cryoprobe designed for 1.2 GHz instruments, leveraging optimal control pulse sequences to enhance performance. Our results demonstrate up to a 56% increase in sensitivity and more than a twofold reduction in experimental time compared to the small volume cryoprobes in use at the moment. Additionally, we systematically optimized the experimental conditions to fully exploit the capabilities of GHz-class magnets. To further extend the benefits of our approach, we developed a library of optimal control triple resonance experiments tailored for the large volume cryoprobe, enabling boosted sensitivity for advanced NMR applications.

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A singlet-filtered NMR method for analysis on complex mixture samples

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Proton nuclear magnetic resonance (^1H NMR) spectroscopy presents a non-invasive detection method for molecular structure elucidation and composition determination. However, ^1H NMR experiments generally confront with the challenge of spectral congestion in practical applications on complex mixtures. Herein, based on the signal filtering mechanism of endogenous spin singlet states, we propose a 1D general NMR method, termed SF-TOCSY, to overcome the challenge of spectral congestion and individually record the signals of targeted composition, thus suitable for high-resolution detections on complex chemical and biological mixture samples. Nuclear spin singlet states can be generated from a pair of coupled protons. According to the signal filtering characteristic of singlet states, we can selectively preserve the singlet state signals of the desired coupled proton pair and filter out other undesired background signals. Additionally, the TOCSY block is further combined with the singlet-filtered module in the SF-TOCSY to transfer the full correlation from the selected coupled proton pair to the whole molecule, thus applicable to component identification and mixture separation in complex chemical and biological samples. The applicability of the SF-TOCSY method is demonstrated by diverse practical samples, including biological samples of in vitro pig brain tissue suffering from intrinsic magnetic field inhomogeneity, human serum containing abundant metabolite compositions, and a protein molecule consisting of various amino acids. These experiments suggest that the proposed method allows one to perform targeted probing of chemical compositions for analyzing complex chemical solution mixtures containing crowded NMR resonances, even biological samples with abundant metabolites and intrinsic field inhomogeneity. Therefore, this method may provide a promising detection tool for component separation and determination in complex biological and chemical mixture samples.



Affinity Ranking of Ligands by ^1H NMR at 1.2 GHz: Targeting Long COVID-Associated Proteases

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Long COVID is suggested to be caused by an amplified activation of the complement immune system, leading to extreme fatigue and other symptoms that severely impact 5% of SARS-CoV-2-infected patients. Proteases Factor B and D play a crucial role in regulating this response and are therefore suitable targets for drug discovery. We aim at identifying and characterizing new ligands for these two proteases by combining ^1H - and ^{19}F -NMR fragment-based drug design (FBDD).

The recent introduction of 1.2 GHz high-field spectrometers opens opportunities for developing novel approaches for widely applicable ^1H -NMR, paralleling the advantages of ^{19}F -NMR in FBDD. The enhanced resolution at 1.2 GHz allows screening of larger mixtures, thus increasing throughput, and with perfect echo-based PEARLScreen experiments, high binding contrast can be achieved. For the estimation of affinity, we are developing ^1H CSAR – a generalization of the ^{19}F CSAR method for ^1H at high fields, allowing for efficient relative affinity ranking no longer limited to fluorinated ligands (CSAR: Chemical Shift-anisotropy-based Affinity Ranking). Additionally, estimates of dissociation rates (k_{off}) for ligands can be derived from PEARLScreen and $T1\rho$ experiments at high field. The two new affinity ranking methods will be compared with quantitative KD determination by conventional titrations. For the most promising ligands identified by these methods, protein-ligand complex structures will subsequently be determined. We intend to accelerate this process with the aid of deep learning-based analysis of the experimental NMR data.

By targeting Factor B and D, this project aspires to contribute to treatment options for Long COVID. The established efficient drug-discovery workflow, based on newly refined ^1H -NMR techniques, can eventually be applied to other medicinal targets, advancing early-stage drug discovery.



Measuring Translational Diffusion of Peptides and Molecules Encapsulated in Lipidic Cubic Phases (LCPs) Using Band-selective Excitation Short Transient (BEST) ^1H PGSE NMR

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Molecular translational diffusion properties not only provide insights into the physical and functional properties of molecules, but also yield an understanding of the morphology of the media through which the molecules diffuse. Over the years, Pulsed Gradient Spin Echo (PGSE) NMR has developed into an indispensable methodology for the experimental quantification of molecular translational diffusion both in vitro and in vivo. In the absence of isotopic labelling, translational diffusion measurement of peptides or molecules using conventional ^1H PGSE NMR in a membrane environment is, however, rather difficult due to the dynamic range issues caused by the intense ^1H resonance(s) of the lipids. The present study reports the use of a band-selective short transient (BEST) PGSE sequence,¹ which avoids the perturbation of ^1H spins of both lipids and hydration water, for measuring diffusion of molecules encapsulated within the lipid acyl chains and the aqueous channels of Lipidic Cubic Phases (LCPs).²

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Enhancing NMR Quantification: The Advantages of qHSQC for Complex Mixtures

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NMR-based dereplication is a powerful approach for identifying compounds in complex mixtures, enabling unambiguous structural elucidation through interpretation comparison of 1D and 2D spectra. However, a major improvement would be quantifying the constituents of natural extracts simultaneously. While ^1H 1D NMR are widely used for quantitative analysis, they are limited by peak overlap. Quantitative HSQC (qHSQC) addresses these issues by spreading spectral information over two dimensions, reducing overlap while maintaining high sensitivity. However, signal distortions can hinder quantitative accuracy due to relaxation effects, J-coupling modulation, and differential polarisation transfers.

This study aims to integrate qHSQC into the CaraMel workflow, an NMR-based dereplication method, enabling structural identification and accurate quantification of metabolites in natural extracts. Rather than developing new pulse sequences, we evaluated existing qHSQC experiments to determine the most accurate and reproducible technique. Acquisition and processing parameters were optimised to ensure robust quantification while maintaining reasonable acquisition times.

Preliminary results showed that quantitative HSQC required careful optimisation of key parameters such as J-coupling, T_1 relaxation times. Under optimised conditions, quantitative results were obtained for small aromatic compounds, confirming the potential of the qHSQC approach for reliable quantification in complex natural matrices.

Integrating qHSQC into NMR-based dereplication will provide quantification information in addition to structural elucidation, improving the reliability of metabolite identification. This advancement will have broad applications in metabolomics, pharmaceuticals, and food sciences, for which precise quantification of complex mixtures is crucial.



High-gradient NMR for understanding solvation patterns of alkali metals in ionic liquids

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Recent studies in sustainable chemistry demonstrate the exceptional ability of ionic liquids (IL) to form battery electrolytes with alkali metal halides (MX). Frequently, water is introduced as the secondary solvent to improve the solubility of the inorganic salts. To understand the corresponding solubility patterns and thus, further broaden the range of applications of such ternary IL-water-MX electrolytes, fundamental knowledge about the structure and composition of the M^+ solvation environment is needed.

This contribution reveals the benefits of using high-gradient nuclear magnetic resonance (NMR) spectroscopy to understand structural heterogeneities in IL-based electrolytes, especially targeting the first solvation shell of alkali metals. For the first time, self-diffusion coefficients of sodium cations in binary IL-water solvents are reported. Herein, they are directly determined using ^{23}Na pulsed field gradient (PFG) experiments. Due to the higher gradient strength (up to 35-fold) of the diffusion probe, it is more suitable to directly measure diffusion constants of low gamma nuclei like ^{23}Na , offering direct dynamic information.

The excellent agreement of the diffusion coefficients of all determined species allows to quantify the number of water molecules forming different environments, and assign them to either a systematically increasing water solvent network or polar nanodomains in the IL network. Finally, the first solvation shell of sodium is precisely characterized, including the ratio of water and BF_4^- ions as a function of time and water content.

The presented results not only provide new insights into structural heterogeneities in the modern ternary electrolytes but also benchmark high-gradient diffusion NMR as a methodology suitable for determining trends in the intramolecular interactions of low-gamma nuclei in the IL-water-MX solutions.



Managing an NMR Technical Service and Supporting Chemistry Research in Academia

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NMR spectroscopists can offer a tremendous amount of value to research across a broad range of disciplines. As NMR is such a flexible tool, it has vast applications from fundamental investigations of the physical properties of atoms to the study of entire biological systems. With a background of rigorous NMR training and an open mind, it is entirely possible for a researcher to transition from their niche or application specialty to supporting any field of scientific work. As to a hammer, everything is a nail; to an NMR spectroscopist, every problem is something that NMR can solve!

In this poster, I present a case study of my experience transitioning from a PhD student working in the field of biophysics to delivering an NMR service that supports fundamental chemical research. This experience highlights two main challenges everyone working to deliver a technical service such as NMR should work to overcome: knowledge and access. As someone with a specialism, you should be responsible for sharing what you have learned with others. NMR is often a topic taught on degree courses, but often it is not necessary to teach the topic beyond its applications focused on assignment. However, novel synthetic products are not always as simple as those presented in the classroom. Providing students with the knowledge to work with complex cases can really assist them in their research work. Alongside this, being conscious of seeking out researchers who could benefit from using NMR is crucial to developing a service. Researchers will not always come to you; being an active member of your research community is key to supporting access to the spectrometers in your research facility.

All of this is possible without letting go of your research aspirations. With the right approach, it is possible to remain an active researcher who contributes meaningfully through publication. If this resonates with you, a technical career may just be for you!



Slicing Solvent Signals Out of Mixture Spectra

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We present a method for improving solvent signal suppression in liquid spectra.

Solvent signals can be many orders of magnitude more intense than the resonances of analytes at mM or μM concentrations. Water is effectively 110 M in ^1H and can give rise to radiation damping and other problematic phenomena. This intensity disparity makes it difficult to detect and quantify the solute.

Presaturation by a low-power RF pulse lasting on the order of the solvent T_1 is the most common method of solvent suppression.[1] Such continuous wave irradiation is relatively straightforward to implement, requires no phase cycling, and is much more selective than methods using pulsed excitation such as WATERGATE.[2] Unfortunately, this very selectivity means that for the fringes of the sample where the B_0 field—and thus the Larmor frequency—deviate from the ideal, a residual “hump” of solvent signal will remain, and faraway signal can reside in the baseline.

Presaturation is commonly complemented by multiple hard pulses such as single-increment NOESY[3] or the specifically designed FLIPSY[4] sequence reduce signal from the sample fringes by exploiting the relatively weaker B_1 field in these regions. These methods of volume selection are “plug and play” but require phase cycling and leave the spectroscopist no room to tune the balance between suppression and sensitivity to the solutes.

We instead propose a very short slice-selective spin-echo to achieve suppression in a controllable active volume without the need for prescribed phase cycles. Our method enables reproducible tailored suppression for demanding applications like chemometrics, metabolomics and food analysis.

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Multidimensional NMR measurement by triple resonance with standard 2-channel system.

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[Introduction]

While ^1H and ^{13}C nuclei are widely measured by NMR, incorporating additional nuclei offers valuable structural insights. Traditionally, such triple resonance experiments require expensive three-channel spectrometers, becoming a significant barrier for many users. Our research addresses this limitation through the innovative Multi-Frequency Drive System (MFDS), which enables sophisticated triple resonance experiments on standard two-channel NMR systems without additional RF resources. The MFDS technology allows a single channel to control up to four independent frequency sources simultaneously. When integrated with our newly developed ROYALPROBE™ C+, this system assigns both ^{13}C and X-nuclei (such as ^{31}P , ^{29}Si , or ^{15}N) frequencies to a single low-frequency channel. The combined radiofrequency output is precisely separated by a diplexer system that directs each component to the appropriate probe port, while the high-frequency channel maintains ^1H functionality. As a result, this configuration achieves a triple resonance circuit with two-channel systems.

[Experimental Validation and Applications]

We validated this approach using a standard JEOL JNM-ECZL500R two-channel spectrometer equipped with the ROYALPROBE™ C+. Our experiments encompassed various nuclear combinations. These demonstrated that our system achieves performance comparable to dedicated three-channel spectrometers.

[Conclusion]

The MFDS approach represents a significant step toward making advanced NMR techniques more accessible. This advancement has the potential to significantly expand the application of multinuclear NMR as a routine analytical tool across diverse research environments. The flexible architecture of the MFDS system further suggests opportunities for continued innovation in pulse sequence development and additional multinuclear applications, promising ongoing expansion of NMR capabilities on standard instrumentation.



Study of photochromism by combined in situ UV-vis/NMR spectroscopy

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The opportunity of in situ investigation of photochemical reactions using NMR has been attracting interest in such fields as photocatalysis, molecular machines, and protein dynamics. The first convenient in situ light irradiation setup was introduced in 2013 by the Gschwind group and has found wide application since. A further significant extension was the introduction of the combined UV-visible/NMR spectroscopic technique, which was recently improved by the Thiele group.

In this work, we present a practical application of this combined setup linking optical properties to chemical structure evolution and allowing the optimization of the photoreaction conditions.

The model compound for this study, benzylidene-thiazolidine-2,4-dione (BTZD), is a glitazone derivative that exhibits P-type photochromism and can undergo $Z \rightarrow E$ photoinduced isomerization upon irradiation in the UV range. Like in many other photoswitches exhibiting cis-trans isomerism, the absorbance spectra of (E)- and (Z)-BTZD overlap, which hinders quantitative isomerization and prevents the acquisition of the pure photo-product absorbance spectrum.

To overcome this limitation, we employed the in situ UV-vis/NMR setup to characterise the photostationary states (PSS) and acquire photoisomerization kinetic profiles from the simultaneously measured NMR and absorbance spectra. Comparing the results obtained using the in situ setup with regular ex situ measurements, we demonstrate that the collective in situ data allows the determination of the pure photoproduct absorbance ((E)-BTZD) and can be used for irradiation wavelength optimization.



Experimental Liouvillian Tomography

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Spherical tensor analysis (STA) enables decomposition of the NMR signal into components, which pass through different irreducible spherical tensor operators (ISTO) at any point during an experiment. Such a decomposition is achieved by performing multiple experiments different only in a single pulse sequence element accomplishing a rotation of the spin polarisation through a predefined set of Euler angles.

The main limitation of STA in its original implementation is the large number of experiments required to separate all spherical signal components at the same time. However, often one is only interested in the evolution of a smaller set of operators, allowing the number of experiments to be significantly reduced. Notably, the coherence order of the spin operators remains unchanged under free evolution, within the secular approximation. In multi-spin systems, there may be multiple different operators with the same spherical rank and component, hence, further separation is required to determine the complete state of the system following an arbitrary preparation sequence, a technique generally referred to as density matrix tomography (DMT).

An extension of STA to complete DMT is demonstrated on a strongly coupled two spins-1/2 system and applied to follow its time evolution following multiple preparation sequences. The small number of experiments required DMT permits multiple decomposition steps within a sequence, and thus, measurement of all cross relaxation curves between operators of the same coherence order. Through relaxation measurements of a complete set of operators (16 for two spins-1/2), numerical minimisation enables a purely experimental and model free reconstruction of the complete Liouvillian including both its coherent and incoherent contributions.



Interplay between polymer dynamics and dynamic reaction networks

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Learning from life, dynamic combinatorial chemistry (DCC) facilitates spontaneous emergence of advanced chemical structures and functions from its dynamic covalent/non-covalent reaction networks. However, unlike living organisms where the dynamics of biomacromolecules dictate biological structures and functions, the macromolecular dynamics had been under-explored in DCC.

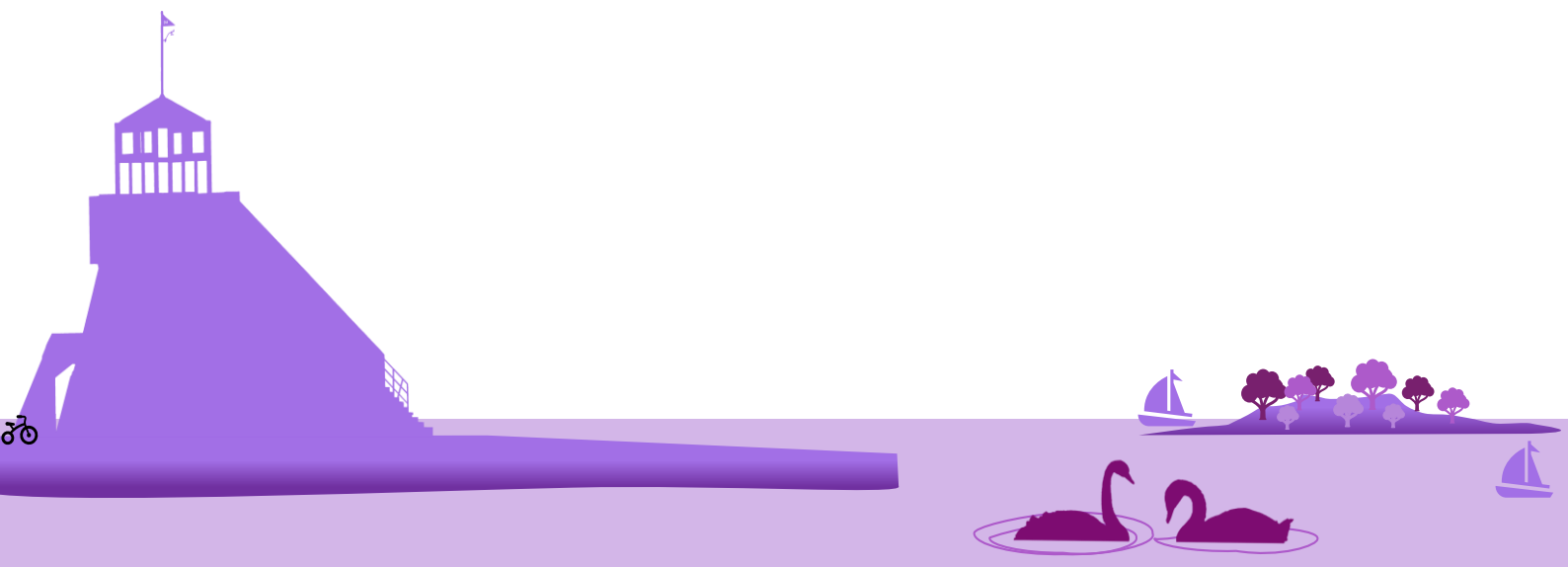
In this study, for the first time, we studied the interplay between polymer dynamics and DCC. Polymer templates were complexed with dynamic combinatorial macrocyclic disulfides (DCMs) via supramolecular interactions, which initiated polymer dynamics and associated control on chemical structure and function. Diffusion/relaxation NMR was used to monitor the polymer dynamics, while HPLC-MS analysis was synchronized to track the corresponding changes in DCMs' chemical exchange.

In one scenario, the polymer-DCM complexation initiated single-chain folding. The DCMs then adaptively restructured in real-time during the folding process, catalyzing it through kinetic intermediates until stabilizing it at the energy minimum, similar to how molecular chaperones assist in peptide folding. This folding could further re-adapt for building high drug-loading content DDSs against drug-resistant cancer.

Conversely, in another scenario, the polymer-DCM complex underwent entropy-unfavourable conformational extension. To compensate for the entropic loss, the DCMs reshuffled into weak-binding species that move fastest when bound to the polymer, resulting in dynamic high-entropy supramolecular self-assembly.

Lastly, a weak-binding kinetic intermediate of DCM initiated polymer dynamics from the shell of polymersome that overcame strong electrostatic and steric repulsion to mediate polymersome fusion, which was akin to the SNARE protein machinery responsible the biological membrane fusion.

Our findings advance DCC to a new stage that utilize polymer dynamics to control the structural and functional emergence.



Fast and flow-compatible 3D diffusion NMR

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Reaction monitoring is essential for studying kinetics and gaining mechanistic insights. Nuclear magnetic resonance (NMR) monitoring provides important chemical information. In particular, using flow NMR to monitor reactions allows chemical synthesis to be carried out with sampling and analysis of the reaction mixture in real time.

Diffusion NMR is an important method for mixture analysis and has been used to monitor reactions. It can help to separate NMR information from different compounds, relying on their different diffusion rates. Classical diffusion-ordered spectroscopy (DOSY), achieved by univariate analysis of signals in 1D spectra, is limited by peak overlap and does not result in "clean" spectra and correct diffusion coefficients. In addition, 1D spectra do not always provide sufficient information, such as correlation information, which is required for molecular structure analysis. For these reasons, diffusion-weighted 2D NMR can be helpful, but in a reaction monitoring context, the experimental time needs to be sufficiently short compared to the reaction time scale. Among existing fast 2D NMR methods, ultrafast (UF) COSY has already been used to monitor reactions. While diffusion-weighted UF COSY has been demonstrated, it is not compatible in its present form with flow NMR because of the lack of solvent suppression and because the method is highly sensitive to motion.

Here we present the convection- and flow-compensated 3D UF COSY DOSY experiment, with the possibility of solvent suppression. Convection compensation was achieved by introducing double-diffusion encoding into the UF 2D pulse sequence with the water suppression enhanced by T1 (WET) block. Diffusion and spatial encoding were performed using gradient axes that are orthogonal to the main direction of the flow. A full 3D data set was acquired in only 3 minutes. The NMR experiment was tested on the mixture of alcohols and L-valine and is promising for analyzing reaction mixtures by flow NMR.



Fully automated analysis of photo-CIDNP NMR spectra for fast fragment screening

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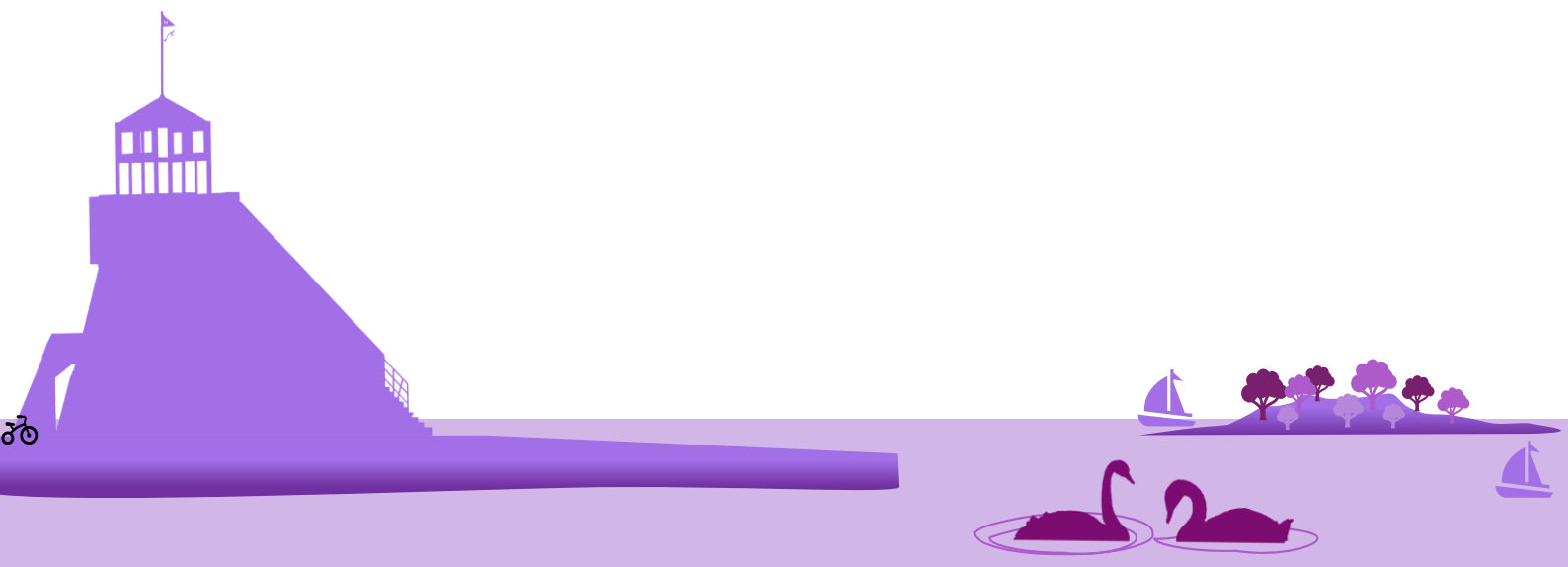
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Recently, photochemically induced dynamic nuclear polarisation (photo-CIDNP) demonstrated its ability to accelerate dramatically NMR-based fragment screening (Torres et Al., J. Am. Chem. Soc. 2023, 145).

Photo-CIDNP NMR can detect weak protein-ligand interactions in aqueous solutions in a low stoichiometric regime, with concentrations of 10 μM ligand and 1-2 μM protein, compared to standard concentrations of 50-200 μM ligand and 10-20 μM protein. The interaction detection is ultrafast: sufficient signal-to-noise is achieved within 2 to 5s, i.e., with a single-scan NMR experiment. However, the advantage of such an acceleration in data production is hindered by a much slower manual data interpretation, remaining as a bottleneck for the successful employment of photo-CIDNP techniques in drug discovery.

To address these issues, we developed a fully automated and expert-free pipeline to identify the occurrence of protein-ligand interaction from photo-CIDNP NMR spectra. Our method combines spectral data and molecular structure knowledge to detect hyperpolarized signals and assign them to the corresponding atoms. Comparing ligand-only and ligand-protein spectra, we are able to automatically recognize and quantify hyperpolarised signal reduction upon binding a small molecule to the target.

This chemical shift-specific signal reduction is then projected onto the ligand structure to produce a binding epitope mapping. The analysis of this epitope mapping classifies ligand-protein pairs as either hits or non-hits, accelerating the identification of potential binders. With our method, data analysis was reduced from days of manual work to minutes of automatic processing.



Ligand Screening Beyond T1 ρ : Reducing the Protein Consumption by One Order of Magnitude with the PEARLScreen Experiment

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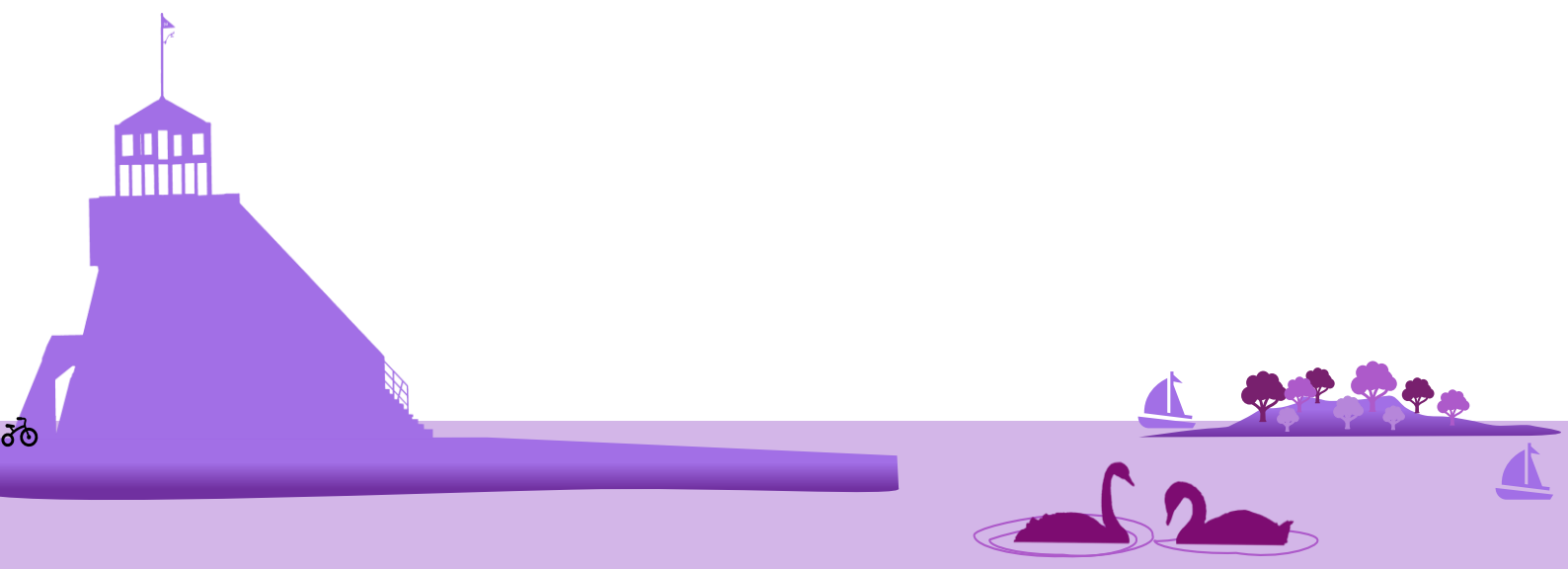
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For initial hit identification in fragment-based drug design, NMR is one of the preferred methods. Still, new screening experiments with higher binding sensitivity are of continuous interest to e.g. reduce the protein consumption or identify ligands for small targets or even intrinsically disordered proteins. Here, we present a more sensitive alternative to T1 ρ , waterLOGSY and STD for ¹H-observed screening: the PEARLScreen.

The PEARLScreen exploits transverse relaxation (R2), like the T1 ρ experiment, and, additionally, exchange effects (Rex) to generate strong binding contrast. Unlike for ¹⁹F, this cannot be achieved by simple CPMG experiments without severely distorting line shapes due to unrefocused homonuclear couplings. Instead, we employed a perfect echo CPMG sequence. As a result, the effect of homonuclear couplings is suppressed, exchange is active, and the length of the relaxation periods can be chosen > 1 s to generate maximum contrast.

We tested our new experiment on 4 different protein targets ranging in size from 12 to 108 kDa. The PEARLScreen allowed a reduction of the protein concentration by up to one order of magnitude compared to the conventional experiments on the same screening setup without losing binding sensitivity. Tests were performed in a magnetic field range between 80 and 1200 MHz, where at the highest magnetic field, hits could be identified in mixtures of 16 compounds with only 1 μ M protein. This corresponds to a 40-fold reduction of the required protein amount resulting from further boosted exchange effects at ultra-high fields.

With the PEARLScreen, we provide a tool to significantly reduce the protein consumption of screens and make use of exchange effects in ¹H-observed screening experiments for the first time. Therefore, we expect it to become the standard experiment for ¹H-detected ligand screening.



NMR of H₂@C₇₀ & 2(H₂)@C₇₀ Endofullerenes in a Magnetically Aligned Liquid Crystal Solution

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Endofullerenes are supramolecular complexes which consist of a small (endohedral) atom or molecule that is confined within a cage of consisting of only carbon atoms (fullerene) [1]. In this work we present a ¹H solution state NMR study performed on the endofullerene complexes H₂@C₇₀ & 2(H₂)@C₇₀, dissolved in the liquid crystal N-(4-Methoxybenzylidene)-4-butyraniline (MBBA). This expands upon previous work conducted on endofullerenes in MBBA using the highly symmetric C₆₀ fullerene (H₂@C₆₀, H₂O@C₆₀ and HF@C₆₀) [2, 3]. By using C₇₀ as our confining fullerene, the symmetry of the system is lowered. The ellipsoidal C₇₀ fullerene can align with the liquid crystal matrix (the icosahedral C₆₀ cannot) imparting preferential ordering of the fullerenes with respect to the magnetic field. Analogous to the case presented here: EPR measurements of N@C₇₀ solution in MBBA liquid crystal display much larger residual dipolar couplings (zero field splittings) than N@C₆₀. [4, 5]

In the case of H₂@C₇₀ we observe two peaks due to one H-H intramolecular residual dipolar coupling. The addition of second hydrogen molecule within the fullerene results in a total of eight peaks for 2(H₂)@C₇₀, due to the intra- and inter-molecular residual dipolar couplings. Observing the 2(H₂)@C₇₀ ¹H NMR spectra requires spectral filtering techniques, as the liquid crystal displays very broad NMR signals in the nematic phase.

We intend to unify our analysis with results from inelastic neutron scattering, aiding in the determination of the translation-rotational states of the endohedral molecules. As each H₂ can exist as two spin isomers (ortho & para), in a state with a total nuclear spin of either 0 or 1, the bis 2(H₂)@C₇₀ species displays rich nuclear spin isomerism structure.

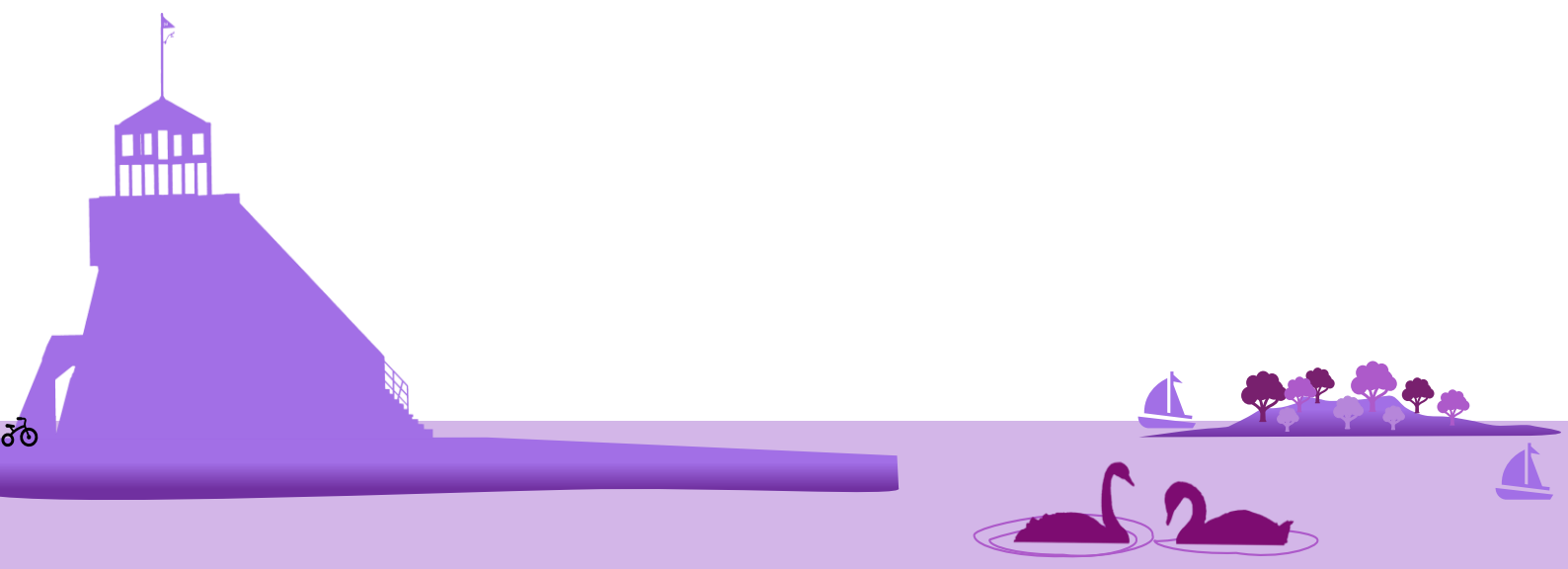
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Photodegradation study of retinylacetate using time-resolved diffusion and NUS HSQC NMR

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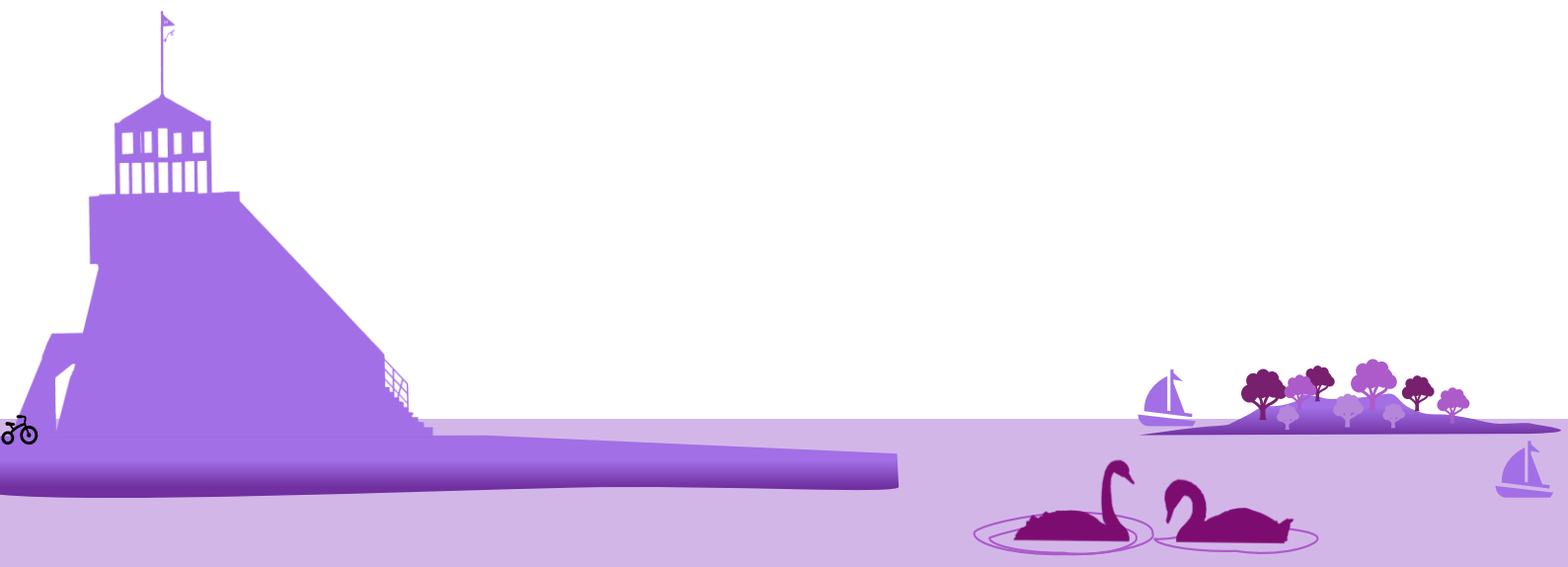
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Aim: Retinyl acetate (RA), an ester of retinol and acetic acid, is a synthetic form of Vitamin A widely used in pharmaceuticals, cosmetics, food fortification, and animal nutrition. However, RA is highly susceptible to photodegradation under sunlight, forming potentially carcinogenic products. Studying its photodegradation is therefore crucial. Chromatographic studies have reported various photo-isomers and dimers, including anhydro retinol (AR), retinyl methyl ether (RME), and other dimeric products in alcoholic solutions. However, NMR-based investigations remain limited. This study explores RA photodegradation using in situ NMR under UV illumination, acquiring time-resolved spectra.

Methods: A snapshot of the process using 1D NMR spectra is the simplest approach for time-resolved studies. However, in this work, we simultaneously acquired two novel time-resolved NMR experiments: TR-DOSY and TR-NUS HSQC. In TR-NUS HSQC approach, sampling is performed according to a NUS schedule in parallel to the chemical reaction. Acquired data are divided into overlapping subsets to form a stack of 2D sets and the reconstruction of missing points is performed. In case of TR-DOSY the similar concept is applied, but with varying pulsed field gradient strength.

The idea of interleaved acquisition datas are collected by jumping between the 2D experiments and 1Ds. The datasets are separately processed and form a stack of spectra allowing to reflect the sample changes in various kinds of spectra.

Conclusion: TR-DOSY provided average diffusion coefficients for all isomers and dimers, while TR-NUS HSQC allowed individual isomer monitoring. Notably, both methods yielded highly consistent average mass calculations, validating HSQC peak assignments. Additionally, HR-MS confirmed the formation of expected isomers and dimers.



Faster and SHARPER fragment scoring

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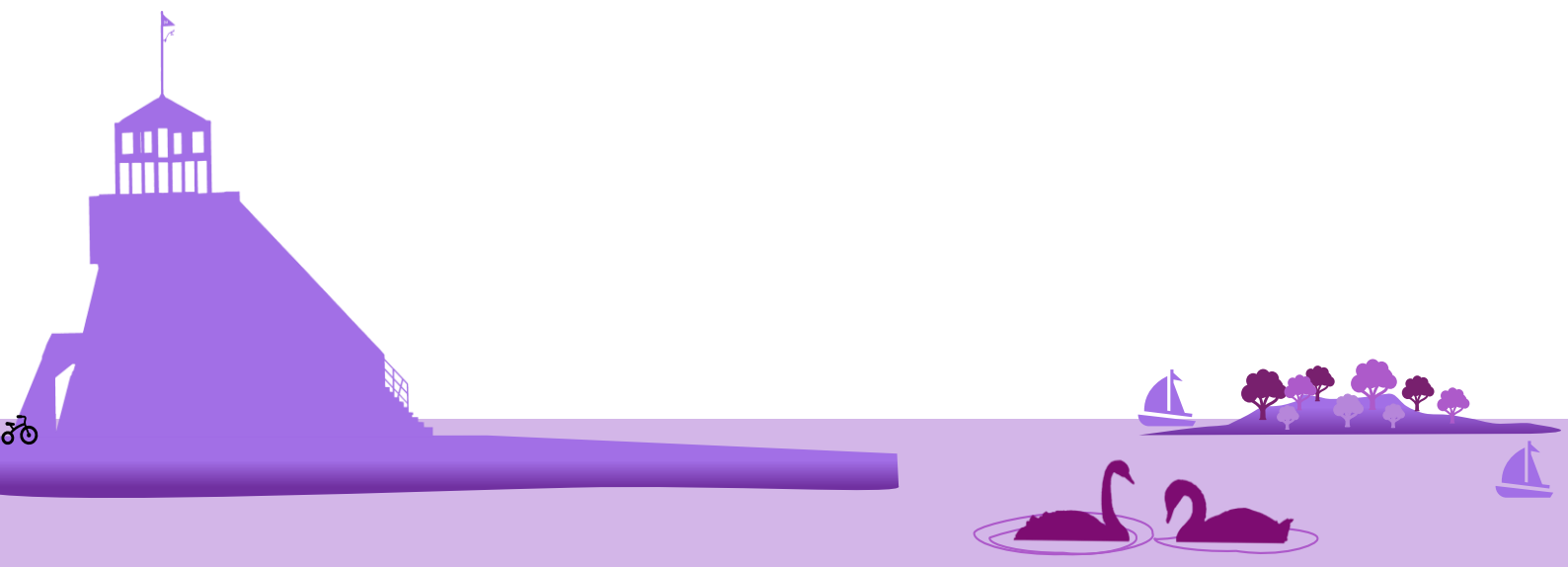
Aims. Fragment-Based Drug Discovery (FBDD) is a powerful strategy, which involves screening of molecular fragments against target proteins. NMR spectroscopy is well suited for this task. However, while NMR is very effective in the initial screening, follow-up NMR experiments to measure KDs, and thus score the fragments, are labour-intensive and time-consuming. This creates a bottleneck in the fragment screening.

Methods. The choice of NMR as the go-to technique for a range of chemist relies on the presence of the chemical shift dispersion and J-couplings in ¹H NMR spectra. However, there are instances, where such information is not required. Removing J splitting of a selected signal e.g., in reaction monitoring (1) or collapsing the whole or a part of the spectrum e.g., in DOSY spectroscopy (2), can lead to signal-to-noise ratio gains of 1-2 orders of magnitude, producing dramatic savings of the material and/or spectrometer time.

Results. Implementing the SHARPER methodology (1-3) into the ligand-observed NMR experiments and combining it with machine learning techniques, can dramatically increase the speed of fragment scoring. Our ML-Boosted ¹H LB SHARPER approach (4) allowed us to determine KDs for up to 144 fragments in 24h on a 600 MHz QCI cryoprobe and our set up. This compares very favourably to only around 5 KDs when using traditional approaches, which also require much more material.

Conclusions. The new fragment scoring methodology will significantly accelerate the drug discovery process by quickly and reliably scoring hundreds of fragments, thus allowing informed decision-making and faster progression from hits to the lead compounds.

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When NMR data is truly big: transformers redefine structure elucidation for small molecules

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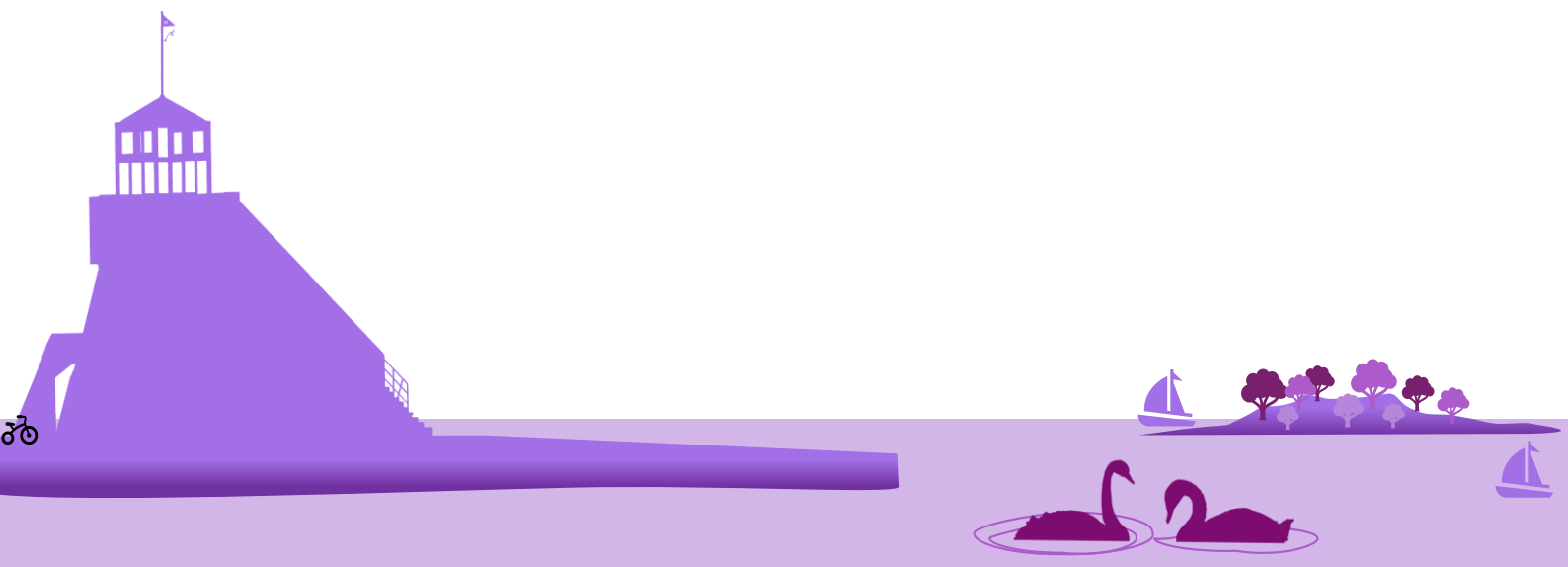
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NMR spectroscopy has a long history in organic chemistry, with millions of spectra to appear in chemical literature and numerous attempts to automate spectral interpretation. Despite this, NMR-based structure elucidation for organic molecules is a challenge that is yet unresolved. While 2D NMR data can be of aid through comprehensive spectral analysis, in many cases - when only 1D spectra are available - the process is still performed manually, leading to a significant time expenditure by experts in industry and academia.

Lately, machine learning, especially generative modeling, has seen remarkable progress, largely driven by transformer-based architectures. Recent efforts to apply transformers for organic structure generation from NMR spectra have shown promise, yet current models still struggle with accuracy. One of the reasons behind it is the scarcity of high-quality training data - an issue to which transformers are particularly sensitive.

To address this issue, we proposed using OdanChem, the largest publicly available database of NMR spectra, to create a transformer-based model for predicting molecular structures solely from 1D ^{13}C NMR spectra. Our approach mirrors the reasoning of an expert spectroscopist by first identifying the key molecular fragments based on the spectrum and then assembling them into a complete structure. Unlike traditional manual interpretation, our model leverages an extensive corpus of spectra from the global chemical literature, significantly improving the prediction accuracy while operating within seconds. This suggests the routine NMR interpretation to soon become fully automated, finally relegating manual spectral analysis to historical textbooks.



NMR in photochemistry – Light-induced selectivity in an exemplary photodimerization reaction.

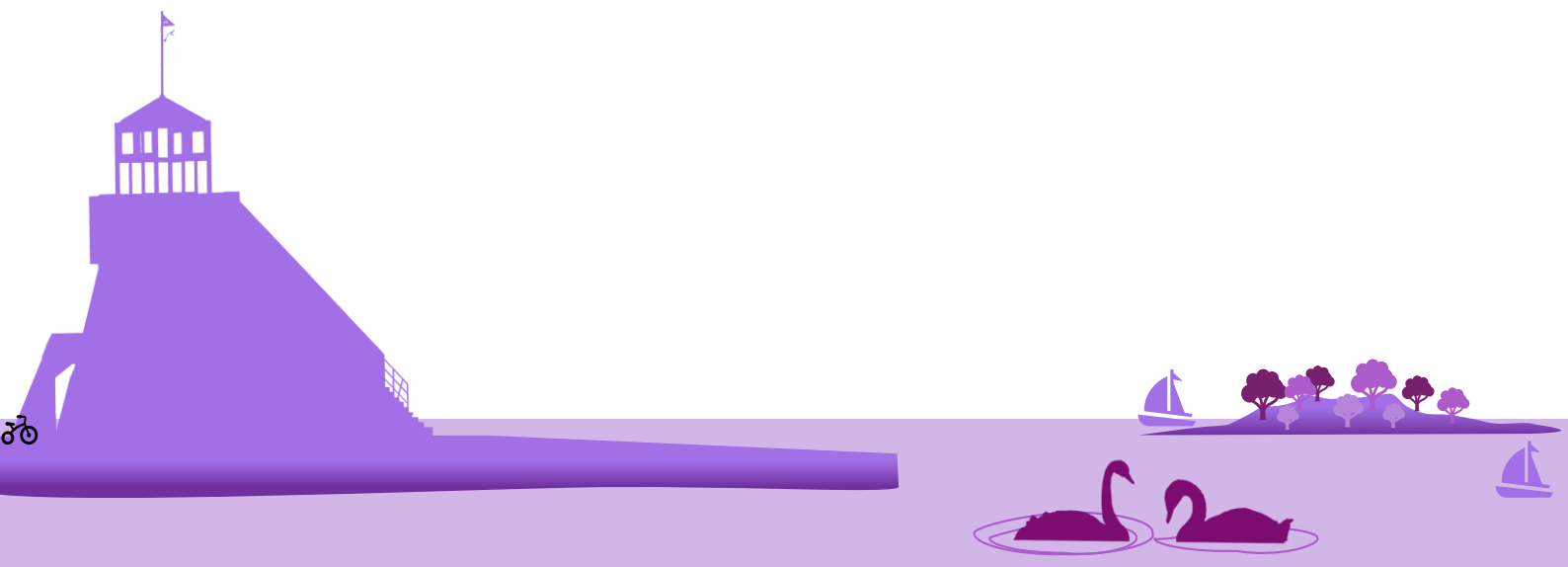
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Light-driven chemical reactions are gaining more and more interest. This includes photocatalytic processes, photodimerization, and photopolymerization. Regiocontrol in such reactions is a key factor, especially if many different photoproducts can be formed. Here, the photodimerization involving 2-azaanthracene (A) and N-methyl-2-azaanthracene (M) is considered. The photoreaction of pure compounds led to mixtures of 4 and 3 regiomers for A and M, respectively, contradicting the literature data. The irradiation of a mixture of only two substrates, A and M, can lead to 12 different products: 4 AA, 4 MM, and 4 AM dimers. Using ^1H NMR monitoring, as well as 2D spectra, we were able to identify all the photoproducts. More importantly, we reduced the number of products by adjusting the irradiation wavelength and the solvent. We have determined the photophysical parameters of A and M and measured the quantum yield of photodimerization using UV-vis spectroscopy. The quantum-chemical calculations in the excited state allowed us to propose a plausible explanation for why only two AM dimers are formed upon irradiation. The presented results indicated that photodimerization among various molecules can be partially controlled, significantly improving the photoreaction selectivity, and NMR monitoring plays a key role here.



NMR-Challenge.com: Shape Your Skills in NMR Assignment

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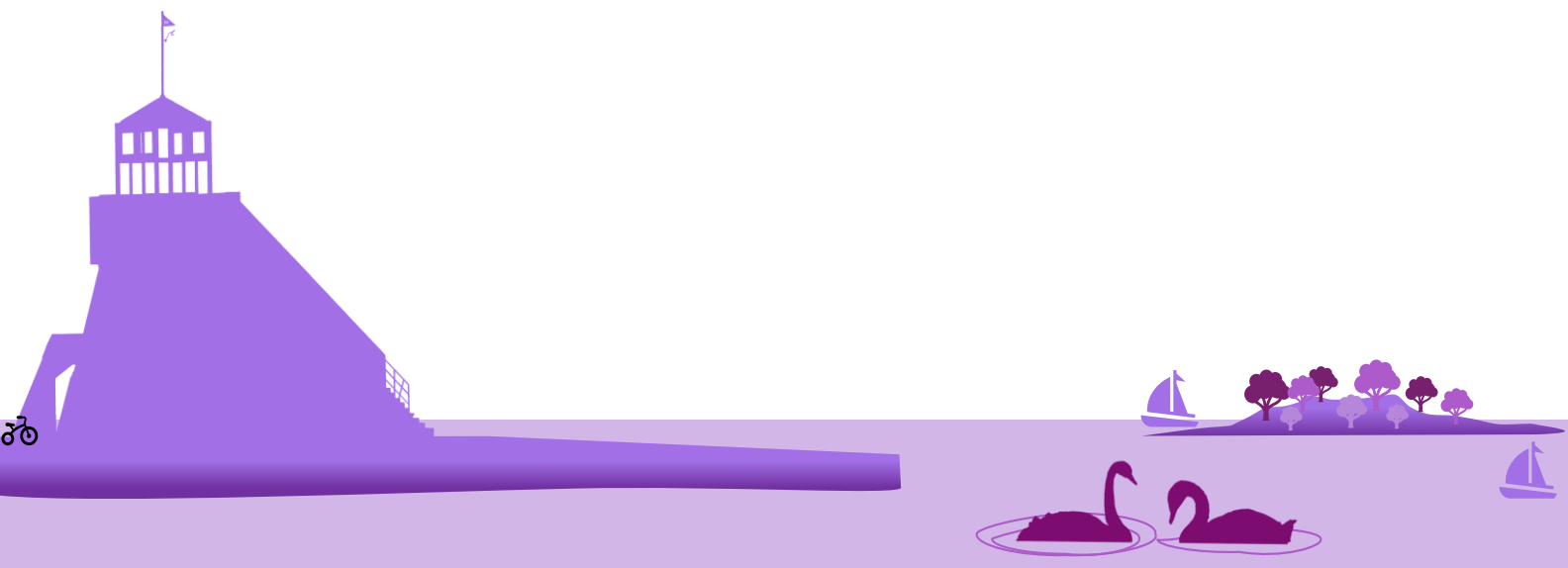
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We present NMR-Challenge.com, an educational NMR website with 200 spectral assignments measured for real samples. Each task contains NMR spectra of an unknown compound, and the students are to determine its structure. Basic assignments contain only 1D ^1H , and ^{13}C NMR spectra; advanced assignments also offer 2D correlation spectra. The web application is enriched with an interactive chemical structure drawing tool, giving users immediate feedback about their proposed structures. Since its introduction in April 2022, NMR-Challenge.com has received over 1,000,000 submitted solutions, making it the most robust dataset of students' responses in the field. The enormous number of students' responses enabled us to observe the current state of NMR knowledge. We identified patterns of the most common mistakes, which might help devise teaching strategies. We analyzed the most abundant spectral misinterpretations: recognition of isomeric esters, the benzene ring substitution, and the influence of an intramolecular interaction on chemical shifts. We also introduce the site's new feature – NMR-Quiz. This tool covers core NMR topics, e.g., structure determination from the limited number of signals, signal assignment, J-coupling, chemical shifts, choice of experiment, etc. The users choose from two to four displayed answers and get an immediate response whether their choice is correct. They also can track the success rate across the topics. The NMR-Quiz is also suitable for touchscreen devices, easing accessibility. NMR education is crucial for attracting newcomers and growing the scientific field. Here, we show how our NMR educational package can help in self-studying and frontal teaching. In addition, we present guidelines for NMR frontal lecturers benefiting from our robust analysis of the most common spectral misinterpretations.

Socha, O.; Osifová, Z.; Dračínský, M., J. Chem. Educ. 2023, 100, 962-968.

Osifová, Z.; Socha, O.; Dračínský, M., J. Chem. Educ. 2024, 101, 2561-2569.



Detection of Pyruvate-Protein Interactions at Low Micromolar Concentrations

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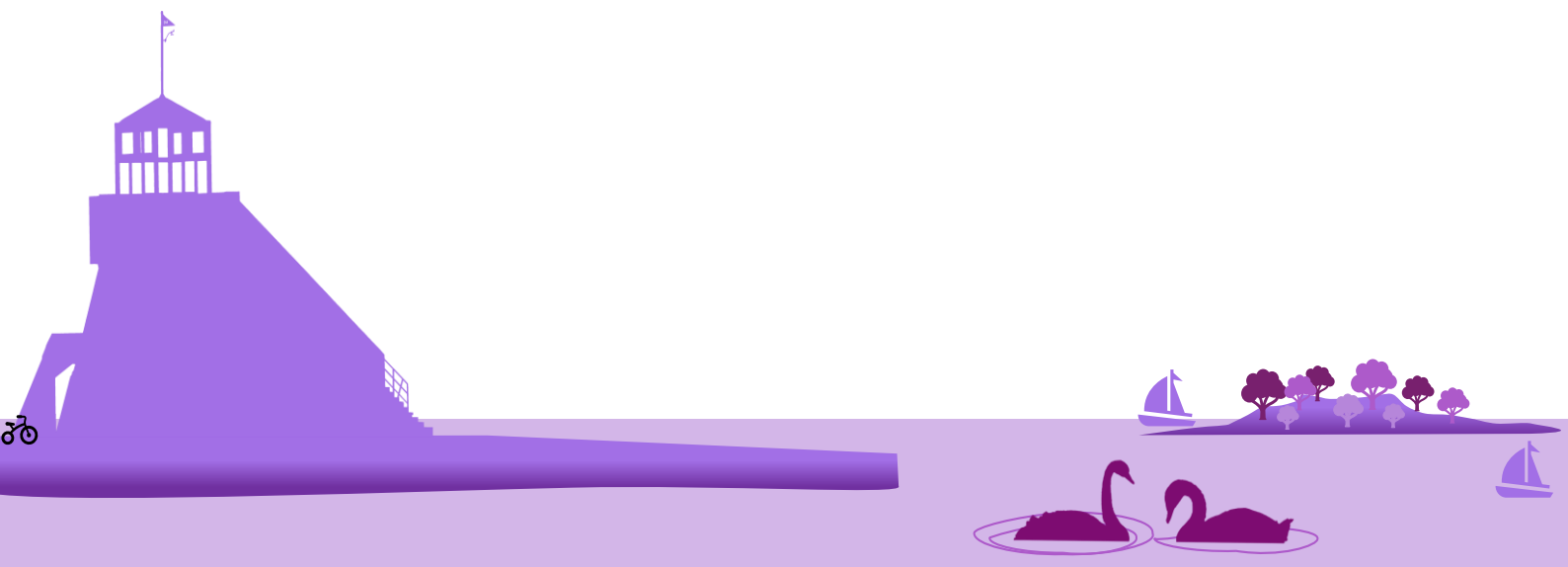
NMR is of central importance for observing ligand-protein interactions, however the low sensitivity of NMR mandates high concentrations of the reporting molecules. The scope of NMR based drug screening could be increased significantly, if the required mass of protein could be reduced. To do so, the primary levers are (i) the limit of detection for the reporting molecules and (ii) the contrast that is available upon binding of the reporter to the protein. (i) Very recently, we have shown how a combination of improved bullet-DNP [1,2] instrumentation, an optimized sample preparation, and a further sensitivity increase via a ^{13}C - ^1H polarization transfer after dissolution enable the observation of pyruvate at concentrations down to 250 nM, corresponding to a substance amount of only 180 pMol [3]. Here we put these sensitivity gains to use for the purpose of studying protein ligand-interactions. To maximize contrast (ii), we use low-field relaxation as a potent contrast mechanism. By keeping the pyruvate-protein solution at a low magnetic field of 1 T for several seconds after dissolution, and pushing it into the NMR tube only for detection afterwards, the presence of the protein is observable at protein concentrations as low as 1 μM , corresponding to a 20-fold saving in protein compared to conventional binding experiments.

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Deuterated Water Accelerated Droplet formation from Reactive Multicomponent Systems

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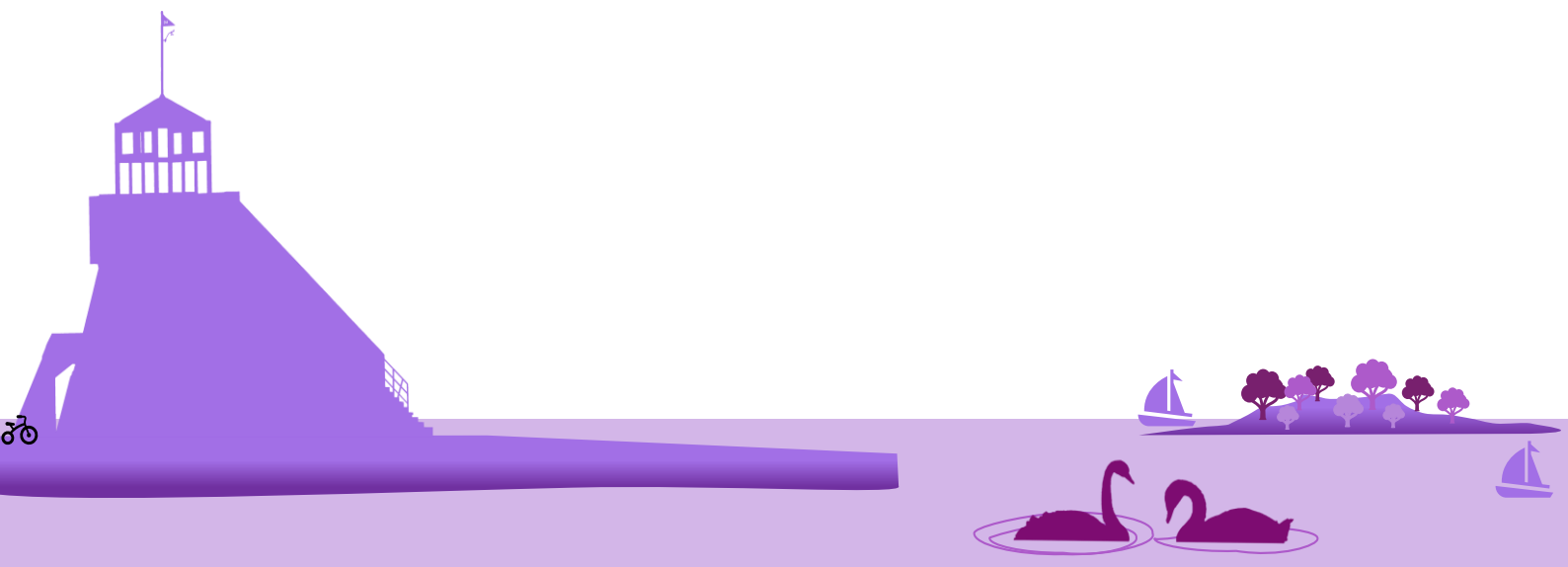
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Liquid-liquid phase separation plays its key role in natural and synthetic systems, providing a viable compartmentalization strategy to achieve multiple inspiring functions and high-order assembly. However, its underlying mechanisms, particularly those influenced by solvents, remain largely unexplored. In this study, we investigated the impact of deuterated water (D₂O) on phase separation in a reactive multicomponent system. Droplets formed more rapidly and abundantly in D₂O buffer compared to conventional buffer (H₂O). The phase separation process was driven by electrostatic complexation between anionic macrocycles (DBCA) and cationic imine surfactants produced via a reversible dynamic reaction. While imine production remained largely unaffected by deuterium, D₂O enhanced various noncovalent interactions, including π - π stacking and hydrogen bonding, which facilitated hydrophobic aggregation and thus droplet formation. The slow diffusion rate and large enthalpy change observed in D₂O further supported accelerated droplet nucleation and growth. Additionally, the Marangoni-type flow of D₂O droplets demonstrated their potential for controlled substance exchange and information transfer. These findings underscore the critical role of isotopic effect in promoting phase separation and highlight new opportunities for leveraging deuterium-enhanced systems in advanced molecular assembly and functional applications.



Discovery of a small molecule degrader of protein kinase CK2 α

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Casein kinase 2 alpha (CK2 α) is a constitutively active serine/threonine kinase frequently overexpressed in cancer. Here, we report the discovery of a novel class of potent small molecule inhibitors of CK2 α . We identify inhibitors with nanomolar affinity and superior cellular activity to known CK2 α inhibitors. In contrast to classical ATP-competitive inhibitors, our compounds induce proteasome-dependent degradation of CK2 α and decrease its thermal stability in cells. Using methyl SOFAST-HMQC NMR on selectively $^{13}\text{CH}_3$ -labeled isoleucine, valine, and methionine residues in a perdeuterated background, we demonstrate that the small molecules bind to an allosteric site distinct from the ATP pocket. The binding event triggers widespread line broadening, indicating a conformational shift toward a destabilized, aggregation-prone state. These findings establish a unique mechanism of CK2 α inhibition via allosteric destabilization and targeted degradation, providing a promising new approach for therapeutic intervention in CK2-driven malignancies



Magnetic Resonance Assays for the Identification of Novel Lectin Inhibitors to Combat Antimicrobial Resistance

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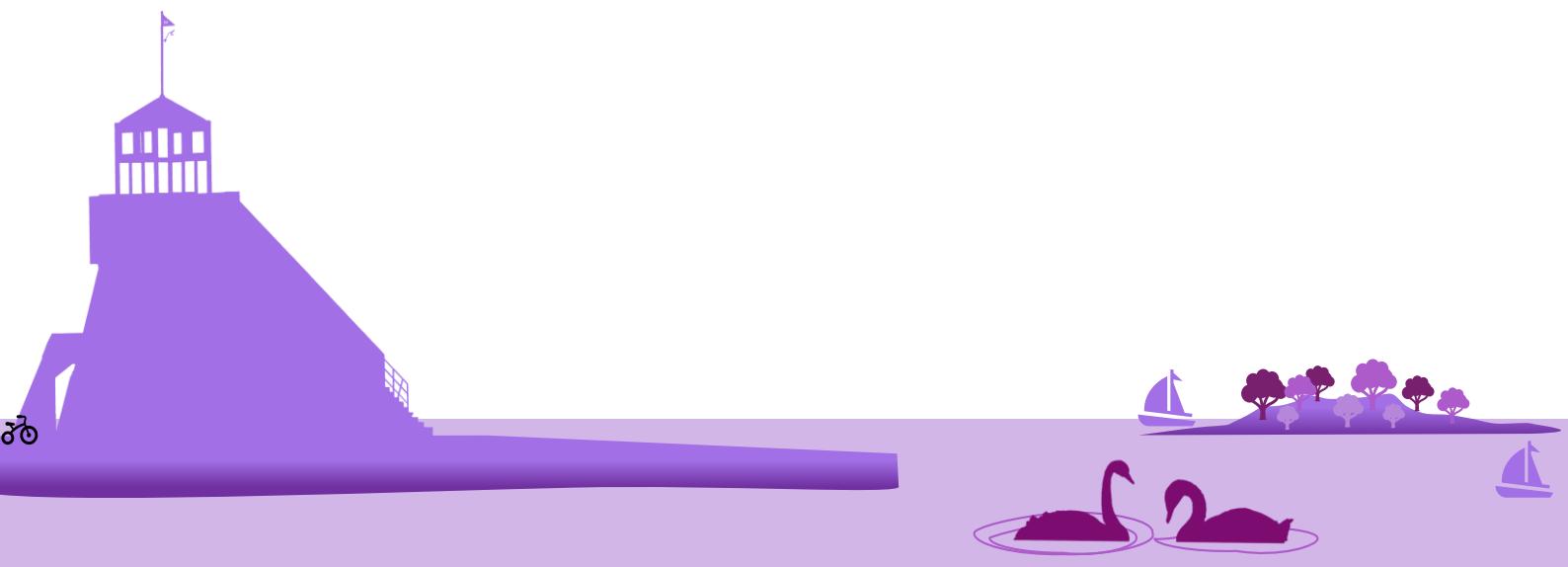
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Antimicrobial resistance is an emerging threat and requires the urgent identification of novel high affinity molecules as potential drugs. Within this project, the proteins of interest are bacterial lectins that bind to glycans on host cells to promote bacterial cell invasion. We propose modified glycans as novel inhibitors for these key virulence factors. To this end we develop innovative NMR and MRI assays that simplify affinity determination and structure elucidation of lectin-glycan complexes.

So far, we successfully employed fluorinated glycans as reporters for the establishment of ^{19}F -based NMR assays for different target proteins of *Vibrio cholerae* and *Pseudomonas aeruginosa* to determine the KD of ligands. The known affinities allow us to identify functional groups of the glycan that are essential for binding, and, conversely, propose groups that can potentially be derivatized. In pursuit of a high affinity compound, we performed small molecule fragment screenings to identify ligands to second binding sites that can be covalently linked to the glycan binding at the primary site. This information, together with the structural NMR-analysis of lectins-ligand complexes, provides a powerful combination for drug discovery. Furthermore, this approach has a dual purpose: (i) with the identified high-affinity ligand, we want to develop efficient and orally available inhibitors to cure bacterial infections, but (ii) we also plan to investigate the feasibility of the small molecule as an active reporter for the diagnosis and localization of bacterial colonies using (^{19}F -) MRI.



Investigation of Manganese Complex Formation and Relaxometric Properties in Conjunction with Silica Nanoparticles

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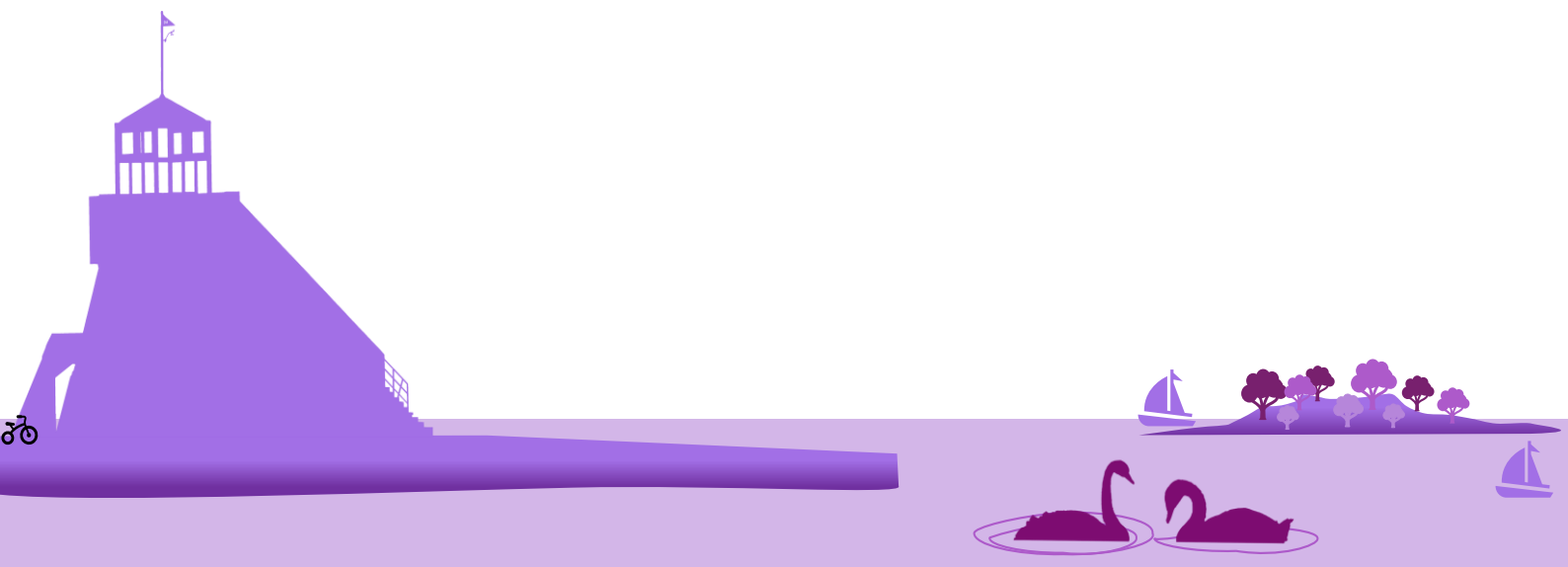
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Manganese(II) complexes are gaining attention as alternatives to gadolinium-based contrast agents for magnetic resonance imaging (MRI) due to their lower toxicity and robust relaxometric properties. This study focuses on the synthesis, equilibrium, kinetic, and relaxometric characterization of Mn(II) complexes with pyridine-containing cyclen (PC2A, PC2A_BF and PC2AM) derivatives. Synthesis of the ligand (PC2A, PC2A_BF) are followed according to the previous work. PC2AM was synthesized by coupling PC2A and Dimethylamine using DIC as a coupling agent. Protonation constants ($\log K_{iH}$) and conditional stability constants ($\log K_{ML}$, pMn) were meticulously determined under physiological conditions, demonstrating that specific ligand modifications significantly enhance the stability and selectivity of Mn(II) complexes.

Relaxometric studies revealed a strong dependence of longitudinal relaxivity (r_1) on pH and ligand coordination environments. Notably, PC2AM derivatives exhibited improved relaxivity profiles attributed to optimized water exchange kinetics and rotational correlation times. Transmetalation kinetics with Cu(II) were investigated, and pseudo-first-order rate constants (k_d) were measured across various pH levels. The results highlighted the superior kinetic inertness of Mn (PC2AM) complexes, with half-lives ($t_{1/2}$) exceeding several hundred hours under physiological conditions.

Furthermore, nuclear magnetic relaxation dispersion (NMRD) studies provided detailed insights into the molecular mechanisms governing relaxivity, confirming the potential of PC2AM derivatives as next-generation MRI contrast agents. This work establishes a comprehensive framework for the rational design of Mn(II) based contrast agents, with applications extending to clinical diagnostics and targeted imaging.



Studies with PBLG at its isotropic-to-anisotropic phase transition

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Poly- γ -benzyl-L-glutamate is a very well studied liquid crystalline alignment medium with apolar solvents like CDCl_3 . In recent years, its behavior near and below the phase transition concentration has been studied using conventional and rheological NMR, as well as MRI. Of particular interest for NMR spectroscopists is the time-dependent co-existence of isotropic and anisotropic regions. The kinetic behavior of the system is studied qualitatively and applications regarding residual anisotropic parameters are looked at in detail.



Towards quantification of Hesperidin in Orange Juice by ^1H -NMR

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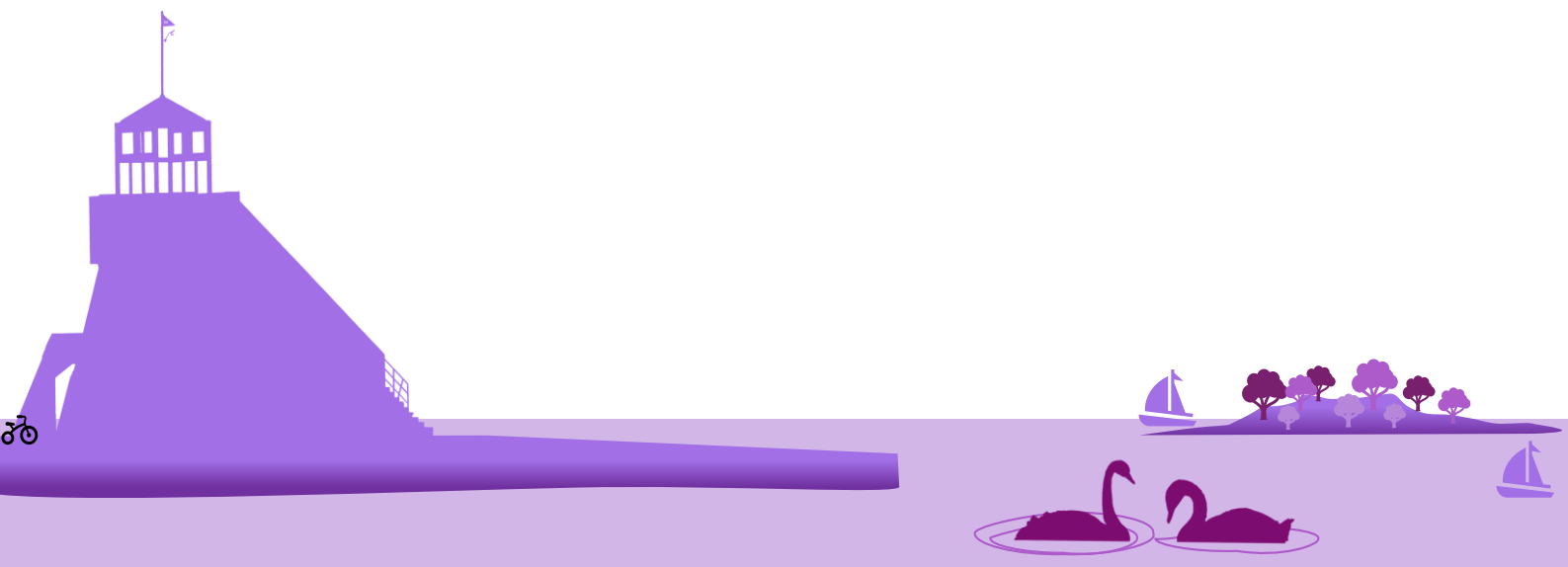
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Hesperidin is the main flavonoid found in sweet oranges (*Citrus sinensis*). It belongs to the flavanones, and is widely recognized for its health benefits, such as antioxidant properties. Hesperidin can prevent cancer and cardiovascular diseases. It also shows antiviral, and anti-inflammatory properties, thus acts as a potential inhibitor of SARS-CoV-2 infection. Sweet oranges are widely cultivated in Brazil, the main world orange juice producer.

In this work we developed a method to quantify hesperidin in sweet orange juice with 1D ^1H -NMR. Fresh orange juice and store-bought orange juices were frozen in liquid nitrogen and lyophilized. The samples were dissolved in DMSO- d_6 , vortexed and centrifuged. Supernatant of each sample was analyzed by 1D ^1H -NMR and 2D HSQC NMR at 600 MHz. To establish the suitability of the hesperidin signal for quantification purposes, an increasing amount of hesperidin (standard extracted from orange peels, <95%, determined by HPLC) was added to the juice samples. The hesperidin content was calculated employing an internal standard, maleic acid, with the integration of the signal at 6.07 ppm for the CH₂ group (s, 2H). We employed the method in freshly squeezed orange juice produced in Brazil and prepackaged commercially also produced in Brazil. The developed method came up with a more easy way to determine the hesperidin content in juices, since the others are based on chromatography, and thus, more difficult to perform.



Optimised Chemical Shift Imaging for pKa Determination: Artefact Elimination and z-Axis Calibration

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Chemical Shift Imaging (CSI) with chemical gradients offers a powerful approach for high-resolution, high-throughput pKa determination. However, robust application requires precise control of imaging parameters. We report method development that overcomes key challenges in CSI for pKa measurement. We demonstrate that artefacts resulting from refocusing of multiple quantum coherences—caused by the interaction between phase encoding and excitation sculpting gradients—can be eliminated by synchronising the sign of the excitation sculpting gradients with that of the phase encoding gradients throughout the acquisition, ensuring consistent, artefact-free chemical shift imaging across the pH gradient. Accurate calibration of the z-axis sampling to the active coil region was achieved through direct measurement and adjustment of sweep width parameters, ensuring maximum use of the pH gradient present within the NMR receiver coil and avoiding resolution loss or data clipping. Integration of fast excitation sculpting for water suppression minimised pH gradient evolution during acquisition. Together, these developments allow the acquisition of high-resolution chemical shift images with 128 spatially resolved titration points from a single sample, greatly enhancing the speed and precision of pKa determination. These advances form the foundation for fully automated, user-friendly CSI workflows suitable for deployment in industrial and research environments.



The role of NMR in the Discovery of Reversible Small Molecule Multivariant Ras Inhibitors that Display Tunable Affinity for the Active and Inactive Forms of Ras

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Ras is a high-profile cancer targets that was once dubbed ‘undruggable’, but two irreversible mutant selective KRasG12C irreversible inhibitors have achieved FDA approval.¹ Ras has multiple mutations and as a GTPase it exists in an inactive state (Ras.GDP) and an active state (Ras.GTP). Other recent publications show reversible inhibitors for pan-KRas in the inactive form,² and for active Ras in complex with cyclophilin A.³ In this work we started from fragment screening, and using biophysics and an integrated drug discovery approach compounds were evolved into nanomolar reversible KRas binders.⁴ Further optimisation showed that for the first time compounds could be tuned to be selective for the inactive and active state of KRas.

NMR played a key role in developing the fragment hits into compounds that could then be crystallised. NMR provided screening information, affinity data, physico-chemical properties of compounds and information on key protein-protein interactions. This data along with the X-ray crystal structures were used to drive the Structure Based Drug Design process.

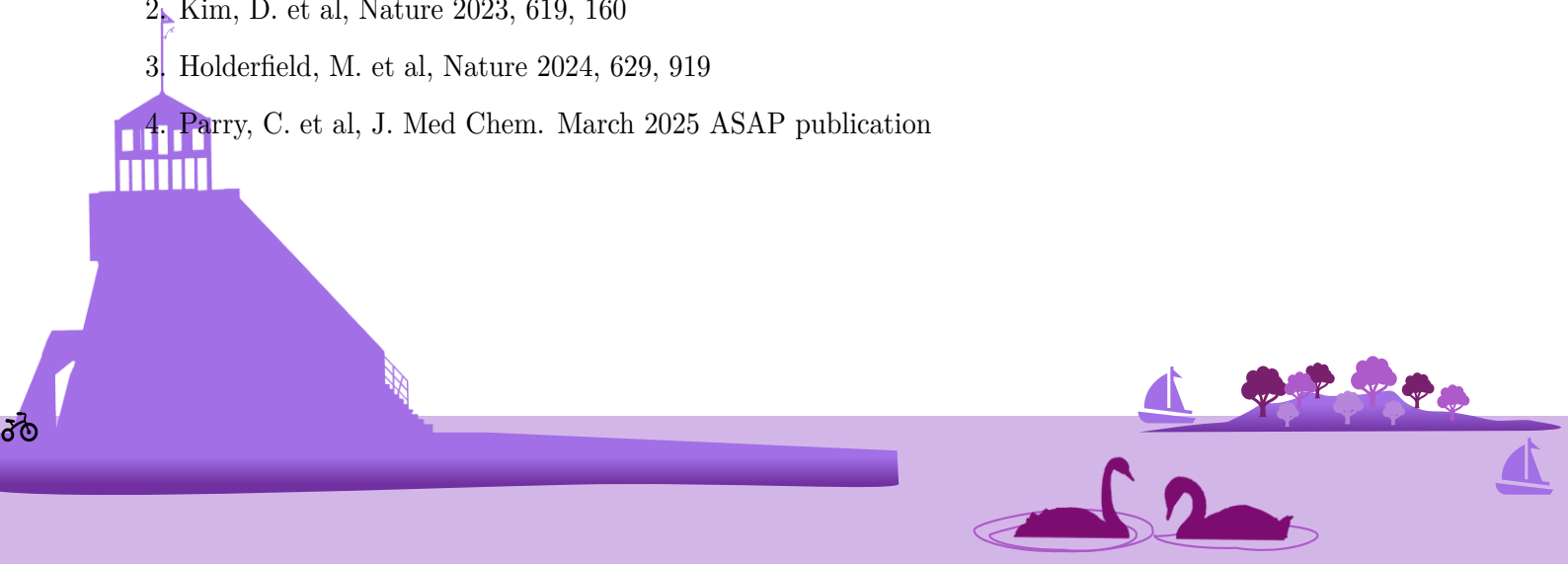
As the project progressed affinity of compounds were improved such that slow exchange was observed by NMR for both active and inactive KRas.

The demonstration of direct competition between active KRasG12D and RafRBD shows that the compounds are expected to be functionally relevant. Biochemical assays were able to demonstrate the functional relevance of the compounds with a Ras-Raf HTRF assay.

Correlation of data across NMR, SPR, biochemistry and the structural biology was key to the progression of compounds. This led to the discovery of compounds that opened a new subpocket gave compounds with a selectivity of active over inactive KRas.

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Elucidating Oxazolidine Photodegradation

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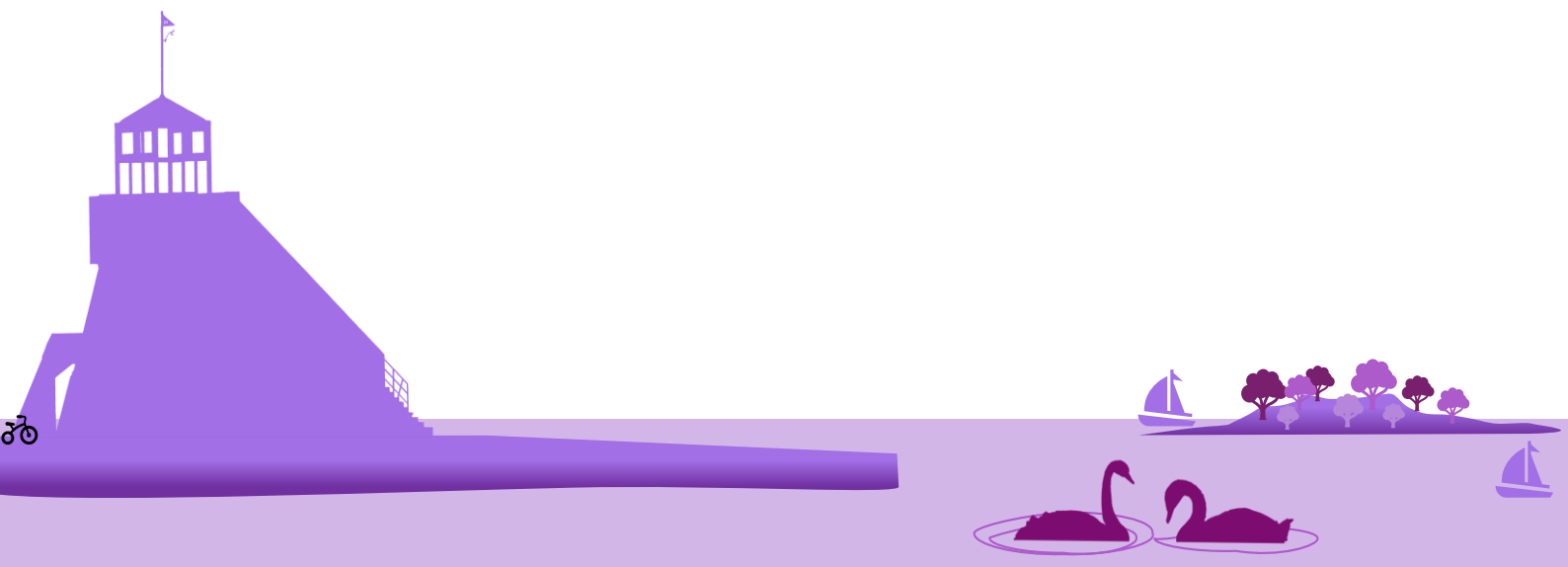
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Designing antibiotics with a built-in self-destruct mechanism could significantly aid the fight against multi-drug resistant bacteria. Our goal is to develop photodegradable antimicrobials that break down after use, via wastewater treatment (human use) or sunlight (veterinary use), minimizing environmental impact. To achieve this, we must understand their photodegradation mechanisms and identify the possible products. LED-NMR provides valuable insights into both. Oxazolidine-based compounds, with their antibacterial activity and potential for rapid decomposition, were selected as model for this study. We investigated whether integrating an oxazolidine moiety into a biologically active molecule enables controlled degradation under UV light. Compounds were irradiated at 310 nm, and their decomposition was monitored using LED-NMR, HRMS and MS/MS. These techniques provided mechanistic insight into their photochemical behavior and degradation profiles.

Oxazolidine photodegrades into ethanolamine via methanediol elimination, which, in the presence of oxygen, gradually oxidizes to formic acid. Ethanolamine then further degrades into two major and several minor degradation products. Structural elucidation of these species enabled us to propose a detailed degradation mechanism. Additionally, a second compound, dioxazepine, was tested under the same conditions. It degraded via the sequential loss of two methanediol units, yielding ethanolamine and proceeding through the same downstream degradation pathway.

Degradation mechanisms for two potential self-destruct functionalities were proposed based on the structures of photodegradation products formed under UV irradiation. The findings underscore the inherent instability of the oxazolidine moiety in aqueous environments, limiting its practical application. However, they also highlight ethanolamine and dioxazepine as promising motifs for incorporating controlled, light-triggered degradation into antimicrobial design.



Challenging Structural Elucidation of APIs: Advanced Solid-State NMR studies of Fostamatinib Crystallography

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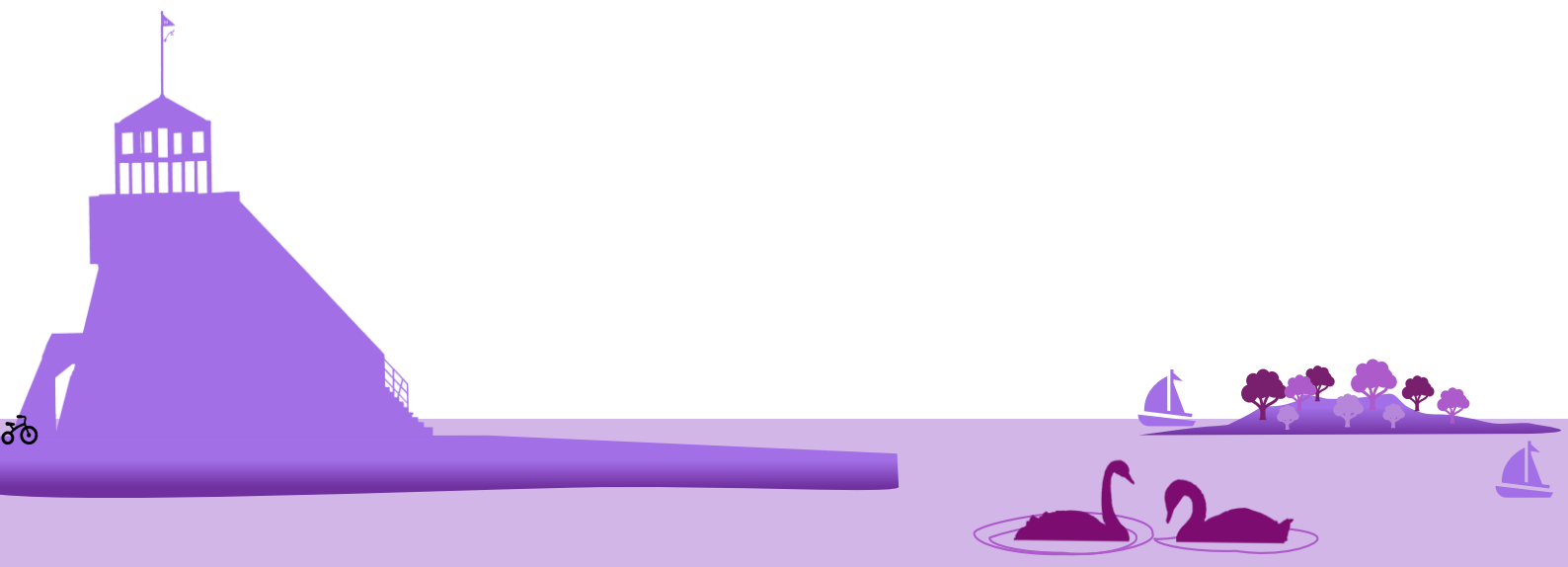
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Fostamatinib, a kinase inhibitor used in autoimmune disease treatment, is known to crystallize as a disodium hexahydrate, yet its precise solid-state structure remains unreported. Our study presents a novel NMR crystallographic approach to elucidate this complex structure using advanced ^1H -detected solid-state NMR techniques. The inherent structural challenges—high molecular flexibility and multiple independent species per asymmetric unit—were addressed using distance measurements from REDOR experiments combined with ultra-fast magic-angle spinning (UF-MAS) and minimal sample consumption.

^{13}C CP-MAS NMR spectra acquired at 9.4T and 14.1T confirm the chemical integrity of the sample and suggest a unique $z' = 1$ value. Complementary experiments (short contact time CP, NQS, hCH, hNH) enabled partial carbon–hydrogen and nitrogen–hydrogen assignment, while theoretical calculations will support full assignment. Crucially, ^{23}Na MAS and MQ-MAS NMR revealed two distinct sodium environments, each with characteristic quadrupolar coupling constants, further informing the lattice model.

This integrative strategy not only enables the generation and refinement of candidate structural models using experimental distance constraints, but also significantly advances NMR crystallography. The methodology is especially impactful for expensive APIs, enabling structural studies with limited material. Beyond fostamatinib, our work lays the foundation for broader applications in pharmaceutical development, influencing drug stability, solubility, and formulation strategies.



Application of ultra-broadband ^{19}F -pulses in 1D/2D NMR experiments for the analysis of perfluorinated substances

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Owing to their unique chemical and physical properties, comprising both a lipophobic and a hydrophobic behaviour, per- and polyfluorinated substances (PFAS) have found widespread industrial application as surfactants or coatings. However, the perfluorinated carbon framework possesses a high chemical stability, therefore, PFAS are prone to bioaccumulation and pose health and environmental concerns.[1] As a consequence, some representatives are regulated internationally through the Stockholm Convention on Persistent Organic Pollutants.

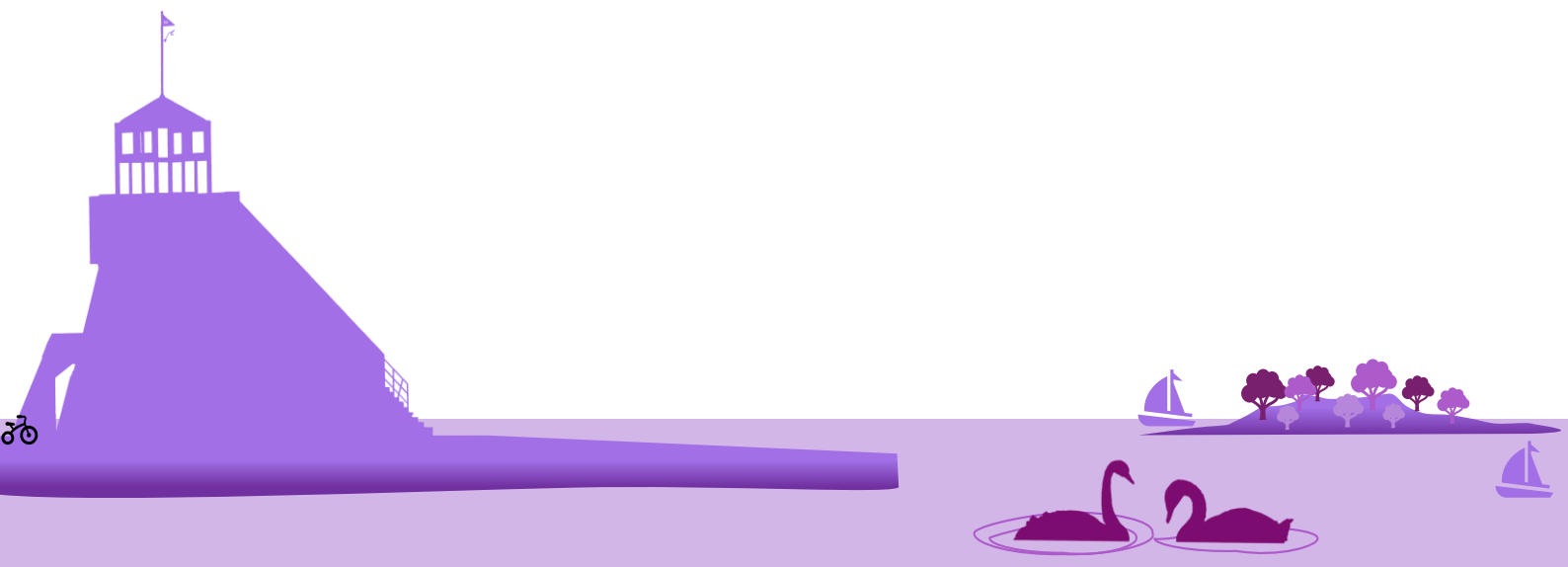
The analysis of PFAS representatives in different environmental and biological matrices remains difficult using standard mass spectrometry, since fragmentation methods are hardly addressable. In contrast ^{19}F NMR spectroscopy offers an effective alternative, owing to the high sensitivity of the ^{19}F nucleus and the ability of a virtually background-free detection.

More recently ultra-broadband ^{19}F -BURBOP[2] and OPERA[3] pulses were introduced, that are able to cover the entire chemical shift region of resonances emerging from PFAS and allow for their uniform excitation. Herein, we report our attempts in incorporating these broadband pulses into standard 1D/2D NMR experiments to obtain the corresponding broadband ^{19}F - ^{19}F -COSY, ^{19}F - ^{19}F -TOCSY, ^{19}F - ^{19}F -NOESY, ^{19}F - ^{13}C -HSQC and ^{19}F - ^{13}C -HMBC experiments. These are currently executed on a Bruker Avance III 600 MHz NMR Spectrometer equipped with a helium cooled four-channel- ^{19}F CryoProbe (QCI-F) and applied to establish a PFAS library, including some of the most prominent representatives. In the near future, we intend to establish a reliable protocol for the ^{19}F NMR analysis of PFAS in a variety of environmental, biological and industrial samples.

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PULCON combined with flow correction factors for the accurate quantification of multinuclear FlowNMR spectra

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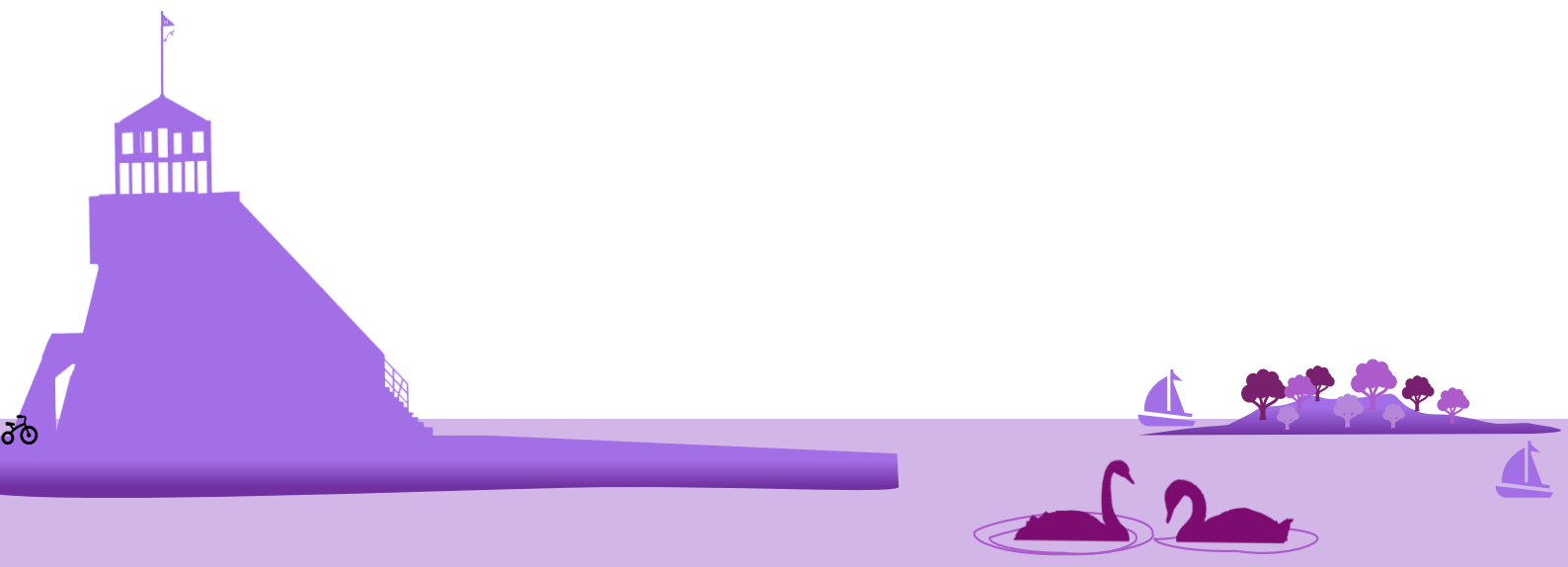
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FlowNMR is currently a widely used technique for reaction monitoring. Quantitation is normally achieved via the use of internal standards, using flow calibration factors to account for signal losses brought about by the effects of continuously flowing the sample (1). However, difficulties can arise if the internal standards are chemically incompatible with (or insoluble in) the reaction matrix, or if their NMR signals overlap with peaks of interest – which may not always be known in advance.

PULCON is an established method for measuring absolute concentrations of NMR samples without the use of internal standards (2). In this work, we demonstrate that PULCON can be successfully applied to FlowNMR studies, and when modified to include flow correction factors the quantification is as accurate as internal referencing, giving results within 1% of absolute reagent concentrations. The technique has been verified for ^1H and heteronuclear (^{31}P and ^{19}F) detection.

PULCON is ideally suited to FlowNMR studies since one FlowNMR tube is typically used for all reactions studied, so eliminating any variations between tubes. It has specific advantages for reaction monitoring studies as it removes the need for solvent compatibility screening, and eliminates the possibility of peaks of interest (e.g., reaction intermediates) overlapping with the resonances for the standard. It is also fully compatible with reaction monitoring at variable temperatures. This approach offers a valuable alternative to quantitation via internal standards for the FlowNMR reaction monitoring community.

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Comparing the binding sensitivity of an LLS-based experiment to state-of-the-art ligand-observed NMR experiments

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Long-lived states (LLS) are spin states whose relaxation times can be far longer than the longitudinal relaxation time (T_1). [1] LLS lifetimes can also be more sensitive to binding between ligands and proteins than T_1 . [2] Therefore, the use of LLS has been proposed for protein-ligand screening.

In this work, we compare the sensitivity of LLS-based experiments with other ligand-observed NMR experiments used for drug screening. Such experiments rely either on intermolecular Nuclear Overhauser Enhancement (NOE), as in the saturation transfer difference (STD) [3] and WaterLOGSY [4] experiments, or on the monitoring of changes in relaxation rates of the ligand. Here, a perfect echo CPMG (peCPMG) [5] was used to measure the transverse relaxation rates of ligand protons and spin-lock induced crossing (SLIC) [6] was used to monitor the LLS relaxation rate.

The sensitivity of these four experiments was compared for a system of (Trifluoromethyl)benzamidine and trypsin. The metrics used for evaluation were the contrast [2], ligand score, and difference-to-noise ratio (DNR). [7] Both relaxation-based experiments outperformed the standard NOE-based experiments. The peCPMG and SLIC experiments performed at a similarly high level, with comparable ligand scores. While the contrast was higher for the SLIC experiment, the DNR was lower. The LLS-based experiment has a very high sensitivity to binding in this system, and its DNR might be boosted by combination with hyperpolarization.

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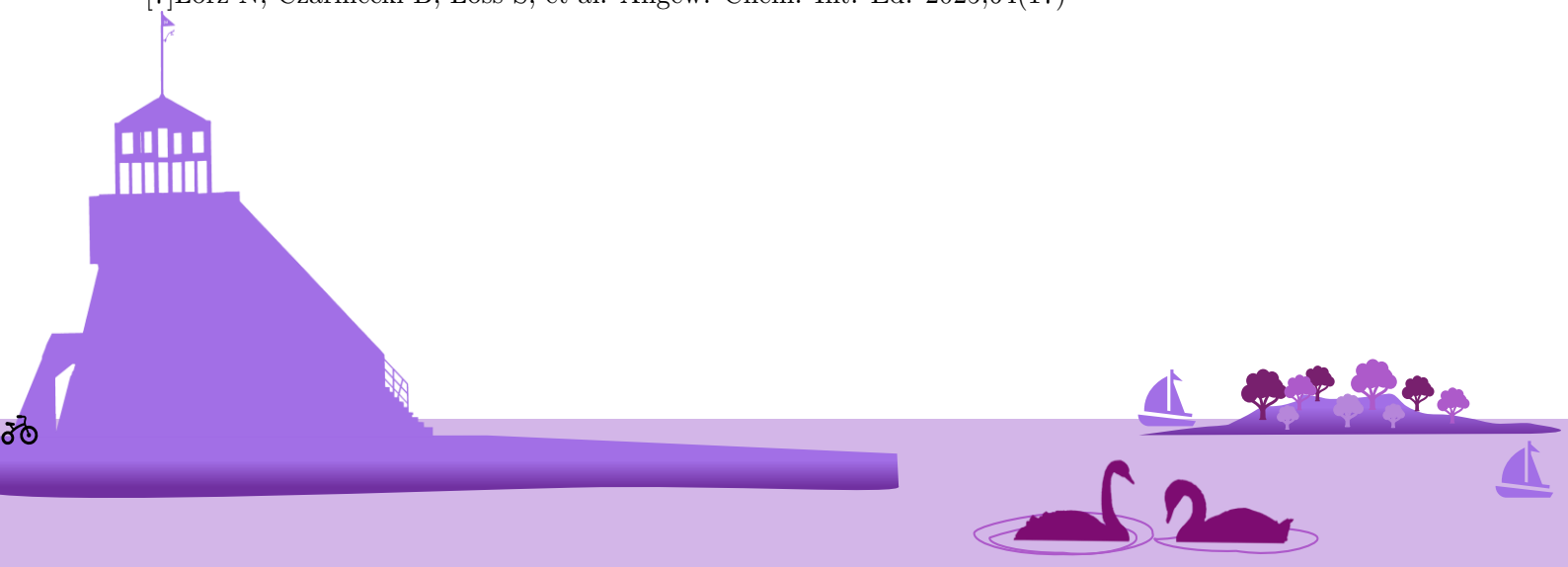
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High-Resolution NMR and MRI Characterization of Catalytic Reactor in Gas-Phase Enabled by Hollow Alumina Supports

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Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) provide non-invasive tools for operando characterization of catalytic reactors. However, their application is limited by low sensitivity, which hinders the study of samples with low concentrations, such as gases or intermediates involved in catalytic reactions. The presence of conventional solid catalyst supports in the sensitive region exacerbates this problem by causing magnetic field inhomogeneities that broaden and distort NMR signals.

Our research addresses these challenges by employing hollow alumina spheres as catalyst supports. Their low solid content and spherical geometry minimize magnetic field distortions, thereby preserving spectral resolution and imaging quality. Experimental results showed that the developed system minimally distorts the uniformity of the magnetic field, unlike traditional beads, and can be utilized for catalytic research in gas phase. Hollow alumina spheres with supported rhodium nanoparticles were tested in the propene hydrogenation reaction under operando conditions. The sensitivity was sufficient to obtain spatially resolved spectra and MR images with good resolution. Spatially resolved NMR spectra showed the distribution of reactant and product throughout the catalyst bed, while the conventional NMR spectrum gave volume-averaged conversion. Similar experiments using parahydrogen-induced polarization (PHIP) revealed that hyperpolarized propane is located at the beginning of the reactor and its amount decreases along the catalytic layer due to relaxation. The magnetic field uniformity proved sufficient to perform chemically selective MRI, enabling the tracking of the reactant and product spatial distribution within the reactor.

Thus, hollow sphere supports enable spatially resolved ^1H NMR and high-quality MR imaging of gas-phase reactions, greatly enhancing characterization precision.

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Highly Parallelized Inhibition Screening Accelerated by Compressed Sensing 19F-MRI

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We present a novel approach for high-throughput screening of non-fluorinated drug candidates using 19F-MR imaging of a fluorinated reporter ligand in competitive binding mode. This strategy enables direct monitoring of ligand–protein interactions within a physiologically relevant environment, preserving both conformational dynamics and steric accessibility.

Traditional MR methods often detect binding events but struggle with spatial multiplexing in a shared signal environment. To overcome this, we developed a spatially resolved assay using a custom honeycomb sample holder with 61 well-isolated wells, allowing simultaneous screening of 55 candidate compounds. Binding-induced changes in transverse relaxation (R_2) of the reporter ligand served as the readout for hit identification.

To enhance throughput, we applied compressed sensing undersampling, leveraging the redundancy in MR images to significantly accelerate acquisition without sacrificing image quality or diagnostic precision. This method offers a robust, scalable platform for rapid hit validation in fragment-based and competitive drug discovery workflows.



Rapid high-resolution T2 mapping via multi-shot multiple overlapping-echo detachment imaging

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Aims: To develop a T2 mapping method with submillimeter spatial resolution, approximately one-minute acquisition time, and high quantification accuracy. Additionally, to develop an associated approach correcting inter-shot phase variations caused by pulsatile cerebrospinal fluid (CSF) flows.

Methods: Multi-shot acquisition scheme along with echo time shifting strategy was adopted for a T2 mapping method termed multiple overlapping-echo detachment (MOLED) imaging. Discontinuous phase jumps were corrected via deep learning leveraging the pattern incoherence between desired MRI signal and undesired artifact. Methodological feasibility and practicality were validated with phantoms and healthy volunteers referring to gold standard.

Results: Phantom/human results exhibited a mean absolute error of 1.11/0.89 ms, and linear regression of phantom results revealed a slope of 0.994 ($R^2 = 0.995$). The impact of CSF-induced phase inconsistency was successfully eliminated.

Conclusions: A T2 mapping method with submillimeter spatial resolution is proposed and validated, and inter-shot phase variations, a frequently-encountered issue to multi-shot sequences, is addressed in a referenceless manner, retaining the quantification accuracy and acquisition rapidness.

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Orientation Dependence of MRF-Derived qMRI Parameters in Human Articular Cartilage

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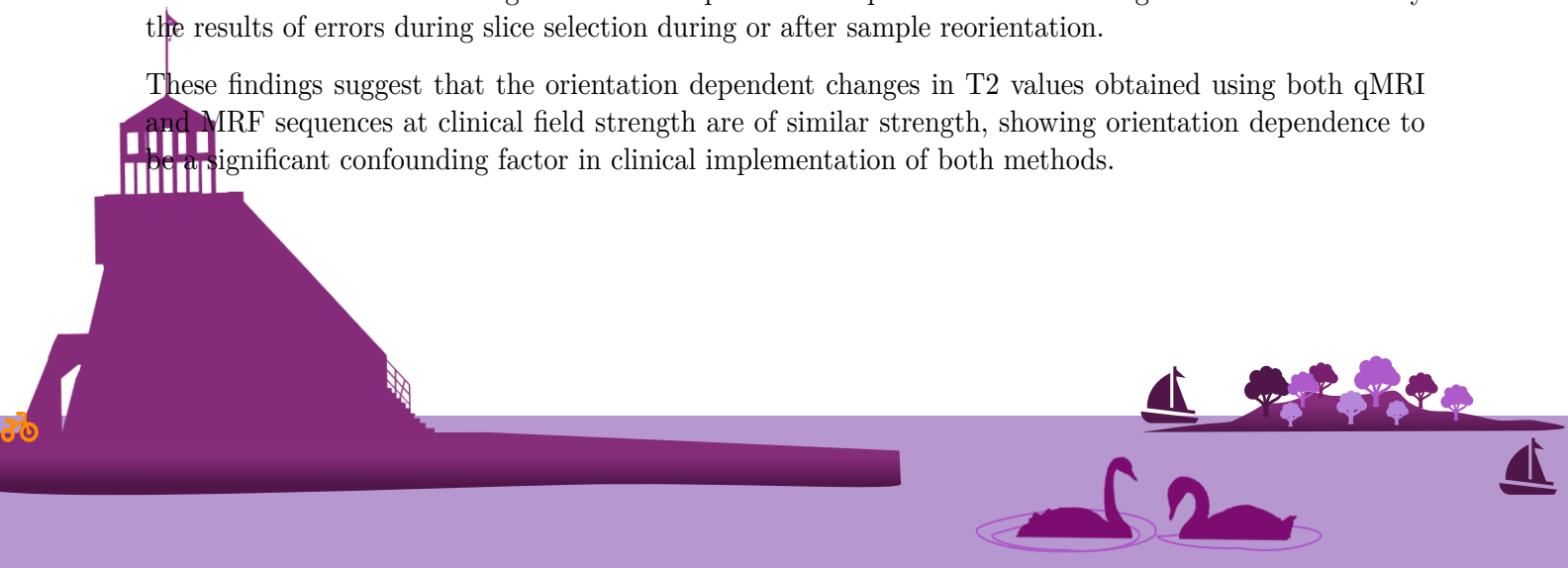
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In articular cartilage certain magnetic relaxation times are dependent on the orientation of the tissue with respect to the main magnetic field of the MRI device. This effect can be used to gain information on the cartilage ultrastructure, but the scan times of applicable quantitative magnetic resonance imaging (qMRI) sequences are often beyond clinical feasibility. Magnetic resonance fingerprinting (MRF) offers a significant reduction in scan times, but open questions remain whether MRF and qMRI sequences are comparable.

The purpose of this study was to measure and compare the orientation dependencies of T1 and T2 relaxation times determined using both qMRI and MRF sequences on a clinical 3 T MRI scanner. To this end, ex vivo human cartilage samples (N=96) were imaged at two orientations (0° and 55°) with respect to the main magnetic field. T1 and T2 maps were obtained using conventional sequences (IR-T1 & MESE-T2), followed by MRF. Bulk cartilage T1 and T2 values of each sample were compared between orientations.

Both qMRI and MRF-derived T2 values displayed significant degrees of orientation dependence. The mean differences in T2 values between the orientations were 47.1% for qMRI and 32.3% for MRF. The MRF-derived T2 values were found to be lower than their qMRI counterparts. T1 values obtained using both qMRI and MRF also displayed small albeit significant orientation dependence. The mean differences in T1 values between the orientations were found to be 7.6% for qMRI and 3.7% for MRF. MRF-derived T1 values were found to be higher than their qMRI counterparts. The small changes seen in T1 are likely the results of errors during slice selection during or after sample reorientation.

These findings suggest that the orientation dependent changes in T2 values obtained using both qMRI and MRF sequences at clinical field strength are of similar strength, showing orientation dependence to be a significant confounding factor in clinical implementation of both methods.



Magnetic Resonance Pore Imaging using Simple Echo Pulse Sequences

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Pulsed Gradient Spin Echo methods, as introduced first by Stejskal and Tanner [1], have constantly evolved over time, became more versatile and sophisticated. Examples include structural investigations in porous materials, soft matter research, or biomedical applications. More recently, Diffusive Pore imaging [2] aka Magnetic Resonance Pore Imaging (MRPI) [3] uses extended gradients to accumulate the phase of the NMR signal as an average over a long diffusion path, thus tracing out pore structures. Achieving the long-time limit for these extended gradients is crucial, else artefacts, such as blurring, may occur.

Practical implementations for MRPI are challenging since the magnetisation needs to be maintained in the transverse plane for extended periods of time. This holds for samples which induce internal gradients due to their heterogeneous internal pore structure. This has been mitigated in the past by MRPI pulse sequences similar to CPMG [3; 4]. While this preserves magnetisation it also introduces complex coherence pathways and requires frequent and rapid gradient switching. Other approaches have used conventional spin-echo sequences [5], owing to a favorable sample structure and alignment in the B0 magnetic field.

Here, we report on the use of conventional spin-echo sequences applied to diffusion in glass capillaries with diameters of 20 μm . Even at echo times on the order of seconds, we observed sufficient signal-to-noise ratios. Depending on the specific properties of the sample being examined, this experimental simplification could expand the applicability of sequences that rely on long diffusion times to media containing closed pores.

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The Geo-Energy Magnetic Resonance Imaging (GEM) Facility at ETH Zurich

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Magnetic resonance imaging (MRI) is a non-destructive and elementally-specific technique that can probe complex geofluids and materials in diverse media. The Geo-Energy MRI (GEM) facility in the GEG group at ETH Zurich will implement both high- and low-field MRI for reactive transport analysis of geofluids in custom 3D-printed porous and fractured media. The primary instrument uses a cryogen-free 4.7 T magnet, with a homogeneous field-of-view over a 20 cm DSV. Further, a custom low-field ca. 36 mT MRI device will be implemented as a portable and cost-effective modality, while also probing unpaired-electron paramagnetic species. This latter facet can also leverage dynamic nuclear polarization (DNP) to enhance low-field ^1H and ^{13}C signals by factors of 660 and 2600 in their theoretical limit, respectively, offering experimental time savings of several orders of magnitude. Both devices offer multinuclear capabilities including the detection of ^1H , ^7Li , ^{13}C , ^{19}F , ^{23}Na , ^{29}Si , ^{31}P , ^{129}Xe , etc., which is critical for analyzing multicomponent geofluids (e.g., H_2O , brine, H_2 , CH_4 , etc.). Unique additive manufacturing strategies are used to 3D print polymers and ceramics that represent scaled-down geological reservoirs. These 3D-printed reservoirs can be studied by MRI and subject to the same conditions found in the Earth's crust (i.e., $>100^\circ\text{C}$ and $>100\text{ bar}$) for in-situ analyses of velocity fields, temperatures, and pressures.

In this presentation, we will discuss plans for the GEM facility and show preliminary results of velocity-field and temperature data acquired for 3D-printed fractured and porous media on 3 T commercial hospital MRI scanners. Researchers from other institutes are encouraged to enquire about using the GEM MRI facility for their unique experiments.



Deuterium Magnetic Resonance Imaging for early monitoring of Cancer treatment using Deuterated Water and 1.5 T MRI

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Aim: A precise and noninvasive evaluation of tumor response to treatment, beyond traditional anatomical imaging techniques, is critically needed. Deuterium magnetic resonance imaging (DMI) has emerged as a promising approach for visualizing cancer metabolism using deuterium-labeled compounds in high-field MRI. This study aimed to apply tissue deuterium labeling combined with deuterium MRI at clinical field strength (1.5T) to visualize tumors and evaluate the therapeutic effects of three anticancer treatments in a pancreatic cancer mouse model.

Methods: BALB/c-nu mice bearing either MIA PaCa-2 pancreatic carcinoma xenografts were used. Mice were given 30% D₂O in drinking water, and serial deuterium MRI scans were conducted over 10 days to monitor ²H distribution across tissues. In MIA PaCa-2-bearing mice, changes in ²H accumulation in tumors were evaluated following treatment with irradiation, bevacizumab, or gemcitabine. Complementary assessments included proton MRI, ex vivo hyperpolarized ¹³C-MRS, and histopathological analysis.

Results: Whole-body distribution of deuterium became detectable within one day of D₂O administration and progressively increased in intensity over time. Although tumor size remained unchanged at 1 and 3 days post-irradiation, ²H signal intensity within the tumor significantly decreased. Similar reductions in tumor ²H signal were observed following treatment with bevacizumab or gemcitabine. The therapeutic efficacy was also confirmed by ¹³C hyperpolarized MRS with ¹³C pyruvate, proton MRI, and ²H-NMR spectroscopy.

Conclusions: Deuterium MRI at clinical field strength (1.5T) enabled noninvasive tracking of ²H distribution in mouse tissues during D₂O intake and revealed enhanced deuterium accumulation in tumor xenografts. This approach provides a feasible and promising method for the early evaluation of radiotherapy and chemotherapy responses in cancer.



Instrumental distortions in quantum optimal control

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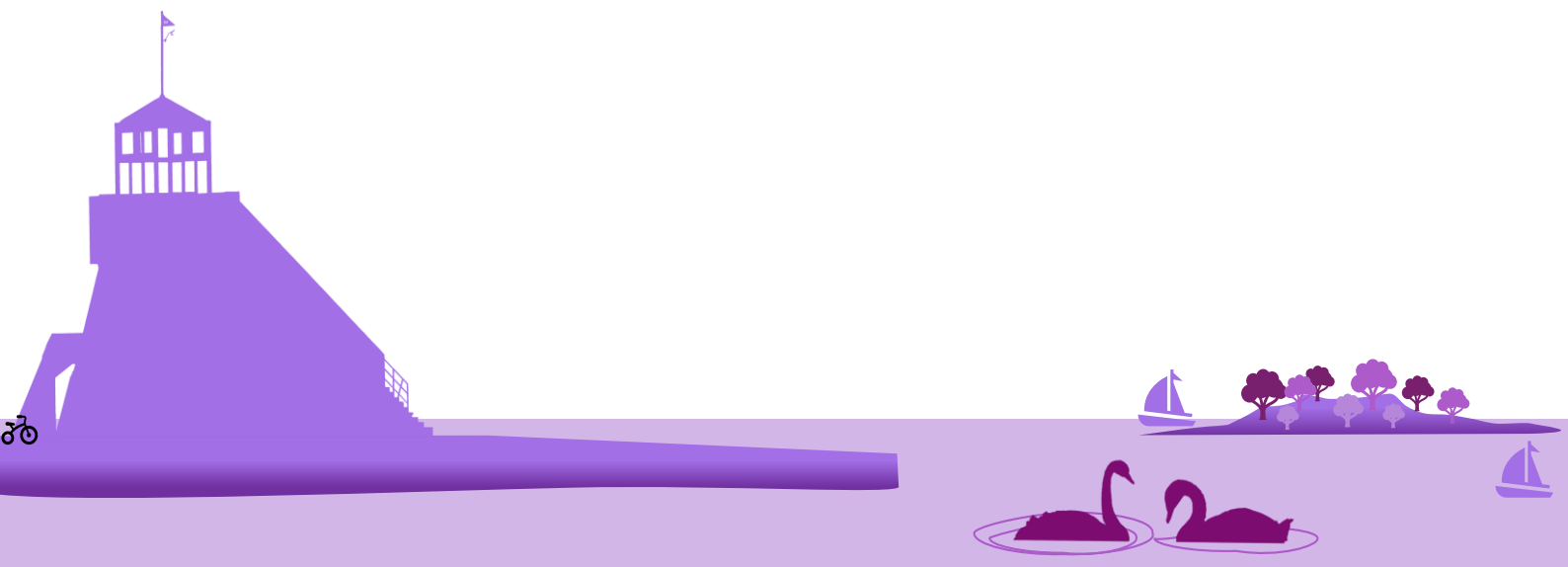
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Quantum optimal control methods, such as gradient ascent pulse engineering (GRAPE), are used for precise manipulation of quantum states. Many of those methods were pioneered in magnetic resonance spectroscopy where instrumental distortions are often negligible. However, that is not the case elsewhere: the usual jumble of cables, resonators, modulators, splitters, amplifiers, and filters can and would distort control signals. Those distortions may be non-linear, their inverse functions may be ill-defined and unstable; they may even vary from one day to the next, and across the sample.

Here we introduce the response-aware gradient ascent pulse engineering (RAW-GRAPE) framework, which accounts for any cascade of differentiable distortions within the GRAPE optimisation loop, does not require filter function inversion, and produces control sequences that are resilient to user-specified distortion cascades with user-specified parameter ensembles.

The framework is implemented into the optimal control module supplied with versions 2.10 and later of the open-source Spinach library; the user needs to provide function handles returning the actions by the distortions and, optionally, parameter ensembles for those actions.



Atomistic structures of early-stage prenucleation clusters by hyperpolarized NMR-validated, quantum mechanical calculations

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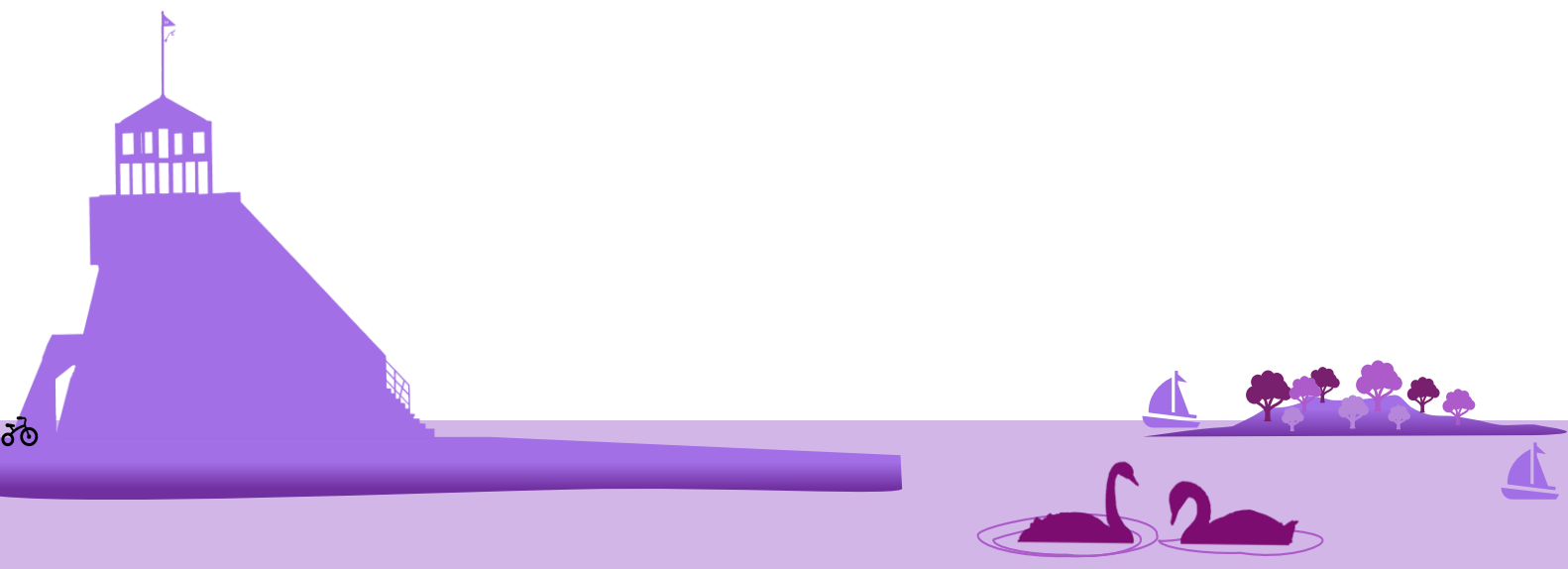
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The discovery of early-stage solution-state precursors of solid materials and biominerals challenges classical nucleation theory (CNT). It opened research avenues, particularly in the study of pre-nucleation species (PNS) and their related non-classical precipitation pathways (NCP). Understanding PNS promises pathways to control material formation mechanisms, advancing rational materials design. One promising method for investigating these early stages is dissolution dynamic nuclear polarization (dDNP)-enhanced nuclear magnetic resonance (NMR) spectroscopy. With dDNP, signal enhancements, often exceeding 10,000-fold, enable real-time tracking of fast processes. Recent applications of dDNP to calcium carbonate (CaC) and phosphate (CaP) systems have demonstrated the feasibility of identifying PNS on timescales much shorter than 10 s [1]. However, the structural details remain difficult to resolve due to experimental limitations. Here, we integrate hyperpolarized NMR of PNS with molecular dynamics (MD) simulations and quantum mechanical calculations to access atomistic insights into CaP PNS. By simulating the PNS structure, computing chemical shift parameters, and comparing these to hyperpolarized NMR ‘fingerprint’ spectra, we demonstrate how to derive models of solution-state structural ensembles of PNS, even when very short-lived. Our method not only extends the methodological scope of hyperpolarized NMR but also sheds light on the structural underpinnings of non-classical pathways in material formation, particularly under conditions that have been experimentally underexplored. These insights could lead to new approaches in the design and synthesis of functional materials.

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MolDETR: A chemistry-informed deep learning model for next-generation analysis of molecular spectra

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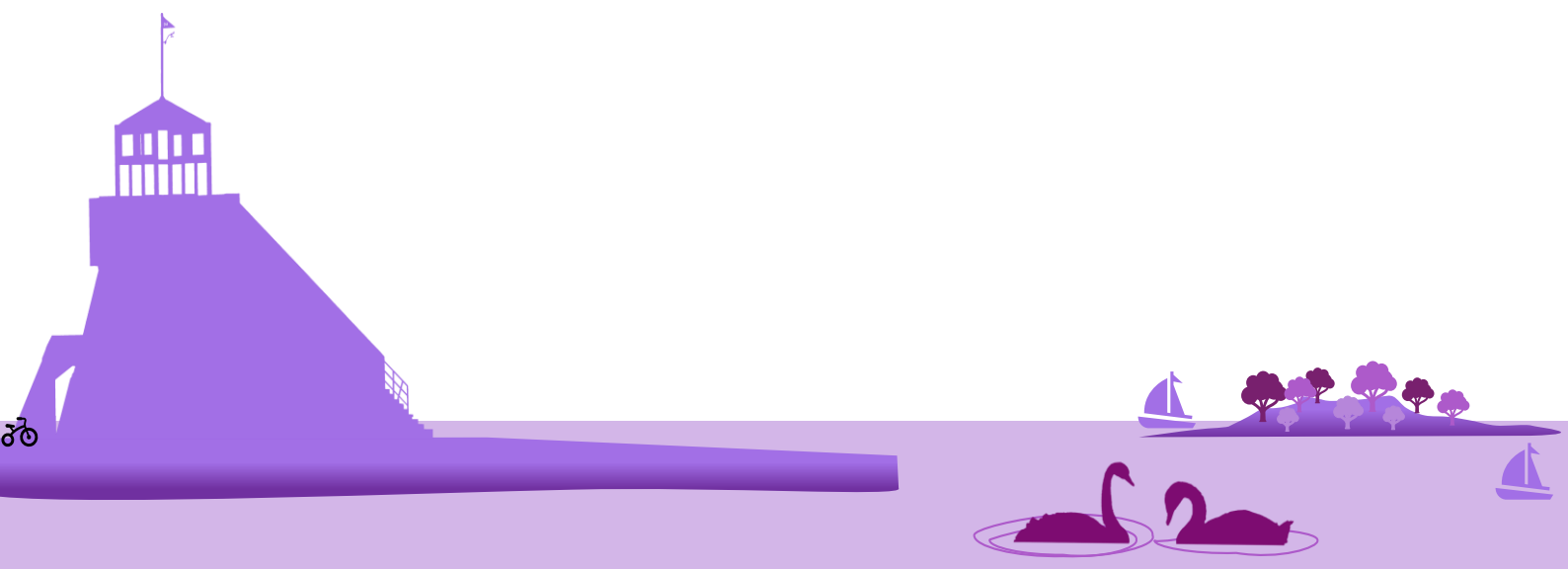
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Automated spectral data analysis is crucial for understanding molecular properties across scientific disciplines. However, current automated methods for interpreting difficult nuclear magnetic resonance (NMR) spectra - a core technique in molecular sciences - struggle with signal overlap, strong coupling patterns, and spectral distortions. These challenges require extensive expertise and prior sample knowledge, limiting scalability and full automation. To address these issues, we introduce MolDETR, a deep learning framework for chemistry-informed molecular analysis inspired by detection transformer models. Trained on synthetic data from quantum mechanical simulations augmented with experimental distortions, MolDETR performs a comprehensive analysis of spectral and molecular features, emulating expert spectroscopists. Applied to difficult experimental one-dimensional proton NMR spectra of small molecules, MolDETR achieves a median absolute error of 0.89 Hz for chemical shift, 0.20 Hz for coupling constant, and 93.5% accuracy in absolute proton count predictions. It outperforms existing methods and expert analysis, allowing for accurate and quantitative interpretation without the need for reference standards or prior knowledge of the compounds or spin systems. Its flexible approach could be extended to other spectral modalities, such as mass spectrometry, X-ray diffraction, and infrared spectroscopy, making it a versatile analytical and computational chemistry tool. This framework could streamline the characterization of unknown drug degradation products in pharmacokinetics, identify bioactive compounds in natural products research, and uncover biomarkers in metabolomics-based clinical diagnostics. MolDETR effectively addresses previously unsolved challenges in spectral data interpretation, enabling more efficient and coherent analysis across these critical scientific and medical domains.



Magnetstein: Web Application for Quantitative NMR Mixture Analysis

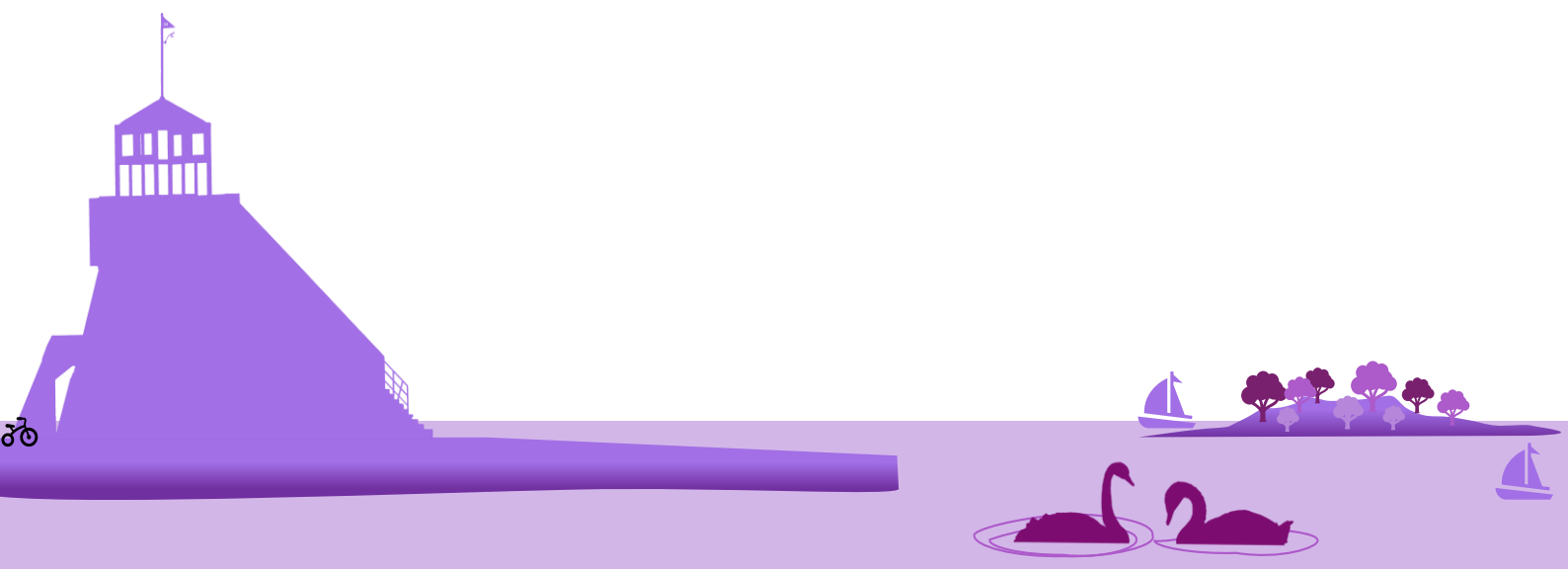
Moszyński, Antoni (1); Goldstein, Antoni (1); Domżał, Barbara (1); Startek, Michał (1); Gambin, Anna (1); Kazimierczuk, Krzysztof (1)

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We present a novel web application for the quantitative analysis of nuclear magnetic resonance spectra. At its core is the previously published Magnetstein algorithm [1], which solves the problem of spectra deconvolution, i.e. determines the relative amounts of components in a mixture. Our innovative approach to this problem, based on the mathematical concept of the Wasserstein distance, makes the algorithm robust to common challenges such as distorted line shapes, peak shifts and low resolution. Thus far, Magnetstein has been available solely in an open-source Python package. Now, by creating the web service with a graphical user interface, we make our software available for a broader scientific community. The usage of our web application requires no knowledge of Python nor other programming languages, is open and free of charge for academics.

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Rela2x: Analytic and automatic NMR relaxation theory

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There is a need for tools that simplify and make the complex theory of NMR relaxation more accessible to a wider audience. Deriving the so-called relaxation superoperator by hand has traditionally been both challenging and error-prone, and previous approaches have relied primarily on numerical methods.

In this presentation, I introduce Rela2x [1,2], a freely available Python-based tool that fully automates the computation of the analytic relaxation superoperator for general spin systems and their interactions. With minimal, human-readable input, the program can compute, analyze, visualize, and approximate the relaxation superoperator, as well as extract all possible relaxation rates within the system. Rela2x serves as a valuable resource for studying and exploring various aspects of NMR relaxation theory, making it more accessible to all scientists in the field.

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[2] <https://github.com/hillaper/Rela2x>



Nuclear spin-state transport in nonlinear kinetic processes

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NMR and MRI techniques are frequently used to study chemical kinetics, particularly in metabolic processes within living systems; many such processes are higher order in nature (e.g. the Warburg effect [1]). Modelling the spin dynamics of such nonlinear reactions has remained challenging because fundamental equations of motion in quantum mechanics for the quantum state [2] and density operator [3] are linear. However, the law of mass action in chemical kinetics [4] and Navier-Stokes [5,6] equations in hydrodynamics are not fundamental; they are statistical approximations and can be non-linear. This incompatibility creates insidious difficulties in theoretical descriptions of systems where quantum processes coexist with chemical kinetics and spatial transport, for example in spin chemistry, magnetic resonance imaging of complex metabolic and hydrodynamic processes, and – our predicament here – nuclear magnetic resonance in microfluidic chips. I will report our progress with this problem in the context of NMR, where good approximate solutions exist under the assumption that nuclear spin states do not influence hydrodynamics or chemical kinetics. We found numerically stable solution methods for problems involving simultaneous non-linear kinetics, diffusion, flow, and quantum mechanical evolution in non-trivial spin systems. The results are used to model magnetic resonance spectroscopy and imaging in microfluidic chips.

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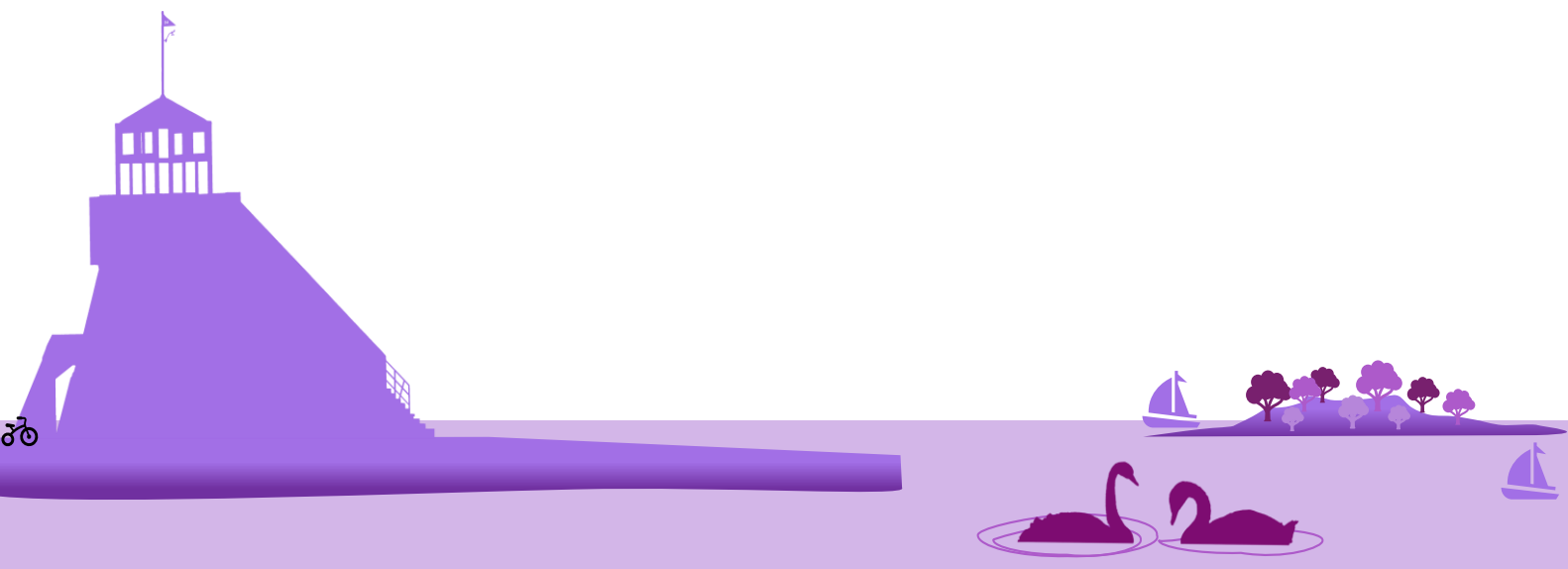
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Bridging experiments with theory: computational solid-state NMR insight into a paramagnetic iron catalyst

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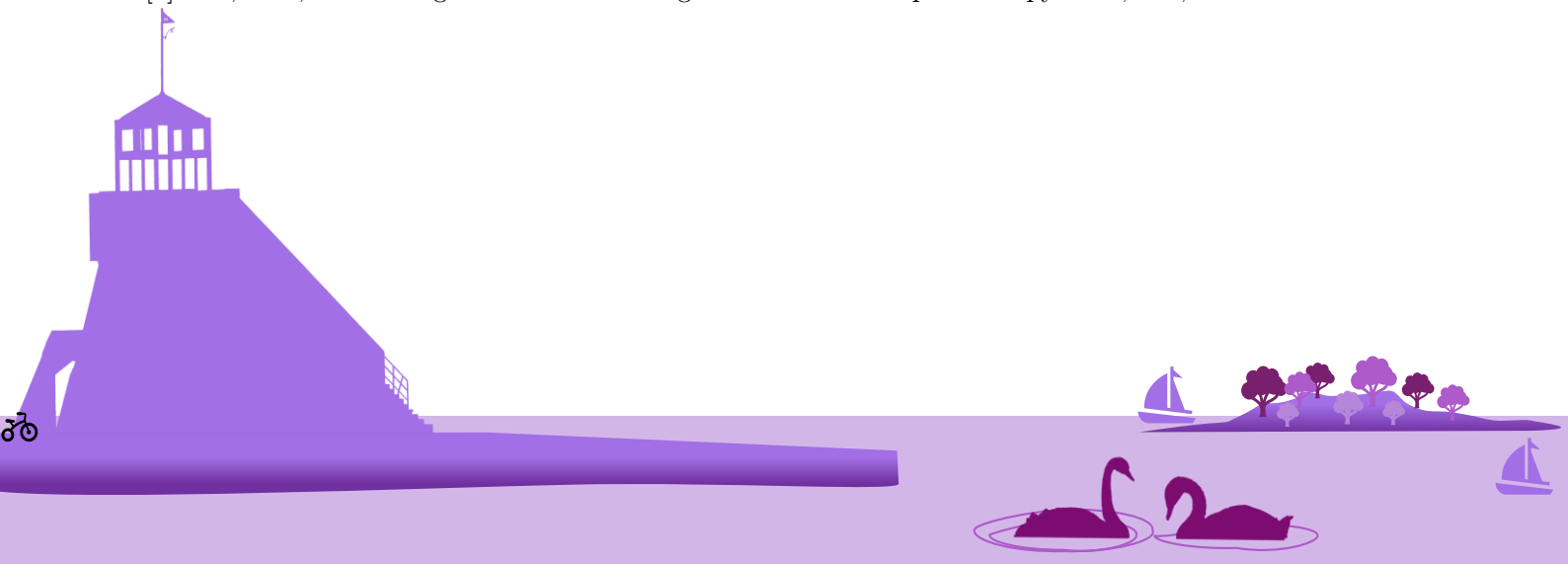
Dimeric Fe(II) tris(tert-butoxy)siloxide is a molecular precursor that catalyses the non-oxidative coupling of methane once grafted at the surface of an amorphous silica [1]. Solid-state MAS NMR is uniquely positioned to bridge the gap between the frozen XRD structure of the crystalline precursor, and the structure of the supported catalyst in operating condition, providing a detailed knowledge of the atomic-scale features of the catalytic centres. However, the very large shifts, shift anisotropies, and short nuclear spin relaxation induced by the strong paramagnetic interactions with the two iron ions drastically complicate the acquisition and the interpretation of the NMR spectra, requiring the development of additional spectroscopic and computational methods [2].

The ^1H and ^{13}C NMR signatures of the precursor were obtained using fast MAS and tailored broadband excitation pulse schemes, incorporated into 1D and 2D experiments. The signals could only be interpreted by computing the relevant NMR and EPR parameters with advanced computational methods models to account for the dynamic motion of the ligands. The assignment provides an unambiguous description of the dimer's structure, electronic structure, and dynamic behaviour distinctly revealing two separate dynamic regimes between the ligands.

The ^1H and ^{13}C NMR signatures of the grafted catalyst reveals a large structural difference with the precursor, with a fast dynamic regime, corresponding to the loss of inequivalence between the ligands. It indicates that the bridge between the iron ions is broken upon grafting, leading to the formation of dispersed single-iron sites, and highlighting paramagnetic solid-state NMR as an avenue for structural characterization in heterogeneous catalysis.

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SimOS: A Python Framework for Simulations of Optically Addressable Spins

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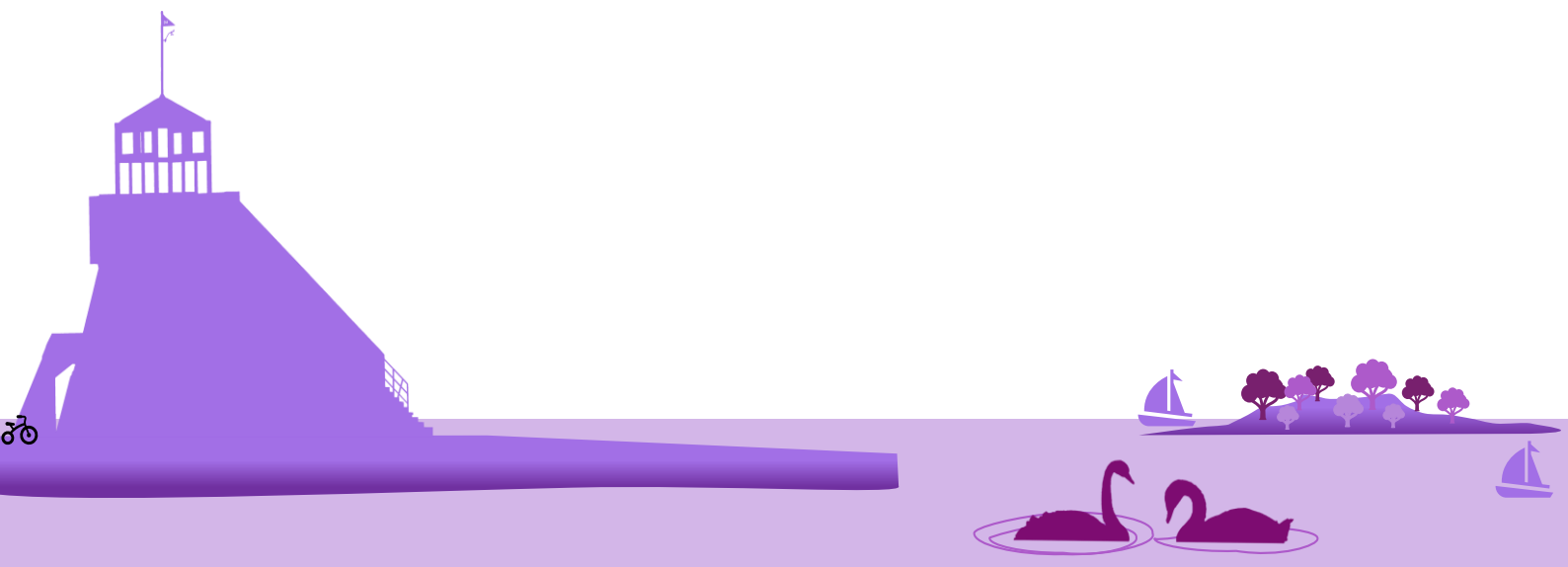
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Optically addressable spins are an emerging platform for quantum sensing, communication, and computing. One important class of optically active spins are point defects in wide band gap semiconductors, e.g., color centers in diamond and silicon carbide. The most well-understood defect in diamond, the nitrogen-vacancy (NV) center, has been investigated for decades, and demonstrates significant potential for applications in nanoscale sensing. Another important class is composed of molecular-based qubit systems, such as photogenerated spin-correlated radical pairs (SCRPs). Here, chemical synthesis offers atomistic control and great versatility over molecular structure, thereby enabling precise tuning of important characteristics of the quantum system.

Here, we introduce SimOS, a simulation library for optically addressable spins in Python. SimOS provides a simple yet versatile interface for the construction, manipulation, and time-evolution of operators and superoperators for quantum systems of spins and electronic levels. The time evolution under combined coherent and incoherent dynamics is formulated in Lindblad form and solver routines facilitate fast implementation and study of complex protocols. As the computational backend, SimOS leverages established Python libraries such as qutip and numpy. To further offload the computational workload, an interface is provided for parallel time integration using batched BLAS (Basic Linear Algebra Subprograms) routines on graphics cards. While developed with simulation of optically addressable spins in mind, SimOS is also a fully-fledged simulation suite for NMR and EPR experiments in Python. Due to its backend-agnostic approach, simulations can and will be easily ported to more frameworks such as PyTorch or CuPy in the future.

Available at <https://simos.kherb.io>



FAIRSpec-Ready Spectroscopic Data Collections - Enabling FAIR publication of spectral data

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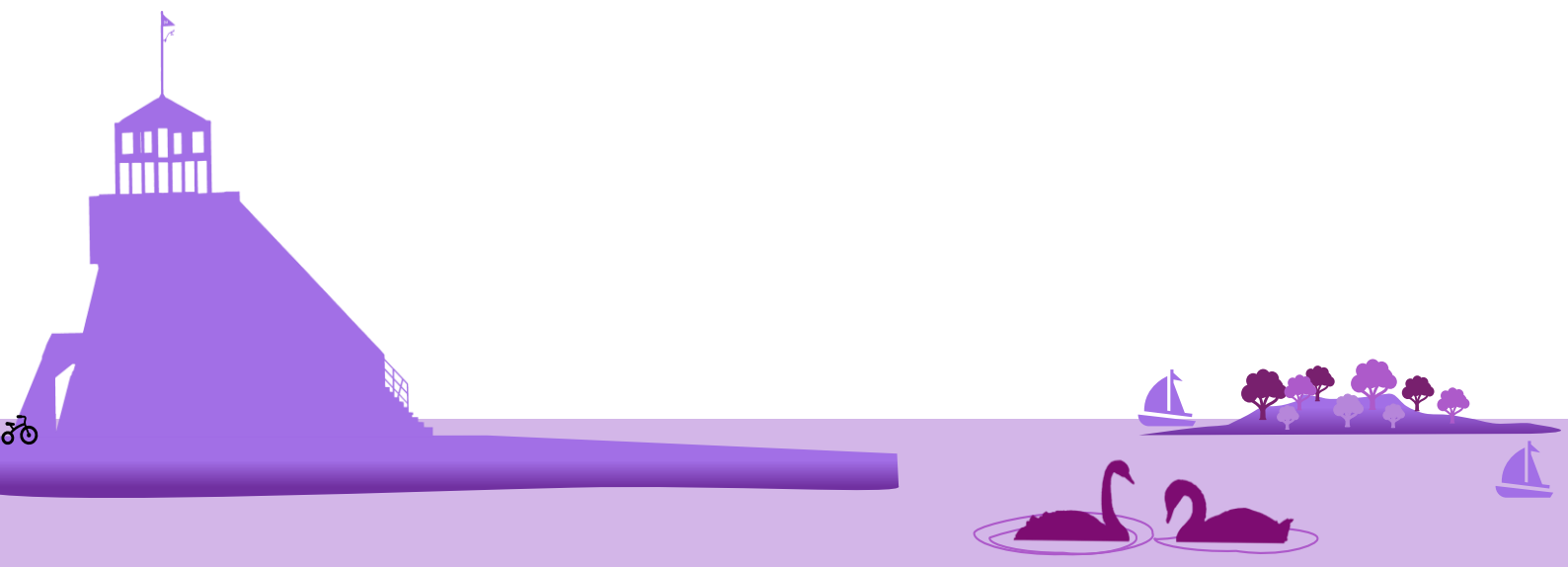
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We introduce the concept of a FAIRSpec-ready spectroscopic data collection – that is, a collection of instrument data, chemical structure representations, and related digital items that is ready to be automatically or semi-automatically extracted for metadata that will allow the production of an IUPAC FAIRSpec Finding Aid. Associating this finding aid with the collection produces an IUPAC FAIRSpec Data Collection. The challenge we set for researchers is relatively simple: to maintain their data in a form that allows critical metadata to be extracted in a discipline-specific way, increasing the probability that the data will be findable and reusable both during the research process and after publication. We focus on a few specific suggestions that researchers can use to maximize the “fairness” of their spectroscopic data collection. Most importantly, following these guidelines ensures that instrument datasets are unambiguously associated with chemical structure. The guidelines promote the inclusion of the instrument dataset itself in the collection and describe ways of organizing the collection such that automated metadata creation is possible. In these guidelines we emphasize the importance of systematically organizing data throughout the entire research process, not just at the time of publication. The talk introduces the concept of FAIRSpec-ready collections and the Finding Aid. It discusses the guidelines and shows their practical application.



SLEEPY: Simple simulation of relaxation and dynamics in NMR

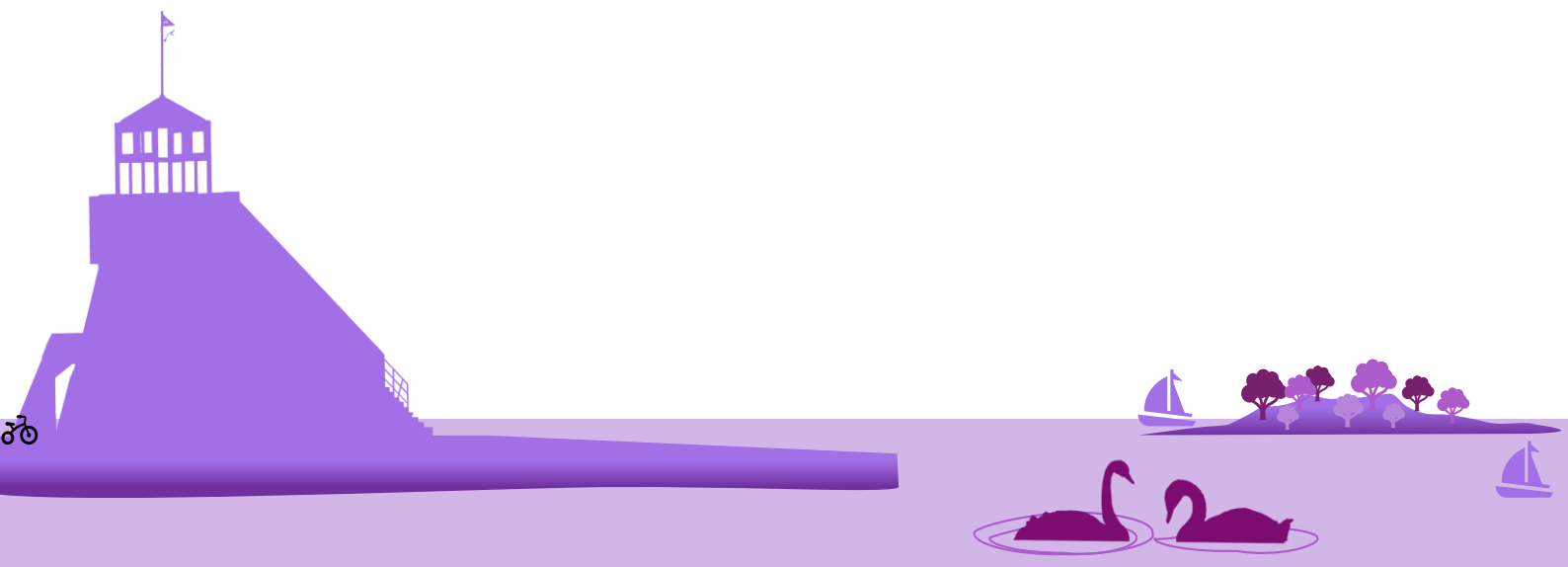
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NMR is a key experimental method for dynamics characterization, where measurement of relaxation behavior and residual couplings can be used to perform detailed dynamics analyses. NMR is uniquely flexible, but highly complex, with nearly endless possibilities for pulse sequences to select different pathways for magnetization. Relaxation studies have largely been limited to very simple pulse sequences, because it is not trivial to predict correlation time dependence of more complex sequences, requiring simulation to determine sensitivity of a given experiment to motion. SLEEPY (Spins in Liouville-space for rElaxation and Exchange in PYthon) addresses this challenge by providing a package that simplifies the inclusion of motion and relaxation in NMR simulations. SLEEPY is a complete simulation package, allowing arbitrary pulse sequences for solution and solid-state (static and spinning) NMR, in the rotating and lab frames. Object-oriented implementation in Python makes SLEEPY particularly easy to use for non-experts, and also allows it to be run using online resources such as Google Colab with minimal effort.

We present the basics of SLEEPY usage, demonstrate its range of capabilities (longitudinal and transverse relaxation, paramagnetic effects, DNP, etc.), and also highlight an online tutorial, available at <http://sleepy-nmr.org>, which includes various examples that may be explored interactively via Google Colab.



Prediction of ^{31}P NMR Shifts using Graph Neural Networks and Transfer Learning

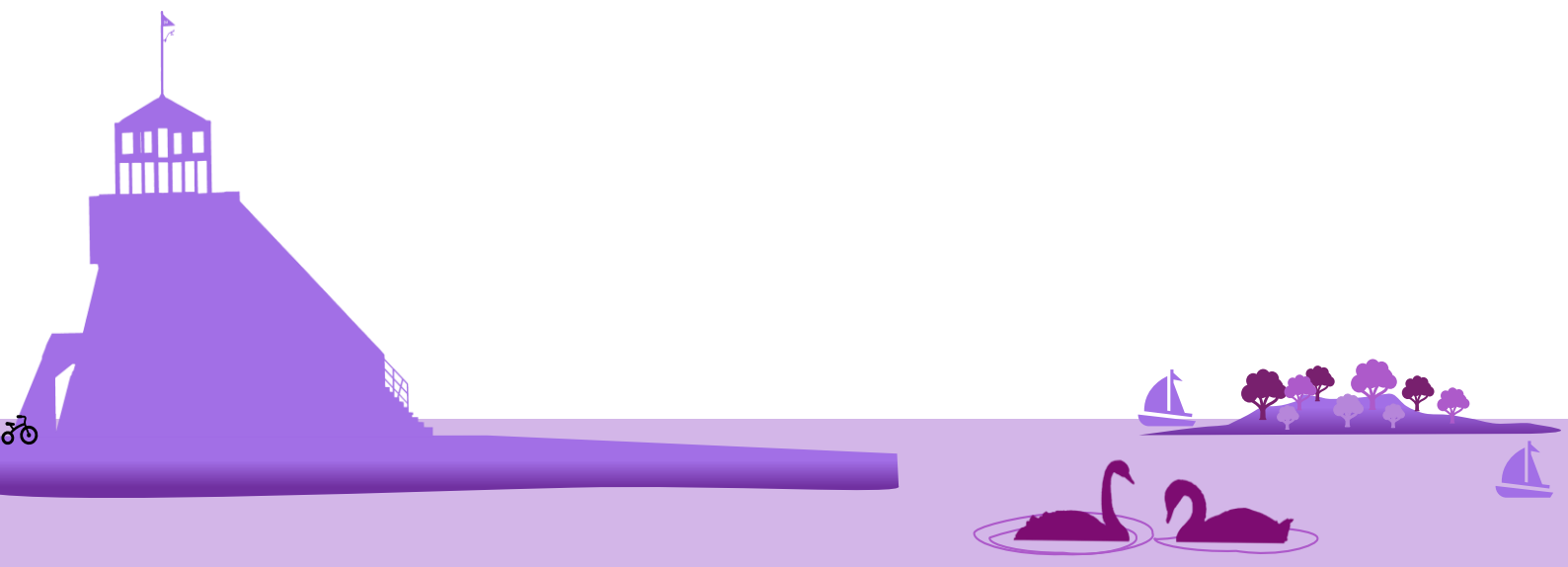
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The accurate prediction of nuclear magnetic resonance (NMR) chemical shifts is essential for structural elucidation and reaction mechanism studies. Traditional quantum chemistry methods, such as density functional theory (DFT), offer high accuracy but are computationally expensive, limiting their applicability to large-scale molecular datasets. As a viable alternative, machine-learning approaches have emerged, capitalizing on large databases that host experimental NMR spectra. Unfortunately, these databases currently mainly focus on ^1H and ^{13}C nuclei.

In this work, we present a machine learning approach utilizing graph neural networks (GNNs) with transfer learning to predict ^{31}P NMR chemical shifts efficiently and accurately. Our model is initially trained on the abundant ^1H and ^{13}C data before being refined on our Ilm-NMR-P31 database. By leveraging molecular graph representations, the GNN captures local and global electronic environments that influence the chemical shift, while the transfer learning approach allows us to improve the prediction accuracy without the need to collect large amounts of novel ^{31}P data. Our results demonstrate a significant improvement in accuracy over traditional heuristic-based methods, achieving a mean absolute error (MAE) within the range of DFT calculations. We discuss the implications of this approach for accelerating NMR-driven molecular discovery and its potential extension to other nuclei and molecular systems.



Exploring Molecular Conformer Distributions in Solution using NMR Tensor Driven Molecular Dynamics

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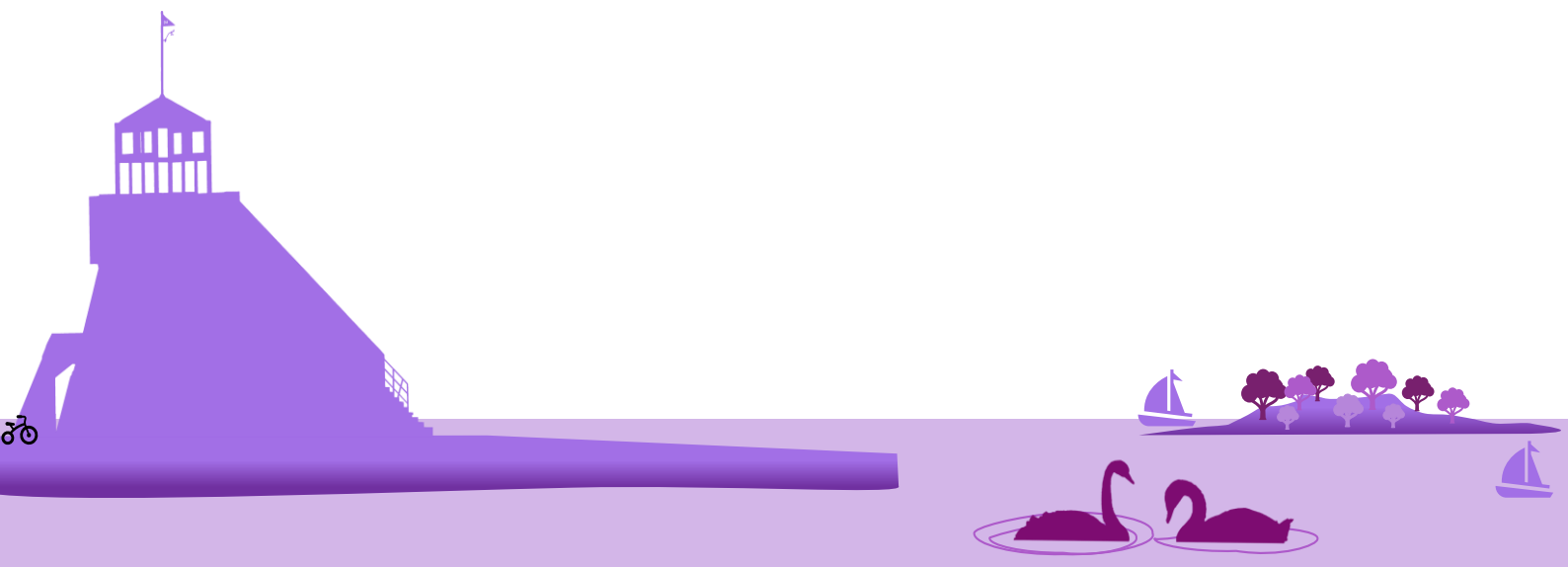
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Traditional NMR structure investigations aim on molecular structures that represent energy minima and fulfil to the same time NMR constraints as for instance NOE distances or J couplings. But NMR constraints are measured mostly at ambient temperatures and the structures are not only governed by energies but by Gibbs free energies that include the entropy term $-T\Delta S$. Most molecules possess several conformers that lead to averaged constraints over the NMR timescale.

By introducing a new type of constraints derived from interaction tensors like RDC it is possible reorient and rotate molecules and molecular parts (MDOC - Molecular Dynamics with tensorial Orientational Constrains). As demonstrated in numerous papers this new method overcomes the former limitations and allows to elucidate conformer equilibria in solution and thereby fulfil readily NMR constraints.

Often RDC are hard or even impossible to measure in abundance. We overcome this problem by introducing ^{13}C - ^1H and ^{15}N - ^1H RDC for all CH and NH bonds and set all tensor components of the dipolar tensors to zero. With these zero tensor constraints we simulate the molecules in solution.

A pseudopeptide with six lysine-like side chains at the amid nitrogens is presented as first example of the new method. In a former NMR structure investigation, it was demonstrated that the most stable form of the pseudopeptide should possess an all-cis backbone. Using our new method, we investigated what happens at ambient temperatures. We obtained in the MDOC simulation a 2:1 distribution of cis and trans forms. In regular MD investigations cis-trans jumps are rare events. Averaging over the 40 nano second trajectory the NOE distances have a 99.99% probability for a 2:1 cis-trans distribution compared to the static minimal structure with the all-cis backbone. Conformers or transient structures that show up at ambient temperatures are mostly reactive and biological active species.



Removing Magnetic Field Noise from NMR Spectra - Reference Deconvolution Revisited

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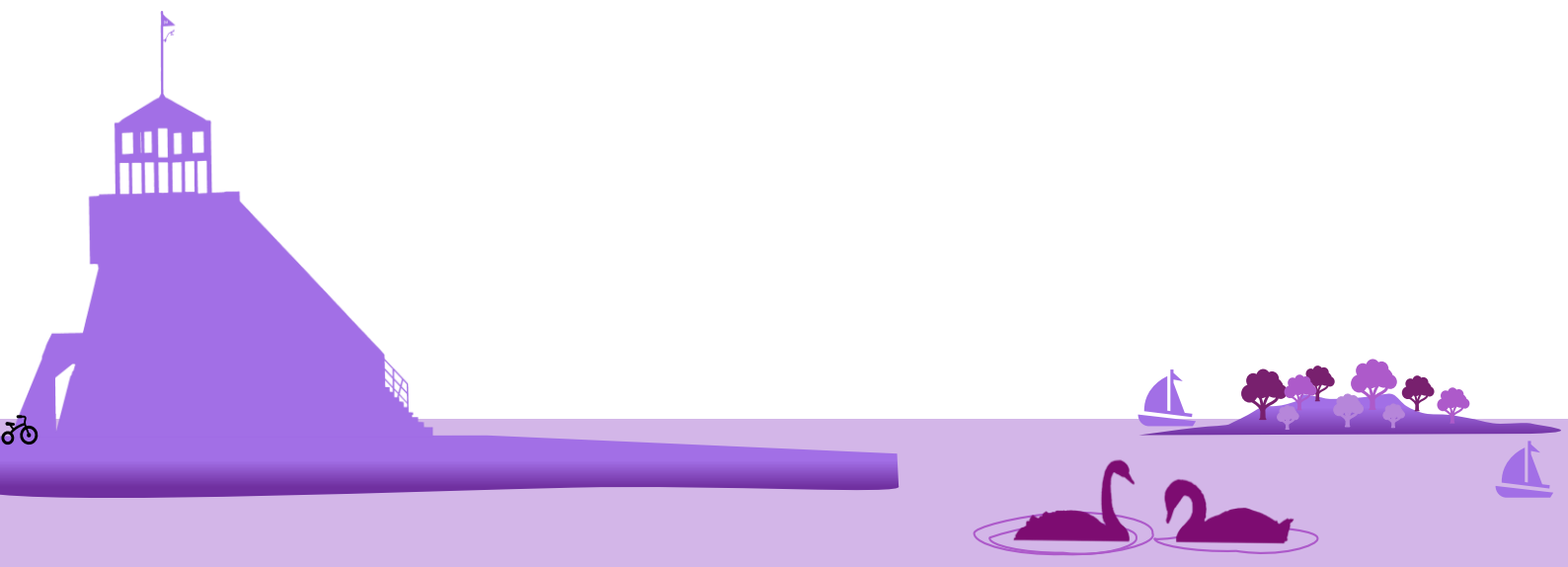
In high-resolution NMR spectroscopy, it is crucial to obtain spectral data free from the effects of static and dynamic magnetic field perturbations. In cases where standard hardware tools (shim coils, field-frequency locking, shielding, filtering, etc.) are unavailable or ineffective, post-acquisition mathematical correction of spectroscopic data is an attractive solution.

One of the most powerful mathematical tools for removing the effects of static and dynamic imperfections of the main magnetic field from NMR data is reference deconvolution. This multiplies the time domain data by a correction function deduced from the experimental reference signal, for which the ideal form, for example, a simple singlet, is known.

In our presentation we will describe the mathematical basis of reference deconvolution and illustrate its efficiency in the presence of strong static and dynamic magnetic field perturbations. The former may be caused, for instance, by poor shimming, or imperfect orientation of the probe, the latter by cold head operation in cryogen-free superconducting magnets, fast field drift due to joints resistance, or environmental disturbances such as those found in busy magnet-producing companies, DC based elevators and underground transport services.

To illustrate the practical efficiency of reference deconvolution for strong field imperfections, we have made a series of single- and multi-scan experiments. These involved relatively simple chemical compounds containing the basic features of typical multi-spin liquid-state systems. All experiments were carried out on a cryogen-free superconducting magnet, built by our company (Cryogenic Ltd). The corresponding experimental evidence for one- and two-dimensional NMR will be presented.

This work is an extension of our previous studies on hardware and software noise suppression in multi-scan/multi-dimensional high-resolution liquid state NMR.



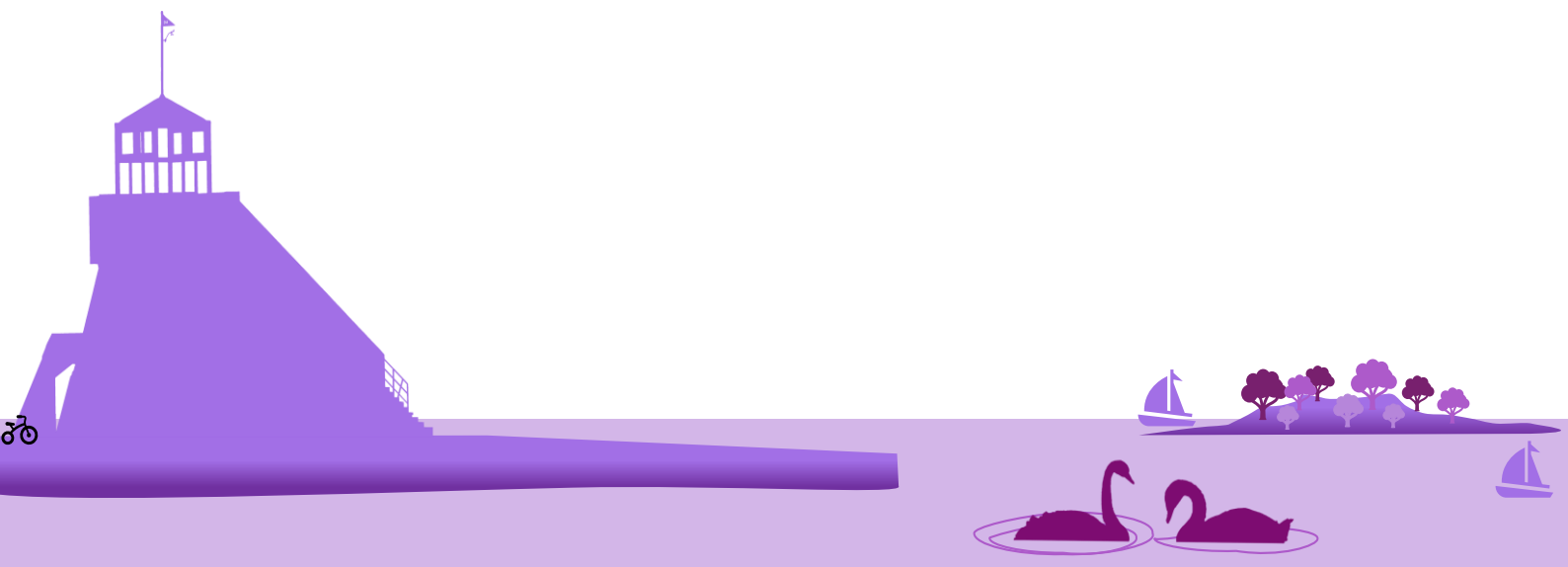
HQSpectrum: Interactive Analysis of Spin-Resolved NMR Spectra

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Analyzing complex NMR spectra remains a significant challenge. The task of performing an assignment can be enhanced with an accurate, quantum-mechanical simulation of the spectrum. This is particularly the case for benchtop spectrometers: while the devices are comparably inexpensive and compact, overlapping peaks and broad multiplets often render spectra more complicated than those from high-field instruments. We present HQSpectrum, a cloud-based software for spectrum simulation and analysis with an intuitive web frontend. In its backend, the platform utilizes a solver that calculates an accurate NMR spectrum for each given set of NMR parameters. Performing a quantum-mechanical spectrum simulation is a non-trivial task, as the computational effort of a naive implementation scales exponentially with the number of spins, making calculations unfeasible for many molecules of practical interest. In our implementation, a clustering approximation and symmetry considerations permit spectra of molecules with tens of spins to be computed with negligible truncation errors in a matter of minutes. Contributions of individual spins to the overall spectrum are available as part of the solution, permitting even complicated, overlapping multiplets to be disentangled. Our demonstration makes use of chemical NMR parameters computed from an in-house density functional theory workflow, involving a rigorous sampling of conformers. The HQSpectrum web frontend permits chemical shifts, J-couplings and broadenings to be adjusted interactively to fine-tune the agreement of the simulated with the experimental spectrum. This work demonstrates how quantum-mechanical spectrum simulation, combined with computational parameter estimation, can be integrated in a user-friendly solution for spectrum analysis, including but not only for low-field spectrometers.



Computational Xe NMR in carbon nanotubes

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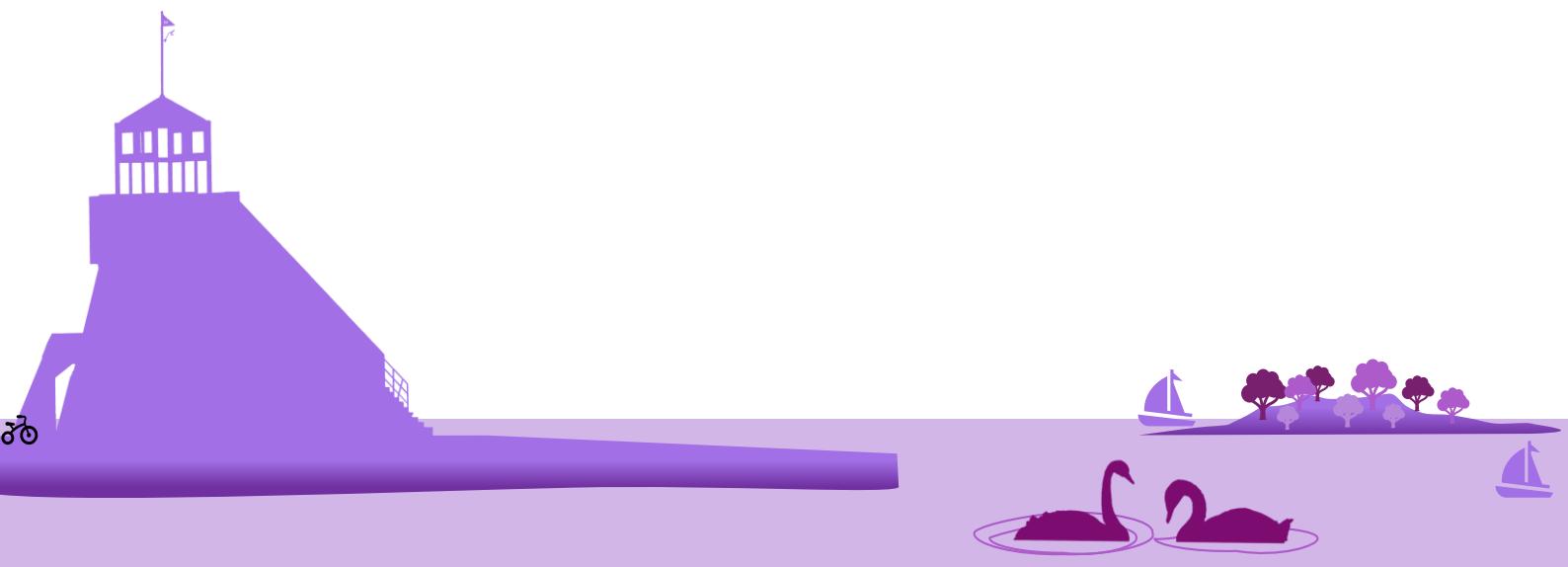
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Carbon nanotubes (CNTs) have been extensively studied due to their unique and fascinating properties. One effective method for investigating porous materials like CNTs is nuclear magnetic resonance (NMR), particularly xenon NMR [1, 2]. Although there have been several experimental studies utilizing Xe NMR on CNTs [3–8], no computational studies have been published to date. In our studies [9, 10], we perform quantum chemical calculations, Monte Carlo (MC) simulations, and molecular dynamics simulations to determine the Xe chemical shift for various CNTs under different conditions. We explore how factors such as diameter of the CNT, the chirality (n, m) and the chirality families defined by $R = (n - m) \bmod 3$ influence the chemical shift of Xe. Furthermore, we analyze the differences among various Xe sites and the effects of Xe-Xe interactions in static systems [9]. The sites assessed are inside and outside a CNT and at the groove between two or three nanotubes. We also consider temperature effects, including Xe-Xe interactions with varying numbers of Xe atoms in the system, both inside a CNT and outside of two-nanotube bundles [10]. Moreover, we observe a temperature dependence of the chemical shift.

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A Deep Learning Approach for Removing Vibration Artifacts in Shuttled NMR Experiments

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High-resolution relaxometry (HRR) measures nuclear spin relaxation rates across a wide range of magnetic fields, using a sample shuttle to move the sample in the stray field of a high-field magnet. HRR combines high sensitivity and resolution of high-field NMR with relaxation at fields down to tens of millitesla. Relaxation dispersion profiles hold information about dynamic processes such as ligand/protein interactions and internal dynamics in biomolecules. Fast shuttle transfer times (<65 ms) are necessary to minimize polarization loss. In motor-driven systems, vibrations can propagate through supporting structures, the magnet's dewar, and detection coils, leading to anti-phase artefacts around the peaks in the spectrum.

We developed a FidNet-based deep-learning model to eliminate vibration artefacts along with well calibrated predicted standard errors for the transformed spectrum. The model effectively removes vibration artefacts and restores signal intensities, providing spectra that can be easily quantified to determine NMR dispersion profiles.

The Deep-Learning model was trained using on-fly data generation where synthetic spectra were modulated with a point-spread function yielding anti-phase artefacts. The network's performance was evaluated using synthetic and experimental HRR spectra through:

RMSD – to quantify deviations between predicted and target spectra.

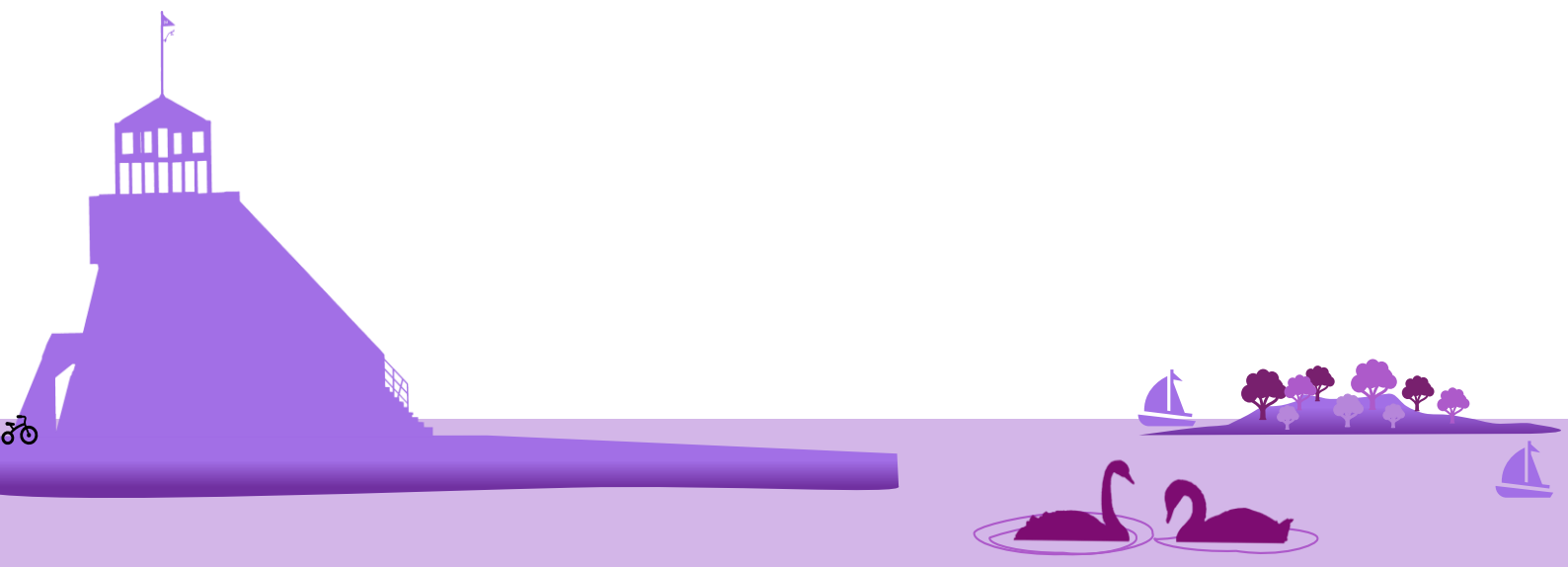
Uncertainty calibration – to ensure confidence intervals accurately reflect real errors.

Spectral integrity metrics – to verify peak positions, linewidths, and intensities.

Quantum simulations – Hilbert space simulations of molecules deposited in the GISSMO database were high-fidelity references.

Experimental validation – HRR spectra confirmed artefact suppression while preserving relaxation parameters.

Our machine-learning framework shows significant potential in accurately processing complex signal data, potentially greatly enhancing the quality and reliability of NMR relaxometry measurements.



Water activity for n-component mixtures from MD simulations with Kirkwood-Buff theory

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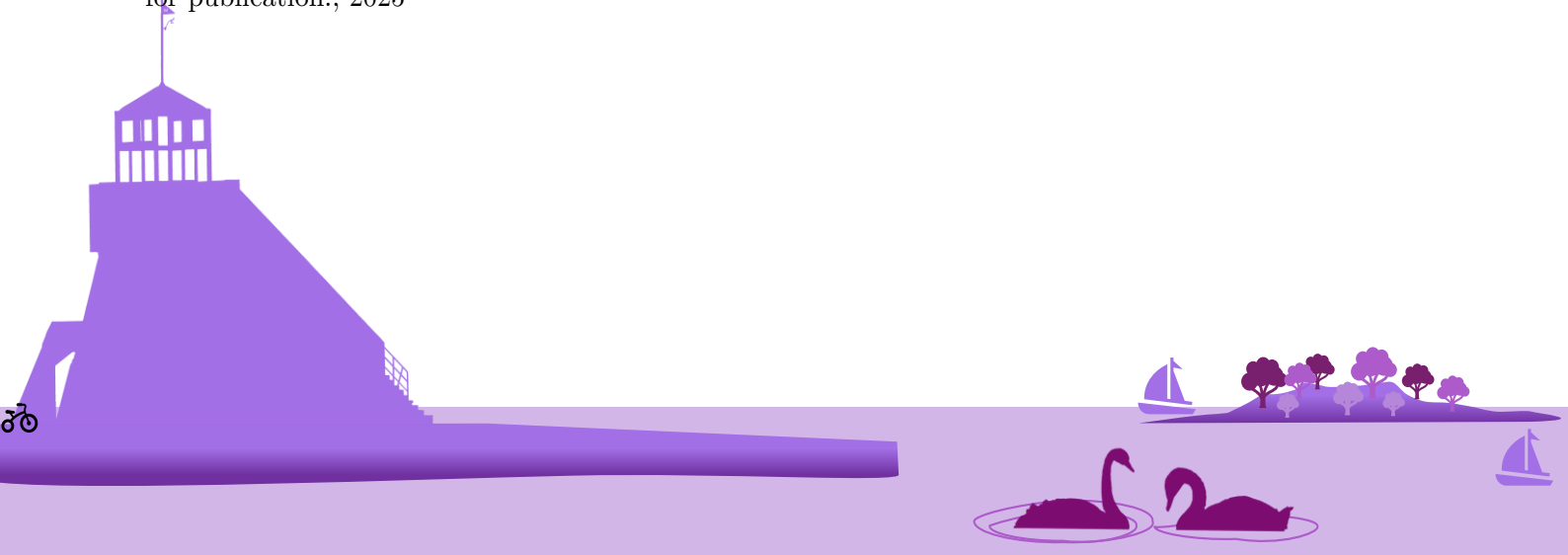
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Kirkwood-Buff (KB) theory (1-3) is one of the most important theories of solutions, which allows one to express experimentally measurable thermodynamic quantities in terms of radial distribution functions, directly obtainable from molecular dynamics (MD) simulations. The KB theory has been successfully applied in many studies (4,5) to obtain molar volumes, isothermal compressibilities, and the activity of solutes and solvents from MD simulations, but usually this is done for binary solutions (6).

This work aims to use the KB theory to compute the water activity for n-component mixtures. This is achieved numerically, allowing one to avoid the cumbersome analytic expressions for solutions with higher order components. The model is tested with the ternary and quaternary systems, water-NaCl and water-NaCl-LiCl, respectively, at high concentrations. Then we compared the water activity from previous work (7), where nuclear magnetic resonance along with climate modelling techniques were used, to probe aqueous systems of sodium decanoate, since these are relevant for climate science.

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Anti-Hermitian Magnetoelectric Interferences in Chiral Molecules

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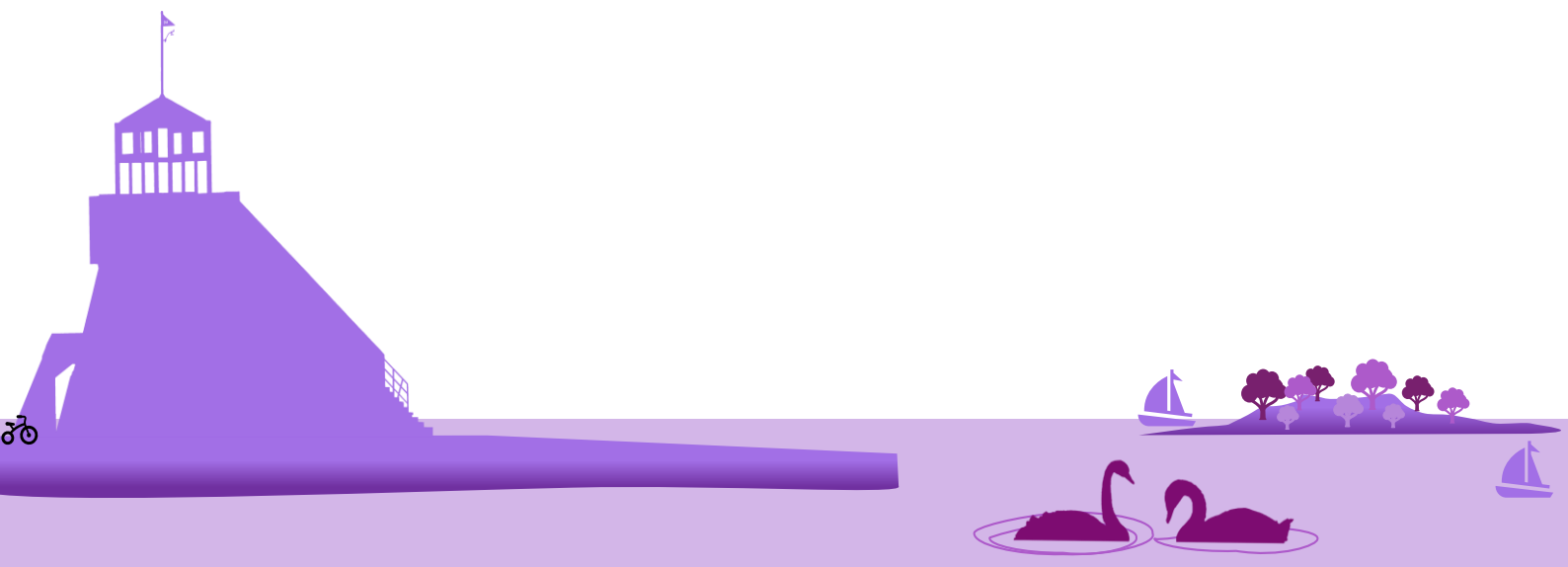
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This theoretical study uncovers a novel mechanism by which chiral molecules exhibit anti-Hermitian behaviour in their spin dynamics, subjected to simultaneous electric and magnetic fields. This effect arises from magnetoelectric interference in dipolar spin interactions and opens new opportunities for chirality-sensitive NMR spectroscopy.

Using the Liouville-space formalism, I derived an effective Liouvillian for a three-spin system embedded in a chiral molecular environment and perturbed by an external electric field. Analysis of the second-order perturbative contributions reveals that chirality, when combined with the partial alignment of the polar molecule by the electric field, induces an anti-Hermitian component in the spin system's evolution. These terms respect parity-time (PT) symmetry, generating coherent yet non-Hermitian spin dynamics. Importantly, the resulting evolution depends on molecular handedness, offering a fundamentally new way for chiral discrimination in NMR.

The effect is particularly pronounced in strongly coupled spin systems, such as the nearly equivalent protons of a methylene group, which are especially well-suited to experimental detection. Under field strengths — 10 kV/cm for the electric field and 10 T for the magnetic field — the estimated chirality-dependent signal reaches approximately one ten-thousandth of a conventional NMR signal.

This work establishes anti-Hermitian spin dynamics as a physically observable phenomenon in NMR. It introduces a new conceptual framework for detecting and characterizing molecular chirality through external field-induced spin interactions.



Water Dynamics in Anisotropic Hydrogels as a Model for Cellular Systems

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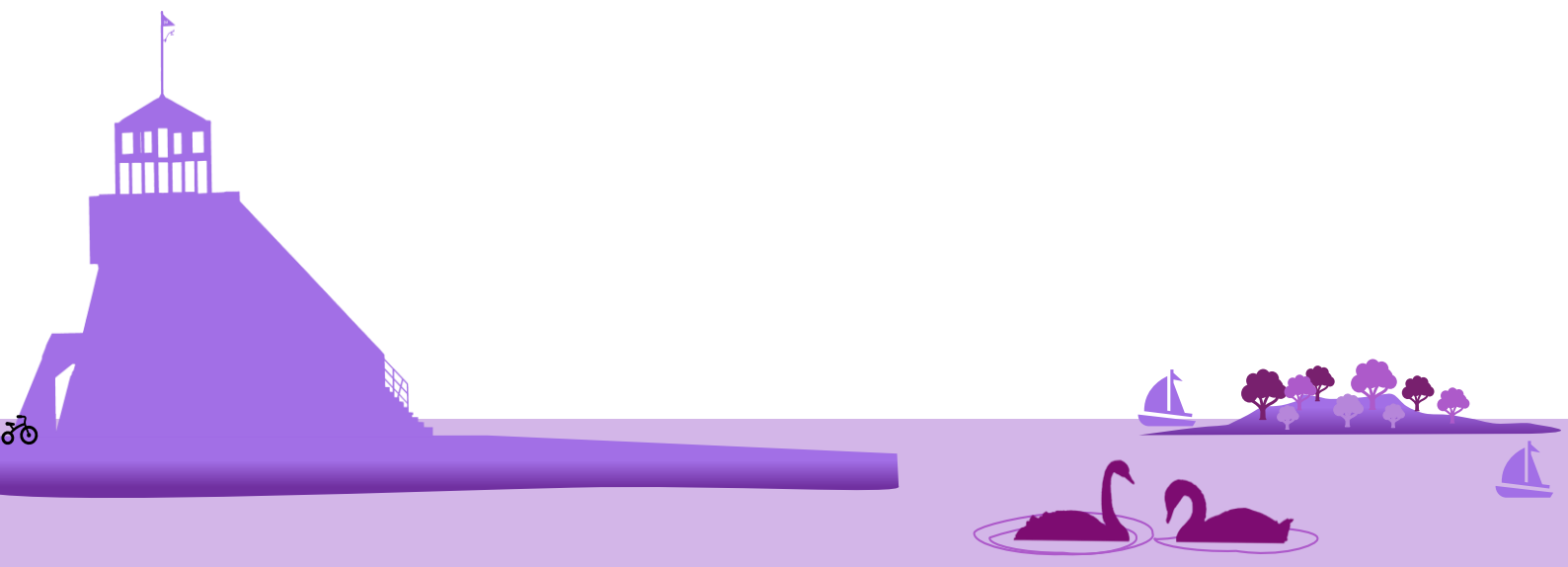
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Water and ions (e.g., $^{23}\text{Na}^+$) play a critical role in living systems. Their mobility and ordering in various tissues differ in ways that are functionally important, and these are identifiable with nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI). To advance our understanding of water dynamics in tissues like tendons, cartilage, neuroaxonal bundles and muscles, we investigated stretched and compressed hydrogels as model anisotropic media. Mechanical deformation of the hydrogels induces motional averaging and alignment effects on embedded quadrupolar nuclear spins, such as ^2H , ^{17}O and ^{23}Na , resulting in residual quadrupolar couplings (RQCs) observable in NMR spectra as additional peaks and splittings, compared with isotropic solutions. Complementary molecular dynamics (MD) simulations provide insights into the dynamics of water in hydrogels including rotational timescales, water exchange rates with the polymer network, and the distribution between free and bound water populations. The data inform a two-site exchange model that was implemented in the Spinach NMR simulation framework, capturing motion between isotropic (free) and anisotropic (bound) environments. Our model successfully reproduces experimental spectra and enables extraction of hidden parameters such as the RQC and rotational correlation time of water in the bound state. To overcome challenges associated with distributions of RQCs, we used one-dimensional slice-selective ^2H NMR imaging along the stretching axis, thus reducing the observed heterogeneity of RQCs and improving spectral resolution. The combined experimental and computational approaches offer a quantitative framework for interpreting water dynamics in partially aligned media. The findings are directly applicable to more complex systems, including protein-bound water/ions and intracellular/extracellular water/ions in high-field NMR and $^2\text{H}/^{23}\text{Na}$ MRI, extends the utility of this model to in vivo environments.



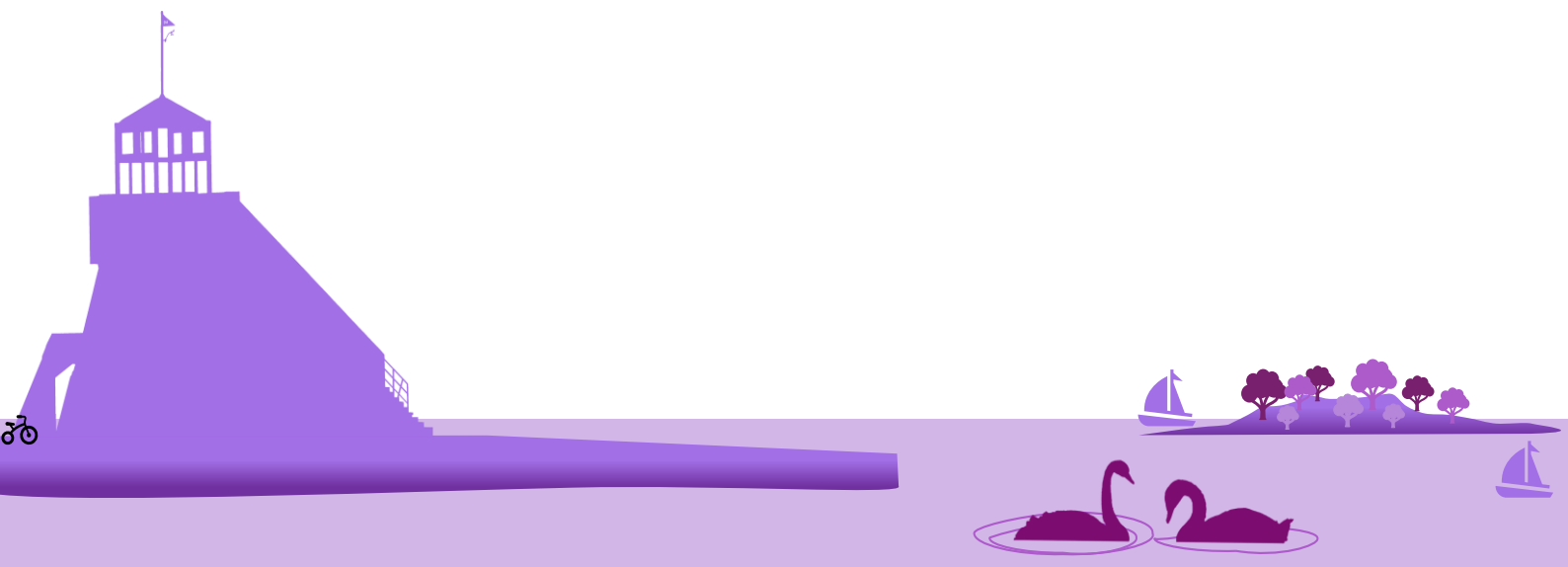
High-Quality Reconstruction for Laplace NMR Based on Deep Learning

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Laplace NMR is a powerful tool for studying molecular dynamics and spin interactions, providing diffusion and relaxation information that complements Fourier NMR for composition and structure analysis. However, the application of Laplace NMR is subject to the performance of signal processing algorithms such as Inverse Laplace Transform (ILT). Due to the ill-posed nature of ILT problems, satisfactory Laplace NMR reconstruction remains challenging. Although the satisfactory outcomes of existing Laplace NMR processing methods, tedious regularization parameter adjustments and lengthy optimization iteration process cause many practical issues remain unresolved. Here, we propose a proof-of-concept deep learning method for Laplace NMR reconstruction. By integrating prior knowledge of the mathematical and physical laws governing one-dimensional (1D) and two-dimensional (2D) decay signals, we construct a forward model to simulate relationships among decay factors, avoiding the need for large amounts of experimental data. The architecture is based on Transformer encoders with multi-layer perceptrons (MLP) and multi-head attention (MHA). For 1D Laplace NMR reconstruction, we treat the domain transformation from raw decay signals to spectra as a language translation task, where MLP extracts molecular features and MHA enhances feature consistency. To address the challenge of handling multi-dimensional data, strategies from Vision Transformers (ViT) are employed for 2D Laplace NMR reconstruction, where 2D decay signals are divided into patches fused with sinusoidal positional embeddings, enabling Transformer encoders to generate a comprehensive feature representation leading to accurate 2D decay factor distributions. Experimental results demonstrate the practical effectiveness of this method for 1D and 2D relaxation and diffusion measurements on commercial NMR instruments. This work provides an advanced and impactful approach to Laplace NMR analysis.



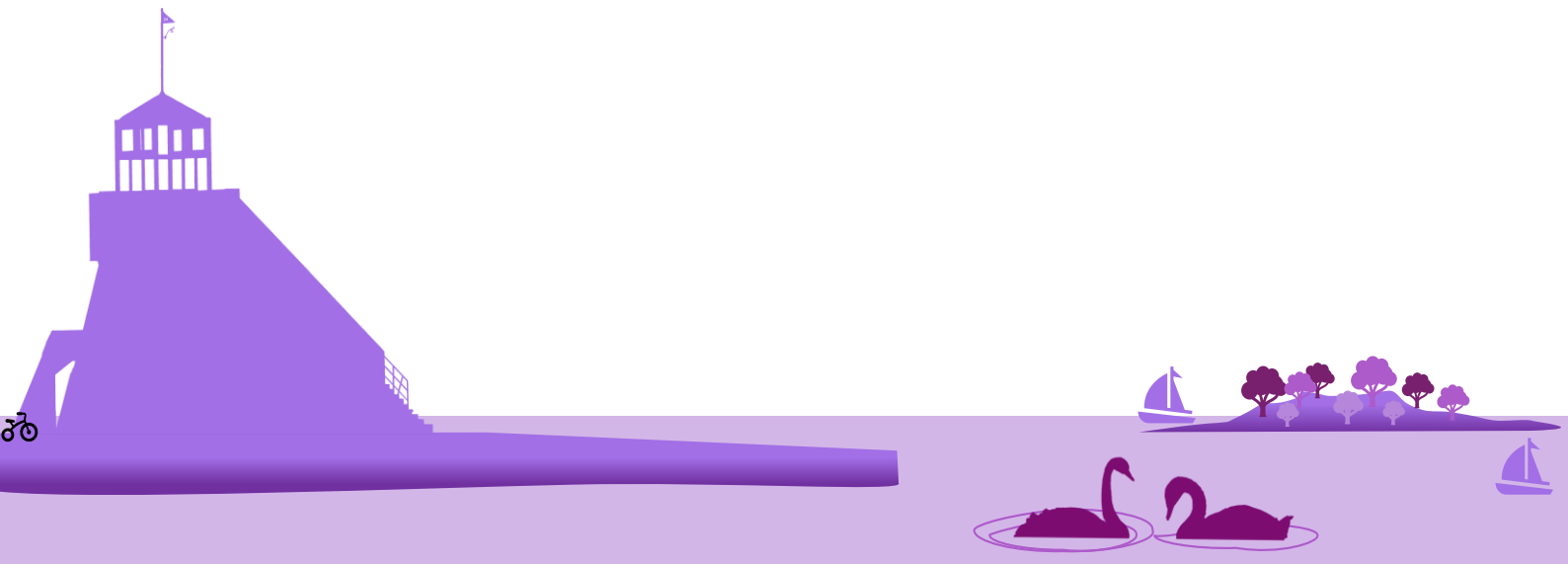
Optimization of magnetic resonance pulse using finite element method

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Optimal control methods are extensively employed in manipulating spin states, such as in the design of universal rotation and selective excitation pulses in magnetic resonance. Gradient ascent pulse engineering (GRAPE) is particularly efficient for gradient computation, and numerous variants have been developed to enhance its performance. In practical scenarios, optimal control models are often designed for ensemble spin systems, accounting for field inhomogeneities, chemical shifts, and other variations. These considerations lead to large-scale models, with computation times ranging from hours to days, even on parallel computing systems. Gradient-based optimization can be accelerated by either improving the efficiency of gradient evaluation or increasing the convergence rate. In this work, we present an approach to accelerate 1-spin pulse optimization by combining the finite element method (FEM) with the method of moving asymptotes (MMA). FEM reformulates the Liouville–von Neumann equation as a linear system, enabling over 10 times faster gradient computation than GRAPE with a relative accuracy loss below 0.1%. MMA further enhances optimization by achieving faster convergence than L-BFGS, with performance comparable to Newton’s method.



Exploring Sphingolipid Membranes with Solid-State NMR, Molecular Dynamics and Quantum Chemical Calculations

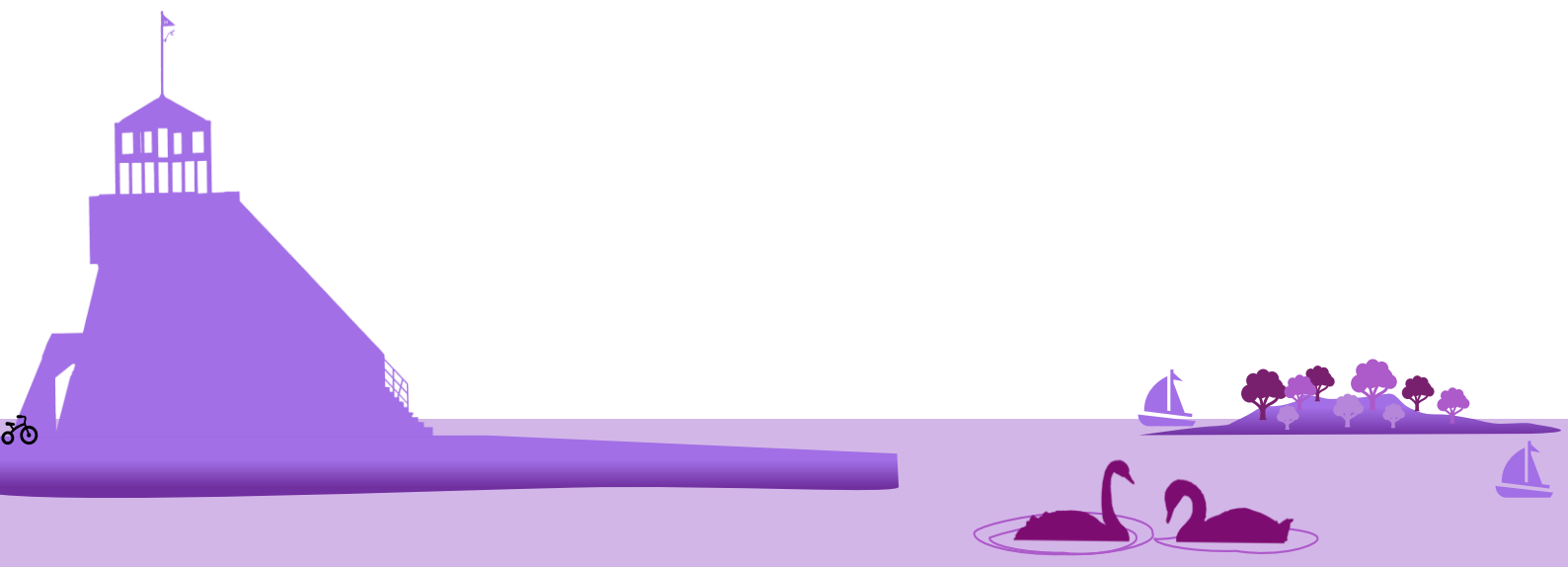
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Computational chemistry is frequently employed to characterise models of biological membranes with atomic resolution, namely through molecular dynamics (MD) simulations. However, the molecular descriptions obtained highly depend on the force fields used, therefore experimental validation of the computational results is crucial. We have been exploring how CHARMM36, one of the most used force fields for all-atom MD simulations, can capture NMR observables such as ^1H - ^{13}C dipolar couplings, relaxation rates and more recently ^{13}C chemical shifts. For the latter, we are testing a novel approach making use of the lipid conformations sampled by the MD simulations to perform quantum chemical calculations of chemical shift tensors that can then be averaged for predicting ^{13}C chemical shift spectra. We show our most recent results for sphingomyelin, galactosyl cerebroside and ceramides.



CHEMeDATA: Driving FAIRness in Chemistry Through Community-Based Initiative

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Achieving FAIR (Findable, Accessible, Interoperable, Reusable) chemical data remains a major challenge, particularly across diverse software platforms. The CHEMeDATA initiative addresses this by introducing a structured, community-driven standard for the exchange, visualization, and reuse of chemical information. CHEMeDATA defines modular data units—CHEMeDATA objects—which encapsulate chemical entities (e.g., molecules, spectra, assignments, transformations) and support hierarchical extension to suit specialized needs. Based on JSON schemas and interactive HTML visualization components, these objects enable seamless integration with existing tools, including web-based viewers and desktop applications like Mnova. The initiative extends the principles of NMReDATA to a broader chemistry context, focusing on granularity, modularity, and human/machine readability. Visualization features support 3D molecular structures, NMR data, and multivariate analysis via embeddable, low-code interfaces. By promoting open specifications, modular converters, and schema validation, CHEMeDATA empowers both users and developers to create and share interoperable datasets, bypassing format silos and enabling AI-ready chemistry data ecosystems. Ongoing developments include integration with public resources such as reinforcing the project's alignment with open science and long-term data stewardship.



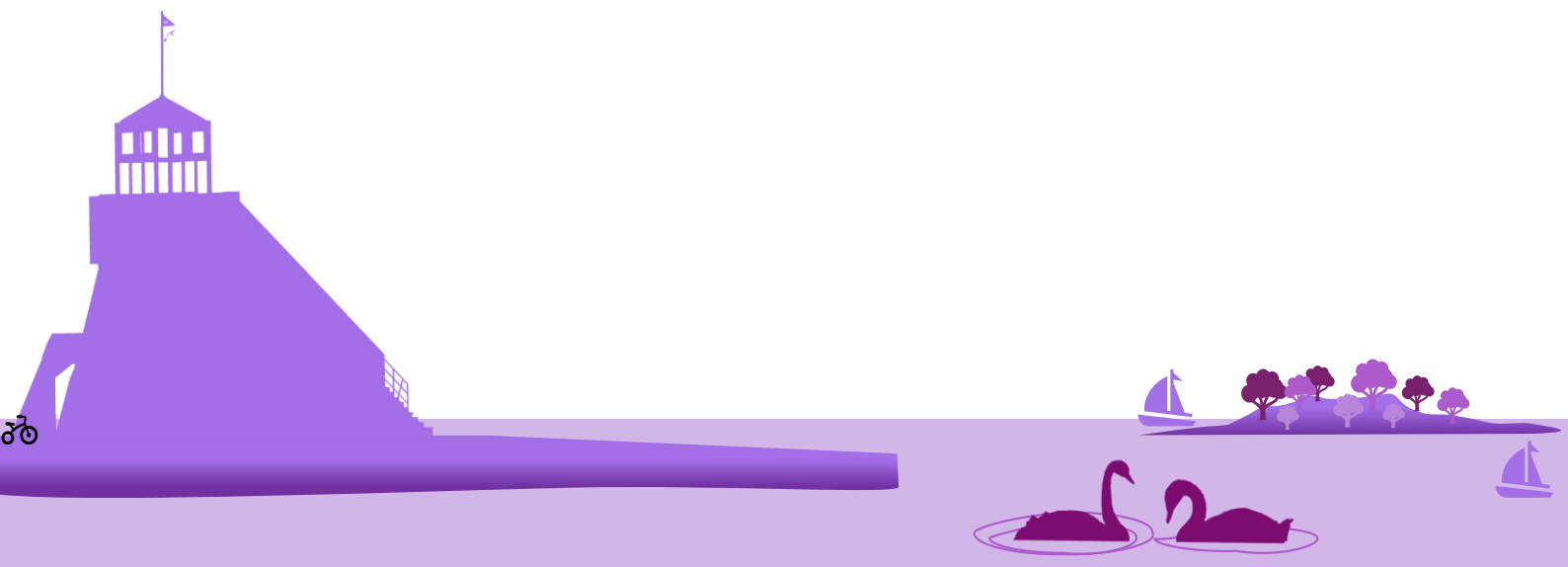
NMRbox: A shared platform for scientific computation, collaboration, and instruction

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In NMR spectroscopy there are many software packages for data processing, analysis, modeling, prediction, and curation available. Managing the discovery, acquisition, installation, and maintenance of these numerous software tools can be challenging for any researcher. To address these issues, enhance reproducibility, and simplify the creation and sharing of complex computational workflows, we have developed NMRbox - a platform for general scientific computing, with more than 270 software packages. By enabling discovery, access, and persistence (maintaining old versions) of software, NMRbox implements “FAIR” (Findable, Accessible, Interoperable, Reusable) standards for software. New features enabling granular control over data sharing, a data lake with instances of multiple public data repositories, and a virtual classroom have added collaboration and instruction to the NMRbox repertoire. The shared nature of NMRbox is both fundamental and empowering: (1) it decreases the effort and cost for individual laboratories to discover, install, configure, and maintain complex software environments; (2) it enables a small dedicated team of professionals to develop a computational environment that is richer and more powerful than individual laboratories can construct, achieving economies of scale and benefiting both large and small laboratories; (3) it fosters collaboration both local and distant; (4) by linking computation and data, it accelerates discovery of latent knowledge only attainable through collective, rather individual studies; (5) it rapidly delivers emerging computational tools to entire communities. In an era of constrained and shrinking financial support for science, the need for collaboration and shared research infrastructure is more pressing than ever. In addition to recently developed capabilities of the NMRbox platform for empowering open science, resources for machine learning and paths to sustaining and enhancing NMRbox are described.



Dependence on Electric Field Frequency of Direct Chiral NMR Effects

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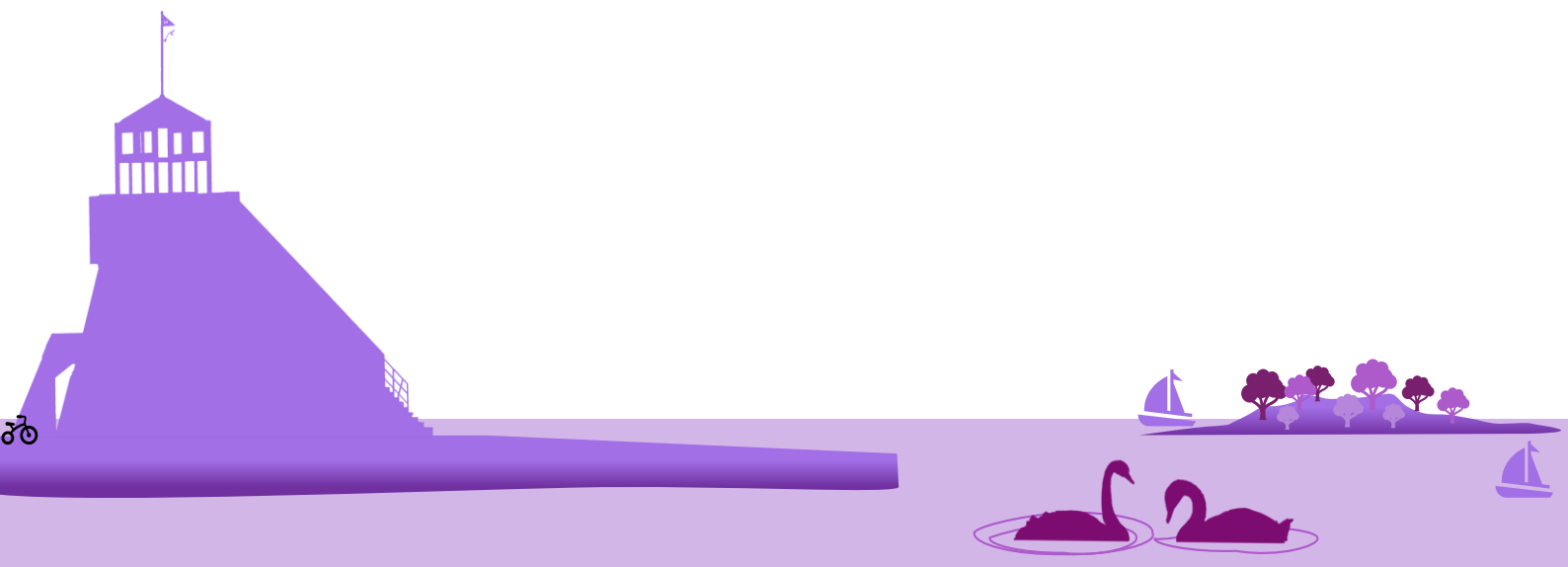
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The application of an external electric field in an NMR experiment prevents the averaging of antisymmetric nuclear properties, resulting in the appearance of chirality-sensitive NMR effects. Such effects, due to their inherent smallness, require the usage of a radiofrequency electric field. However, since the polar molecules in a liquid state cannot follow the E field instantaneously, the phase of chirality-sensitive effects that differentiate enantiomers may be shifted. In order to study the influence of the electric field frequency on the effects phases, we employed molecular dynamics simulations combined with quantum chemistry computations.

Specifically, we focused on the alignment of chiral molecules of 1,1,1-trifluoropropan-2-ol by the electric field of the frequency up to 10 GHz. As the antisymmetric interactions, we chose antisymmetries of nuclear magnetic shielding and indirect spin-spin coupling. Molecular dynamics simulations and quantum chemistry computations were performed in Gromacs and Turbomole, respectively. Obtained data were then used as parameters to compute the time variations of the chirality-sensitive quantum spin states in SpinDynamica using the master equation in the Lindblad form.

We found that the internal friction of the liquid strongly influences the chirality-sensitive effect phase if the electric field frequency exceeds a few GHz. At low frequencies (below 1 GHz), the influence of the frequency on the phase of the signal is moderate, but in a rigorous treatment, it should also be considered. These results highlight the importance of integrating quantum chemistry, molecular dynamics, and spin dynamics computations to capture chirality-sensitive nuclear interactions accurately under realistic conditions.



Agile Processing and Analysis of Bullet-DNP data with pyNMR

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Due to the high degree of automation in bullet-DNP, hundreds of spectra are collected and need to be evaluated in a short amount of time. The processing, analysis and visualisation of the data must often be done individually for every dataset. To reduce the effort of this procedure “pyNMR” can be used.

It combines the processing of data in a fast graphical user interface (based on the pyqtgraph library [1]) with the ability to repeatably and consistently process data using python scripts and/or Jupyter notebooks. Multiple datasets can be processed with the same processing settings and can be changed for single experiments, if needed. The supported formats are Bruker Topspin, RS2D and Tecmag NTNMR.

[1] www.pyqtgraph.org



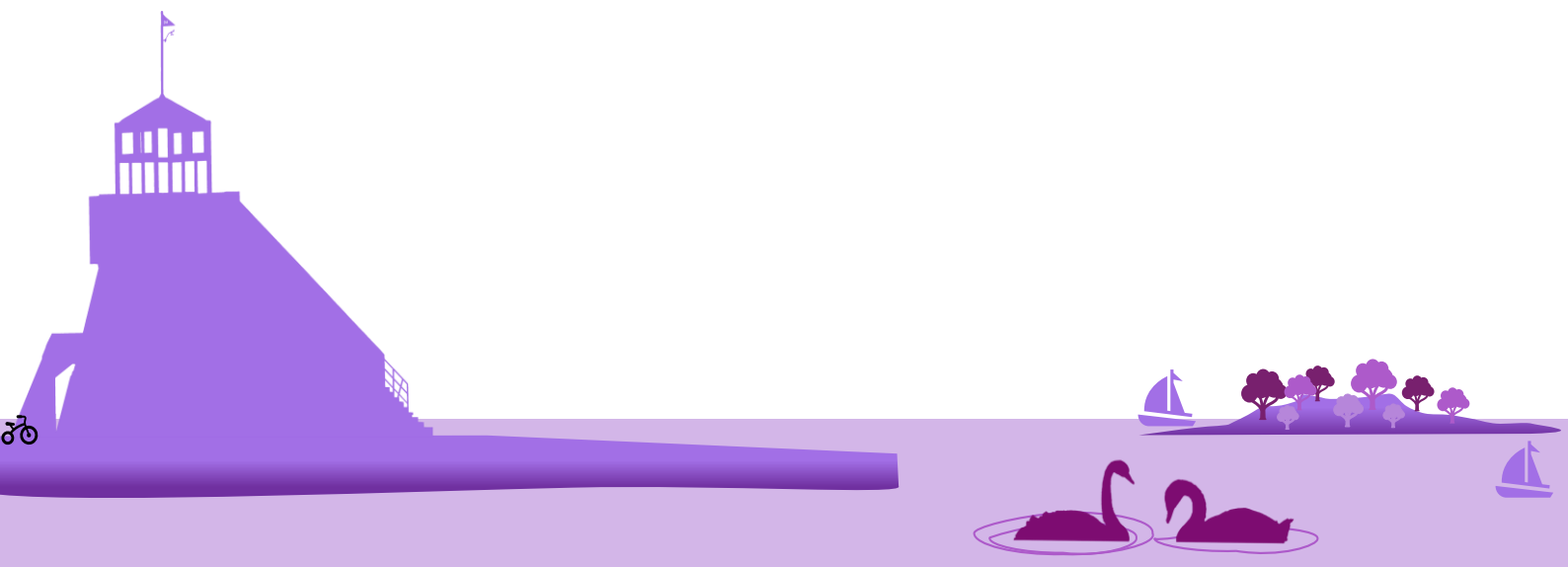
FAIRMD: Molecular Simulations to Interpret NMR Experiments

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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 (Kiirikki et al., Nat. Commun. 15 1136 (2024)), 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 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 simulation 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.



JINEPT: designing pulses with evolution control

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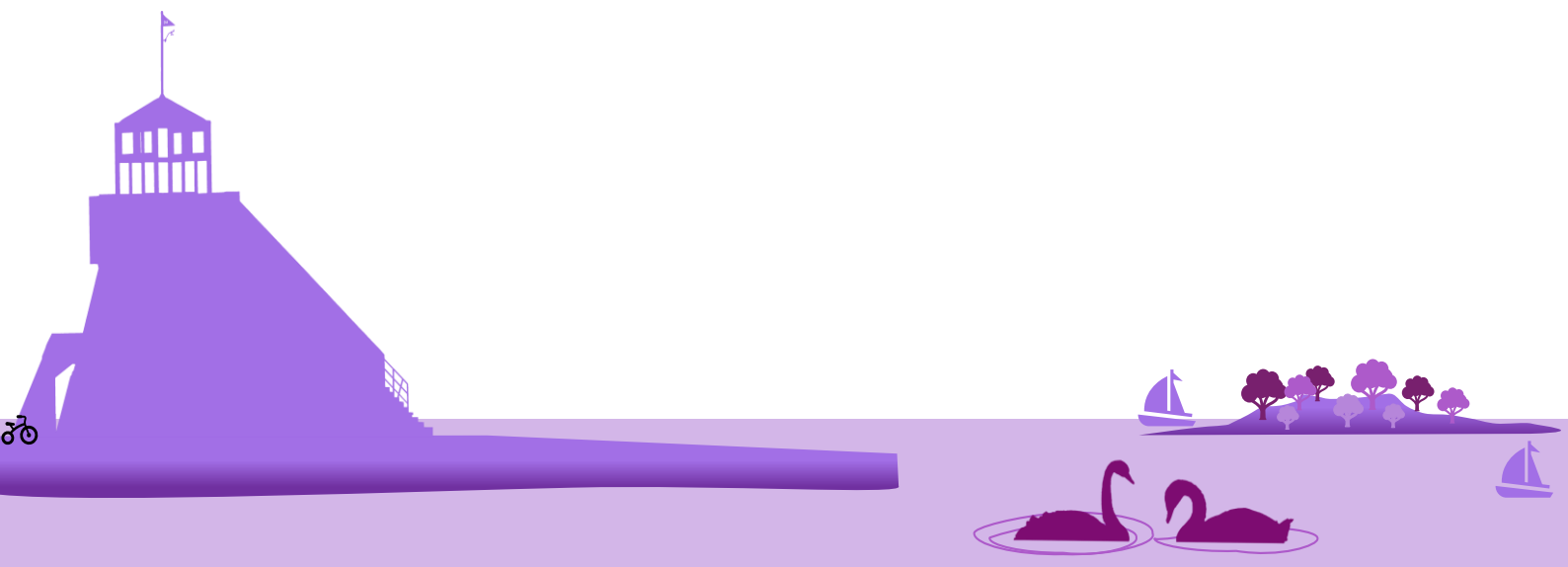
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The current canon of pulses used in NMR including hard rectangular pulses and shaped pulses are typically used with no assumption of evolution of spins during the pulse duration, causing the effective delays in a pulse sequence to be longer than expected. Efforts of back-calculating the equivalent evolution delays to subtract from the surrounding delays start to address this problem, but does not allow for true control. [Lescop et al., 2010] We have used Seedless to design de novo phase-modulated optimal control pulses with inherent evolution periods built-in, which allow us to let the spins undergo effective pulses and delays in sequence whilst truly pulsing continuously. [Buchanan et al, 2025]

The a-Ux-b schematic pulse description of pulses, whereby all pulses can be approximated by an initial period of evolution (a), then a perfect unitary rotation (Ux), and then a final period of evolution (b), is used as the restraint in calculation. The efficacy of this has been demonstrated by using evolution-controlled a90s (pulses with inherent evolution period then 90 rotation) and their time- and phase-reversed counterparts to replace the 90-tau-180-tau-90 on the I spin of an INEPT sequence, to create the so-called JINEPT (Joint-INEPT). [Morris and Freeman, 1979]

This JINEPT was used to replace the first INEPT in an HSQC pulse sequence adapted from the Bruker hsqcetgp sequence, and the equivalence of changing the inherent evolution period of the a90s used to changing the delays in a traditional hard-pulse INEPT has been demonstrated, with a range of total pulse lengths.

In the future, the control of evolution demonstrated by these a-Ux-b pulses may allow for the creation of JINEPT sequences with a tolerance for multiple J values in a sample, rather than having to settle for an approximate, average value. This would allow for increased signal-to-noise for a range of multidimensional experiments, such as the TROSY.



RNA-binding proteins undergoing Liquid-Liquid Phase Separation – Towards building a conformational ensemble using DEER

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Liquid-liquid phase separation (LLPS) drives the formation of biomolecular condensates—highly concentrated, dynamic droplets that compartmentalize proteins and other cellular components without membranes. These condensates play essential roles in processes such as RNA metabolism and stress response, but their structural characterization remains challenging due to their dynamic and heterogeneous nature.

RNA-binding proteins often contain intrinsically disordered regions (IDRs), which enable them to interact with diverse RNA targets but also complicate structural studies using classical techniques like NMR or X-ray crystallography. In contrast, DEER spectroscopy provides distance distributions between spin-labeled residues, offering valuable insights into protein conformations, even within disordered regions.

Applying DEER to phase-separated proteins presents unique challenges in sample preparation and data analysis. Since DEER measurements require cryogenic temperatures, preserving condensate integrity is critical. Conventional cryoprotectants like glycerol disrupt phase separation, so polyethylene glycol (PEG) was used both as a crowding agent to induce phase separation *in vitro* and as a cryoprotectant. Additionally, DEER signals reflect contributions from both the condensed and dispersed protein fractions, complicating data interpretation. To address this, we assessed protein partitioning of hnRNP A1 and local concentration within condensates, finding that 85% of the protein resides in the condensed phase.

Distance distributions were measured for hnRNP A1 in both its phase-separated and dispersed states, providing insights into how protein conformation changes during LLPS. These findings could contribute to a deeper understanding of the molecular mechanisms governing phase separation in RNA-binding proteins.



94 GHz chirp and phase-modulated EPR experiments for spin dynamics analysis and ENDOR spectroscopy

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Over the last decade, shaped microwave (MW) pulses have evolved into valuable tools for EPR spectroscopy. They have been used to improve existing experiments by providing tuneable broadband or band-selective frequency profiles as well as to design new experimental approaches. However, most applications were performed at low EPR frequencies (X- or Q-band) where high MW powers are available, with only rare examples at W-band (94 GHz) frequencies.

Here, we present the implementation of chirp and phase modulated pulses at a commercial Bruker E680 W-band EPR spectrometer using a SpinJet arbitrary waveform generator. Despite hardware constraints including a limited resonator bandwidth (140 MHz) and comparably low Rabi frequency (≤ 20 MHz), new experimental designs become feasible. In particular, we use frequency chirped MW pulses for Fourier transform EPR spectroscopy. Using phase modulated pulses, we perform echo experiments during spin locking.

We apply shaped pulse experiments to the analysis of spin dynamics during long periods of MW irradiation. [1] By combining CHirp Echo Epr SpectroscopY (CHEESY) [2-3] of hole burning profiles with spin lock echoes [4], we monitor the spin state both during and after MW irradiation. From this we gain information on coherent and dissipative processes during spin locking. Additionally, we illustrate how shaped MW pulses can be used to gain new information from Electron Nuclear DOuble Resonance (ENDOR) spectroscopy, otherwise not accessible.

References: [1] M. Lenjer, N. Wili, F. Hecker, M. Bennati, *Magn. Reson.* 2025, 6, 43 – 75. [2] N. Wili et al., *J. Magn. Reson.* 2018, 289, 26-34. [3] M.V.H. Subramanya et al., *Appl. Magn. Reson.* 2022, 54, 165-181. [4] N. Wili et al., *Magn. Reson.* 2020, 1, 75-87.



Sensitivity increase & 2D Deconvolution of ENDOR spectra with chirp pulses

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The hyperfine and nuclear quadrupolar couplings of nuclei close to a paramagnetic center encode unique geometric and electronic structural information for transition metal complexes. Electron Nuclear Double Resonance (ENDOR) spectroscopy uses microwave (MW) pulses on electron spins and radiofrequency (RF) pulses on nuclear spins to probe nuclear frequency spectra via the electron spins. Since typical samples of interest are non-crystalline solids, i.e. powders or frozen solutions, the ENDOR peaks are often broad due to the anisotropy of the nuclear interactions, thus limiting sensitivity in conventional ENDOR spectroscopy.

Here, I report on the use of chirp pulses to enhance the sensitivity of ENDOR experiments and to deconvolute crowded ENDOR, enabled by a home-built X-/Q-band EPR spectrometer with dual arbitrary waveform generators for chirp RF and chirp MW excitation. Chirp RF pulses, which are more broadband than typically used rectangular single-frequency pulses, excite a larger fraction of the nuclear spins and therefore lead to higher ENDOR intensities. This is especially beneficial for broad anisotropic lines of low-gamma nuclei, and we observed signal increases up to tenfold. [1] Switching the detection sequence in Davies ENDOR from a Hahn echo to a chirp echo, enables to detect the complete hole pattern after the RF pulse instead of only the central hole. The resulting additional hyperfine dimension simplifies ENDOR spectra with overlapping peaks from different nuclei by spreading them along two dimensions and has been previously reported for FID-detected ENDOR spectroscopy. [2] The improved ENDOR experiments with chirp pulses are applied to study transition metal complexes of current research interest, where we show the direct detection of Ti(III) hyperfine couplings at natural isotope abundance.

[1] Stropp, J. et al., Magn. Reson., 2025 6, 33–42.

[2] Wacker, T ; Schweiger, A., Chem. Phys. Letters, 1992, 191, 136-141



Enabling EPR measurements in cells utilising reductively stable spin labels

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To understand how biomacromolecules interact and work in their native environment, structural information should be acquired directly within the respective organism to shed light onto the complex interactions that take place inside living cells.

The emerging field of electron paramagnetic resonance (EPR) spectroscopy enables studying biomacromolecular structures in their natural environment by means of site directed spin labelling (SDSL) and e.g. pulsed dipolar EPR spectroscopy (PDS) methods. Triaryl methyl (TAM)[1] or Gadolinium based [2] spin labels that are persistent in reducing environments, will be exploited to study the behaviour of the Yersinia outer protein O (YopO) from Yersinia enterocolitica as well as the Group G protein G, domain B1 (GB1) from Streptococcus sp inside cells. Investigating proteins within cells might give further insights into the effects of molecular crowding, post translational modifications or their interactome on their structure and function.

The effect of different cell types and spin labels on the structure of the protein as well as on the spin label is of particular interest for this work. Here, I will report on experiments with YopO and GB1 screening different spin labels, cell types as well as delivery methods to optimise the resulting PDS data.

Based on the data that was collected so far it could be shown that YopO seems to aggregate in combination with TAM radicals and to a lesser extent with Gadolinium based spin labels. In contrast GB1 with either TAM or Gd spin labels did not aggregate under the examined conditions and is currently being investigated in HeLa and E. coli cells. The acquired data highlights that the combinations between spin label and protein are of essential importance for the stability of the systems and quality of the resulting PDS data.

[1] N. Fleck et al., Angew. Chem. Int. Ed. 2020, 59, 9767-9772.

[2] L. Garbuio et al., J. Phys. Chem. B. 2013, 117, 3145.



High-precision spectroscopy of a single ^{93}Nb nuclear spin in solid

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Enhancing the sensitivity of nuclear magnetic resonance spectroscopy to the single spin level could have a transformative impact in chemistry and biology, and has been the subject of significant research efforts. Here, we present magnetic resonance spectroscopy measurements of an individual ^{93}Nb nuclear spin ($I=9/2$) in a crystal coupled to a neighboring paramagnetic center, with detection performed via a microwave photon counter at 10mK. We demonstrate polarization, readout, and full coherent control of the nuclear spin. Using stimulated Raman pulses, we access all nuclear spin states and measure coherence times of $T_2^* \sim 300$ ms and $T_2 \sim 6$ s for adjacent nuclear spin states. The long coherence of the ^{93}Nb nuclear spin allows us to measure the frequencies of all 18 nuclear spin transitions with a precision of approximately 10 Hz. The methods employed here are applicable to a wide range of systems and the large nuclear spin could be employed for quantum-enhanced sensing of nearby nuclear spins.

[1] Wang, Z et al. Nature 619, 276–281 (2023).

[2] Jaime Travesedo et al. Sci. Adv.11,eadu0581(2025)

[3] James O'Sullivan et al. arXiv:2410.10432 [quant-ph]



Analysing the architecture of PTBP1-RNA biomolecular condensates with pulsed EPR

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The compartmentalization of cellular contents into membrane-less organelles is now understood to arise from liquid-liquid phase separation (LLPS). While originating in a sensitive balance of intermolecular interactions, the detailed understanding of its molecular basis remains elusive, particularly in the context of RNA-binding proteins (RBPs) and the role of RNA. Many RBPs contain intrinsically disordered regions (IDRs) which are enriched in biomolecular condensates but cannot be studied by conventional structural biology techniques due to their dynamic nature.

Pulsed EPR spectroscopy can capture the highly complex and dense environment of biomolecular condensates and provide selective information by clever engineering of paramagnetic labels attached to the biomolecules. In particular, DEER allows for exploration of the conformational space of IDRs and RNA and is sensitive to the structural differences between LLPS and the dispersed state. A newly developed technique called ih-RIDME further expands the realm of possibilities by providing information about the proton density distribution in the local environment of the spin label, allowing a glimpse into the distribution of biomolecules in the dense phase.

The human protein PTBP1, which only undergoes LLPS in the presence of RNA, is our model system for understanding the interplay of RBPs and RNA in biomolecular condensates. Application of ih-RIDME to PTBP1 and RNA in a deuterated buffer allows us to observe contacts between biomolecules in the condensates, showing that certain spin-labelled positions are found in more or less proton-rich environments. Combined with distance restraints from DEER, this provides insights into the inner architecture of PTBP1-RNA condensates.



Combining Orientation selective Light-Induced Pulsed Dipolar Spectroscopy with Molecular Dynamics

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We present a new approach that combines Light-induced Pulsed Dipolar Spectroscopy (LiPDS) techniques with Molecular Dynamics (MD) simulations to comprehensively explore the conformational dynamics of a benchmark system. This employs tetraphenylporphyrin and nitroxide probes separated by a rigid alpha-helical bridge with a defined conformational preference for the two probes. Initial analysis was based on a geometric model of the system that did not take into account the complete molecular structure (J. Phys. Chem. Lett., 2021,12, 3819–3826). To assess if the selected conformations are physically meaningful, we use MD simulations that model the conformational landscape while accounting for the complete molecular structure within its environment, enabling us to complete the structural information extracted from LiPDS traces.

LiPDS is a useful technique to study biological structures by extracting inter-spin distances between paramagnetic centres, where at least one is a photoexcited triplet state, through their dipolar interaction. The resolved anisotropy of the nitroxide at Q-band enables orientation selection by selectively exciting only a small portion of the spectrum. As a result, only limited molecular orientations with respect to the applied magnetic field are excited, allowing us to gain insights on the relative spin-label orientations in addition to a distribution of inter-label distances. Simulations of LiPDS traces based on conformations extracted from MD trajectories are used to calculate a library of time traces, which are fitted to experimental data.

Different fitting procedures result in subsets of conformations that best reflect the experimental results, and comparison of these helps to validate the methods. In each step of our analysis, the comparison of calculated and experimental LiPDS traces obtained with orientation selection introduces further constraints that lead us to determine the dynamic structure of the peptide under investigation.



High-field Magic Angle Spinning EPR of P1 Centers in Diamond

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The low sensitivity of NMR can be overcome by DNP spectroscopy. In DNP, mm-wave irradiation at or near the electron Larmor frequency results in hyperpolarization of neighbouring nuclei, causing the NMR signal to increase by up to four orders of magnitude. The use of EPR spectroscopy improved our understanding of the role of electron spin dynamics in static-DNP and to identify new DNP mechanisms. However, most contemporary DNP is performed under magic angle spinning (MAS), where the insights obtained from static experiments do not apply. Therefore MAS-EPR experiments are needed to understand the role of electron spin dynamics in MAS-DNP. Recently, we reported the first high-field MAS-EPR experiments with up to 3 kHz spinning rate, where we were able to observe the electron spin dynamics on a few microsecond timescales. However, DNP buildup typically occurs over a longer timescale.

Here we demonstrate the first rotor-synchronized high-field MAS-EPR experiments allowing to observe electron spin dynamics on the hundreds of microsecond timescale. We use substitutional nitrogen defects in diamond, reported to efficiently hyperpolarize bulk ^{13}C in the diamond lattice at room temperature. We performed three-pulse, rotor-synchronized stimulated echo MAS-EPR experiments with up to a 37 kHz spinning rate at 7 T. Our results revealed the unique capability of MAS-EPR to separate EPR spectra based on their anisotropy, achieved via the strong dependence of MAS-induced spin dephasing on the anisotropic line width. In addition, by measuring stimulated echo decay over multiple rotor periods, we can now estimate how MAS affects the electron spins apparent T_1 , which is directly relevant to predicting MAS-DNP efficiency. The experiments are accompanied by time-domain simulations confirming the interpretation of the observed changes in the EPR lineshapes.

This work is a step towards a deeper understanding of MAS-DNP mechanisms, aiming to achieve stronger hyperpolarization.



EPR - a tool to improve Supercapacitors performance

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This study highlights the potential of SnO₂-based materials for high-performance supercapacitor applications, utilizing different dopant ions. Changes in the defect structure resulting from introducing dopant elements and the oxidation state were detected using electron paramagnetic resonance (EPR) and photoluminescence (PL) spectroscopy. A comprehensive electrochemical analysis was conducted, where the SnO₂-based materials are was examined as electrode materials in all-in-one symmetrical supercapacitor devices, followed by a detailed performance evaluation.



Application of CW and Pulsed EPR Spectroscopy for the Genetic and Thermodynamic Profiling of Human Transport Proteins

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How would nature engineer and evolve a biophysical transporter that needs to fulfill its task with the highest thermodynamic efficiency? This study combines spin probing and EPR spectroscopy with thermodynamic and genetic binding studies for an in-depth molecular view into the ligand binding and functionality of transport proteins. We studied three isoforms of human cytosolic FABPs and Serum Albumins from various species by loading them with the spin-labeled fatty acids 5- or 16-DSA as model ligands. CW EPR spectroscopic studies and simulations provided binding pocket polarities, ligand dynamics and the concentrations of free, intermediately and strongly bound ligands in equilibrium. The derivation of temperature-dependent binding affinities enabled the analysis of thermodynamics for multiple binding processes. The method revealed complex thermodynamic profiles for FABPs with differences and similarities between the isoforms, suggesting fine-tuned functional thermodynamics. DEER experiments on spin probed Albumins delivered broad, polymodal distance distributions, reflecting the distributions of multiple ligand-loaded binding pockets and enabling the comparison of dynamic Albumin structures in solution. We found significant binding differences between species classes, possibly correlating with nutrition and amino acid sequence similarities. Both studies reveal thermodynamic and genetic adaptations of transport proteins and provide important information about protein-ligand interactions in general. They further establish EPR spectroscopy as a platform to study molecular binding processes and thermodynamics which could be further developed to engineer and tune synthetic transport systems for advanced applications.



Identifying the copper coordination environment between interacting neurodegenerative proteins

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Alzheimer's disease (AD) stands as the foremost common type of dementia and ranks as the 7th highest cause of death worldwide. The prevailing model posits that the buildup of amyloid-beta ($A\beta$) aggregates in the brain, followed by their uptake into cells, significantly influences the onset and advancement of AD. Recently, the cellular prion protein (PrPC) has been identified as the primary receptor for $A\beta$. We propose that the role of PrPC as an $A\beta$ receptor could strengthen through their mutual interaction with Cu(II). This increased affinity for each other stabilizes the complex and likely allows for $A\beta$ to be endocytosed in the Cu(II) dependent pathway. Employing pulsed electron paramagnetic resonance techniques, ESEEM and HYSCORE, we distinguish and identify how Cu(II) interacts with both proteins by observing the interplay between Cu(II) and nearby residues. In this work, we isotopically label PrPC with ^{15}N ($I=1/2$) and utilize natural isotopic abundance for $A\beta$, i.e. primarily ^{14}N ($I=1$), to identify how Cu(II) coordinates with both $A\beta$ and PrPC. Furthermore, varying the relative concentration between Cu(II) and the two proteins indicates that a ternary complex is formed, rather than Cu(II) interacting with $A\beta$ and PrPC individually. Moreover, the methodologies developed in this work provide a framework for assessing the copper environment in other interacting neurodegenerative proteins.



Investigating the Structural Dynamics of the XRCC4-SUMO Protein System Using Electron Paramagnetic Resonance (EPR) Pulsed Dipolar Spectroscopy (PDS)

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The repair of DNA double-strand breaks (DSBs) through non-homologous end joining (NHEJ) is essential for maintaining genomic stability and preventing carcinogenesis 1. XRCC4, a central NHEJ factor, interacts with various proteins, including XLF, IFFO1, and LIG4, and is regulated by small ubiquitin-like modifier (SUMO) chains 2. Understanding the structural dynamics of the XRCC4-SUMO complex is crucial for elucidating the regulatory mechanisms of NHEJ. In this study, we utilize Electron Paramagnetic Resonance (EPR) Pulsed Dipolar Spectroscopy (PDS), specifically Double Electron-Electron Resonance (DEER), to investigate the structural changes within the XRCC4 dimer upon SUMO binding. We have conducted distance measurements within the XRCC4 dimer without SUMO to establish a baseline for comparison. These measurements provide critical insights into the native conformation of XRCC4 before SUMO binding. Our approach involves site-directed spin labelling and distance measurements, which will be integrated into molecular docking and dynamics simulations to refine existing NMR-based models of the XRCC4-SUMO protein system. The baseline distances obtained will serve as a reference to identify and quantify conformational changes induced by SUMO binding in future experiments. This project aims to enhance our understanding of the structural dynamics of the XRCC4-SUMO protein system, contributing to the broader knowledge of DNA repair mechanisms



EPR and DFT studies of the first manganese catalyst for nitrile dihydroboration

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The manganese hydride dimer, $[(2,6\text{-iPr}_2\text{PhBDI})\text{Mn}(\text{u-H})]_2$ (1), was found to mediate nitrile dihydroboration, rendering it the first manganese catalyst for this transformation. Stoichiometric experiments revealed that benzonitrile insertion affords $[(2,6\text{-iPr}_2\text{PhBDI})\text{Mn}(\text{u-NCHC}_6\text{H}_5)]_2$ (2) en route to N,N-diborylamine formation. EPR spectroscopy was used to characterize the electronic structure of 2. Density functional theory calculations reveal the precise mechanism and demonstrate that catalysis is promoted by monomeric species.



Experimental application of multiharmonic analysis on low-concentration spin-labelled proteins in solution and in organelles

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Continuous wave (CW) electron paramagnetic resonance (EPR) spectroscopy is a powerful tool for retrieving dynamic information from proteins and protein complexes in different environments. However, single-digit micromolar sample concentrations represent a challenge for CW EPR. It would be desirable to detect dynamic information on proteins at low micromolar or sub-micromolar concentrations in physiologically relevant environments, as in organelles. In order to overcome the known sensitivity problems of CW EPR with the common lock-in detection schemes, multiharmonic analysis was previously proposed as a method to increase the signal-to-noise ratio by the detection of multiple harmonics generated by signal overmodulation. (1) Only recently, a benchtop hardware implementing multiharmonic analysis became available, which promises an increase of over 10 times on the signal-to-noise ratio of a CW EPR spectrum. (2) Additionally, the detected harmonics with degrees higher than 1 should improve spectral resolution, providing information on possible hidden features of broad EPR profiles.

In this study, we explore the potential of multiharmonic analysis vs lock-in detection on several systems displaying different degrees of complexity: i) EPR kinetics of chemical reduction of water-soluble nitroxide radicals; ii) multi-component EPR spectra of spin-labelled γ D-crystallin forming liquid-liquid phase separation; iii) EPR kinetics of the insertion of a spin-labeled proapoptotic protein Bax into the outer membrane of mouse-derived mitochondria.

In conclusion, we show that coupling a multiharmonic device to a CW EPR spectrometer is a valuable approach to study low-concentration biomolecules carrying nitroxide radicals at physiological temperatures in different environmental conditions.

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In situ Monitoring of Hybrid Redox-active Films Using a Novel Low-cost Setup for CW-EPR

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Redox-conductive polymers are promising alternatives to inorganic-based energy storage materials due to their high energy density and fast redox kinetics.[1] However, their redox mechanisms - involving both polaron/bipolaron species and nitroxide radicals - make the analysis of charge transfer processes challenging. Electron paramagnetic resonance methods (ex/in situ cwEPR) offer direct insight into radical species, reaction mechanisms, and the local environment of redox centers.[2]

Conducting in situ EPR experiments is challenging due to significant dielectric loss at microwave frequencies when using polar solvents and the need to maintain clear electrochemical responses. Traditional “flat-cell” designs, in which the sample is located at a sufficient distance from the electric fields of the resonator, often suffer from high ohmic drop, complex assembly, and poor reproducibility. Alternatively, a standard three-electrode cylindrical cell offers better electrochemical response due to minimised ohmic drop [3]. This design provides experimental flexibility and can be tailored for specific samples.

This work focuses on cost-effective and accessible electrochemical cell designs for EPR experiments in the X-band range (9-10 GHz) using organic electrolytes. The ability to obtain reliable, simultaneous electrochemical and cwEPR responses during the charge/discharge process at various potential sweep rates was demonstrated. Full-spectrum cwEPR measurements enabled accurate assessment of the material's state of charge, while single-field-point measurements allowed precise determination of redox onset potentials. Polymerisation and degradation processes of the materials were also analysed, contributing to a deeper understanding of these transformations.

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Extending ENDOR spin dynamics simulations to large nuclear spin systems

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In recent years, several electron-nuclear double resonance (ENDOR) experiments have been developed or optimized for applications. This allowed to elevate the spectral resolution of ENDOR techniques to a range of a few tens of kHz and even lower.[1,2] At this level, weak spin-spin interactions, which were negligible in classical applications, are also resolved and contribute to line broadening. Consequently, standard models for data analysis and interpretation become limited, and the question of high-precision numeric simulation of the data comes to the forefront.

In this contribution, we introduce the newly developed software for scripted general-purpose pulse ENDOR simulations (SimDyn) and present its upgrade optimized for larger spin systems.

The software SimDyn simulates the spin dynamics through the ENDOR pulse sequence. This enables simulation of both standard and non-standard ENDOR experiments. The built-in functions account for the anisotropy of spin interactions and for orientation selection based on the EPR spectrum. It has been thoroughly tested on systems up to four spins, and it showed good agreement with the experiment.[1,2]

Extension of the simulated spin system is strongly challenged by the exponential growth of the Hilbert space dimension. In this contribution, we overview several exact and approximate strategies to overcome this problem in ENDOR spectroscopy. The presented upgrade of SimDyn's simulation core encapsulates the machinery from the user, which allows for automating the decision making, allowing for choosing the best theoretical models and numerical algorithms to balance between accuracy and performance. While the general framework is in the development stage, we present selected cases of the successful application.

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[2] L.Siellaff, A.Kehl, A.Aden, A.Meyer, M.Bennati, submitted



Pulsed dipolar hyperfine spectroscopy for molecular distance measurements in the angstrom to nanometer scale

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Hyperfine spectroscopy is a fundamental method in biophysical and material sciences to detect nuclear spins in vicinity of paramagnetic centers, leading to molecular structural information. Recently, Electron nuclear double resonance (ENDOR) has been established as a complementary tool to detect nuclei at inter-spin distances up to about 1.7 nm using ^{19}F labels (1). So far, frequency domain ENDOR experiments have been the method of choice to measure ENDOR spectra. However, in this experimental approach, the full magnetic interactions of the ^{19}F spin, including its chemical shift anisotropy (CSA) and nuclear dipolar couplings (NDC), contribute to the spectrum, leading to observed ENDOR line widths of 10 – 30 kHz, limiting the resolution of the experiment.

In this work, we present ENDOR experiments based on nuclear sublevel coherence spectroscopy (2) that push the boundaries of ENDOR sensitivity and resolution (3). We show that the multiplex advantage of Fourier transform permits to record ENDOR spectra of samples at low micromolar concentrations, effectively enhancing the ENDOR sensitivity of about one order of magnitude vs standard FD ENDOR. Building upon these results we introduce a novel time domain experiment that disentangles the hyperfine interaction from other undesired nuclear spin interactions. Using a series of rationally designed model systems, we demonstrate that the method pushes ENDOR resolution by one order of magnitude. Finally, we show that now distance distributions become accessible from ENDOR. This methodology paves the way for new structural studies using ^{19}F ENDOR in biomolecular systems. Moreover, it offers opportunities to access spin dynamics in electron-nuclear coupled spin systems.

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Paramagnetism of NO@C60

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The fullerene C60 is a truncated icosahedral molecule with an internal cavity that is large enough to accommodate guest molecules. Through molecular surgery [1], it is possible to reliably insert small molecules into C60 with high yield. These molecules exhibit features of isolated gas molecules, modified by interaction with the confining potential of the cage.

NO has an unpaired electron in its π^* molecular orbital and the coupling of the spin with the orbital angular momentum produces a rich energy level structure. The ground state is nearly diamagnetic as the orbital and spin contributions to the gyromagnetic ratio cancel out and NO's paramagnetism is due to thermally populated excited states [2]. We present evidence of the Zeeman splitting of the first excited state using THz spectroscopy and X-band EPR measurements, and preliminary observations of the temperature dependence of the susceptibility through the paramagnetic NMR shift of the cage ^{13}C nuclei.

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EPR investigations on in vivo gadolinium release in murine brain tissue after gadolinium-based contrast agent administration

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Retention of gadolinium (Gd) following administration of gadolinium-based contrast agents (GBCAs) for magnetic resonance imaging (MRI) has been reported in various human tissues, including the brain, bones, skin, and liver [1]. Prior work on experimental autoimmune encephalomyelitis (EAE), a common mouse model of multiple sclerosis, revealed that neuroinflammatory conditions can significantly enhance Gd accumulation in the cerebellum [2].

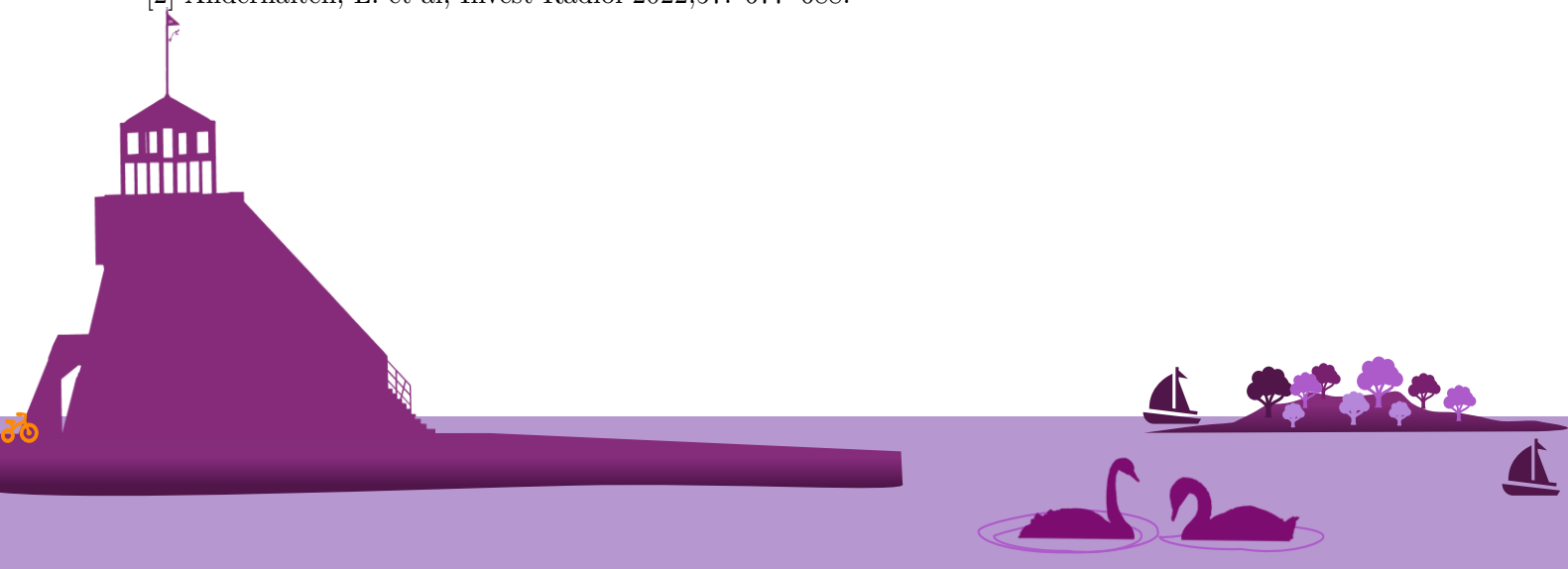
In this study, we applied electron paramagnetic resonance (EPR) techniques to assess how ionic gadolinium (Gd^{3+}) is retained and potentially released after intravenous in vivo injections of either the linear agent gadopentetate dimeglumine (Magnevist) or the macrocyclic agent gadobutrol (Gadovist) in EAE mice. We also extended our investigation ex vivo using inflamed living murine hippocampal slices. High-frequency EPR at 94 GHz (3.3 T) detected varying amounts of Gd^{3+} retention. Comparisons of EPR line shapes from biopsy samples and the aqueous gadopentetate solution suggested that, in vivo, some Gd^{3+} dissociates from its chelating complex.

To further explore the local environment of retained Gd^{3+} , we employed electron-nuclear double resonance (ENDOR). In brain slices treated with either GBCA and the tissue biopsy from the gadobutrol-treated specimen, ^1H ENDOR spectra indicated that Gd^{3+} remained in its chelated form. In contrast, cerebellar samples from the gadopentetate-treated mouse exhibited spectral features consistent with a partial Gd^{3+} release. Supporting this, ^{31}P ENDOR measurements on the biopsy samples showed close spatial proximity between Gd^{3+} and phosphorus nuclei only in the gadopentetate condition, implying binding of Gd^{3+} to endogenous phosphate-containing species.

Support by Deutsche Forschungsgemeinschaft, CRC 1340 Matrix in Vision is gratefully acknowledged.

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Chirality-induced spin selectivity in photosystem I probed by transient EPR and out-of-phase ESEEM

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Chirality-induced spin selectivity (CISS) is the phenomenon by which a local spin polarization is observed in the presence of a chiral potential. The possibility of having large spin polarization even at room temperature offers promising applicability in spintronics and in quantum computing for the initialization and for the readout of qubits [1].

Nevertheless, a complete theoretical framework describing CISS has not yet been developed. Key insight can be obtained by studying simple systems consisting of a photoinducible donor-chiral bridge-acceptor (D- χ -A). In this work we focus on photosystem I, a well-characterized sample that has been reported to show increasing CISS for increasing temperature in a range between 200K and 300K when investigated under conductive atomic force microscopy [2].

Here we present transient EPR (trEPR) and out-of-phase electron spin echo envelope modulation (OOP-ESEEM) data in this temperature range. Electron spin resonance represents a powerful tool to extract information about the electronic spin wavefunction after electron transfer, being able to distinguish between initial singlet state (no CISS) and initial presence of triplet state population (CISS). In particular, the trEPR spectrum, while being well described using an initial singlet state for temperatures below 200K, is expected to include also transitions due to an initial triplet contribution at higher temperatures, due to the increase of CISS efficiency. Furthermore, given its dependence on the initial spin wavefunction, studying the intensity of the OOP-ESEEM as a function of the turning angle of the first pulse represents another method to extract the initial state of the system and infer the CISS efficiency.



Advances in Distance Measurement Throughput and Sensitivity through a Superconducting Resonator Platform

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Pulsed Dipolar Spectroscopy (PDS), a branch of Electron Paramagnetic Resonance (EPR) spectroscopy focused on the measurement of nanoscale electron-electron distances via the strength of their dipolar interaction, has garnered significant interest within the scientific community in recent decades. PDS can provide rich structural and dynamic information on biomolecules that may be difficult or impossible to attain through alternative biophysical methods such as Cryo-EM, NMR, and X-ray crystallography, making it an attractive technique in the field of structural biology at large. However, PDS faces obstacles to its widespread adoption into standard structural biology workflows, such as the steep learning curve to conducting distance measurements and low sample throughput. Here, we present advancements in distance measurement methodology and system automation, leading to higher measurement sensitivity and sample throughput.

These enhancements are enabled by a compact superconducting resonator device, enabling high stability and micromolar sensitivity with small sample volumes and a compact form factor. The stability and sensitivity of the instrument allow for long, uninterrupted data collection with no phase drift, for unattended sequential acquisitions. Such stability is also ideal for advanced distance measurement methods, which can greatly enhance measurement sensitivity. Furthermore, high throughput is facilitated by the automation of sample handling, sample characterization, data collection, and distance analysis. Finally, we exhibit multisample capabilities, with up to five samples being introduced into the cryostat simultaneously for multiple sequential data acquisitions with no input necessary between samples. The work presented here represents important progress in broadening the accessibility and application of EPR into diverse scientific disciplines.



Resolution Enhancement in Solid State NMR of Zeolites through Triple-Quantum-Filtered ^{133}Cs NMR Spectroscopy

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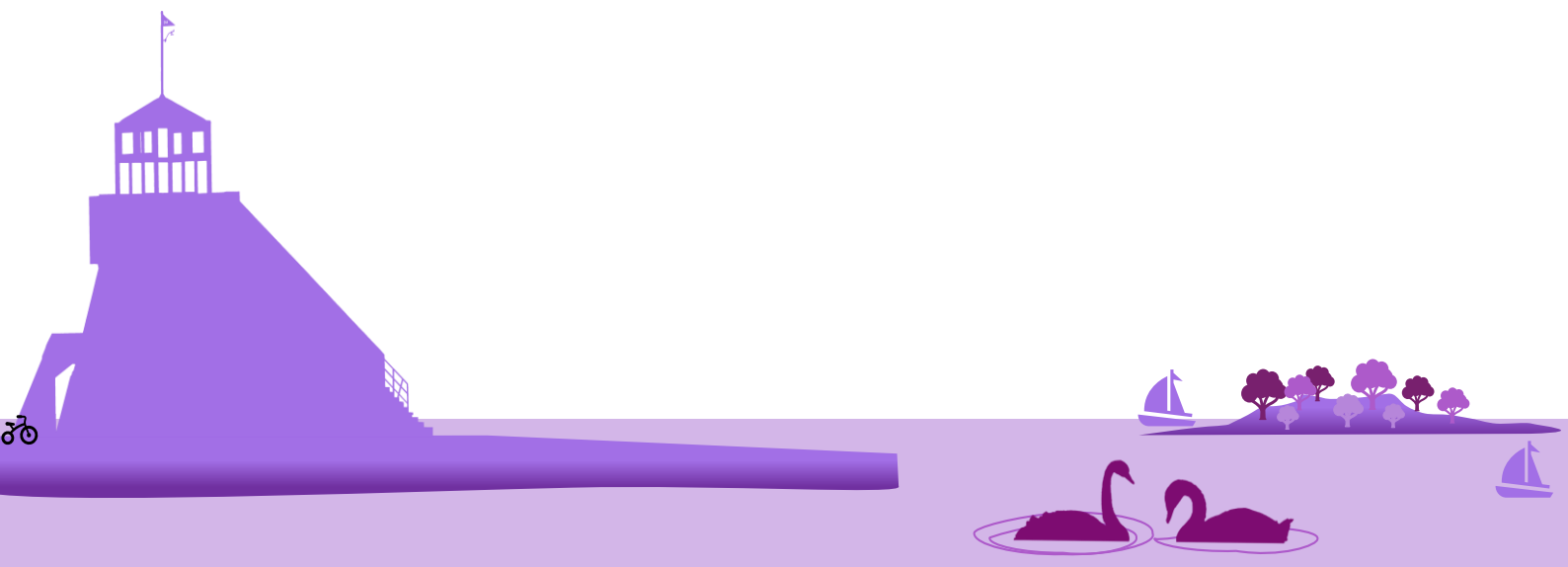
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Geopolymers, made of zeolitic crystalline and amorphous phases, exhibit effective immobilization properties for radioactive low and intermediate-level nuclear waste, and more specifically for the immobilization of radioactive cesium. The identification of the cesium-binding sites and their distribution between the different phases making up the geopolymeric matrix can be obtained using ^{133}Cs solid-state NMR. Once the distribution of cesium inside geopolymers is well characterized, they can be tailored to maximize the binding and residence time of cesium.

Multiple-quantum NMR can be used for investigating complex molecular structures due to the potential increase in spectral resolution. However, its application is often limited due to the inefficiency in the excitation of triple-quantum, and higher-quantum coherences, mainly in nuclei with weak quadrupolar coupling constants.

^{133}Cs is a spin-7/2 with a small quadrupole moment. In this work, we show using various methods that in hydrated zeolites A and X, the quadrupolar coupling constant (C_q) of Cs is small, ~ 20 kHz. We then present excitation schemes, tailored to such small C_q values, that enhance the efficiency of triple quantum excitation of ^{133}Cs , allowing the acquisition of spectra with superior resolution over direct detection. We demonstrate our new excitation schemes by performing two-dimensional triple-quantum/single-quantum ^{133}Cs magic-angle spinning solid-state NMR experiments on Cs/Na-loaded zeolites A and X, and discuss the chemical interpretation of the results. Once such techniques will be applied to the more complex geopolymers, a detailed characterization of cesium-binding sites can be obtained that will guide the synthesis of more efficient immobilization matrices.



Development of an MRI methodology for polymeric films by ex-situ solid-state NMR with a needle-like ferromagnet

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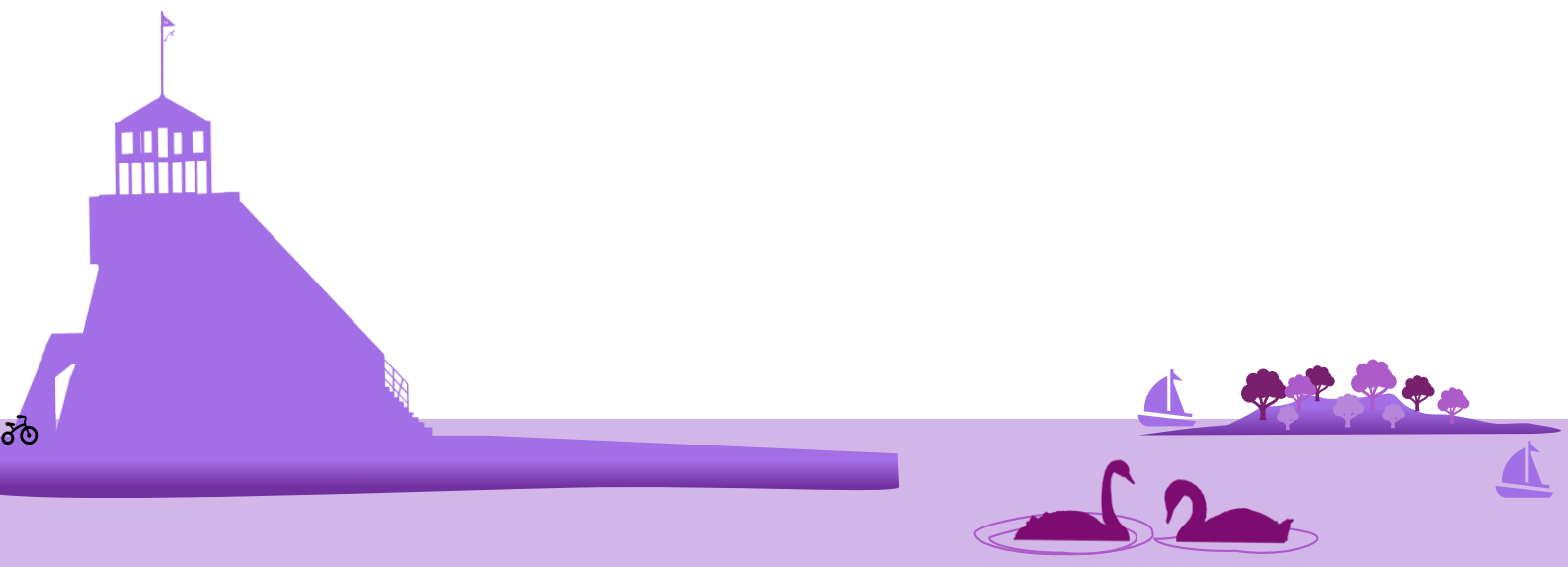
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This study introduces a novel remote sensing and material imaging technique utilizing ex-situ solid-state NMR, focusing on the local static magnetic field gradient generated by a needle-shaped ferromagnet.

The method employs an electromagnet as the static magnetic field source, with a flat sample coil placed between the magnet poles. A needle-shaped ferromagnet positioned above the sample creates the magnetic field gradient. By sweeping the electromagnet's field in discrete steps, depth-resolved imaging is achieved.

To validate this method, one-dimensional F-19 MRI measurements were performed on a three-layer PTFE/Al/PTFE membrane. The resulting spectrum showed two distinct F-19 peaks corresponding to the PTFE layers, with a signal gap reflecting the Al spacer, confirming the method's feasibility for one-dimensional depth imaging of composite membranes.

Additionally, F-19 spin-lattice relaxation rate (R_1) measurements in a 2 mm-thick PTFE sample revealed that molecular motion was more pronounced near the film surface than in the interior. Variable frequency R_1 measurements further demonstrated distinct spectral density functions between the membrane surface and interior across a range of 10 kHz to 27 MHz. The surface data fitted the Cole-Davidson model, while the interior data matched the Cole-Cole model. These findings highlight significant differences in correlation time and molecular dynamics between the sample's surface and interior regions.



Deeper insight into supramolecular hydrogel structure and dynamics through the combined use of solid-state, liquid-state and relaxometry NMR

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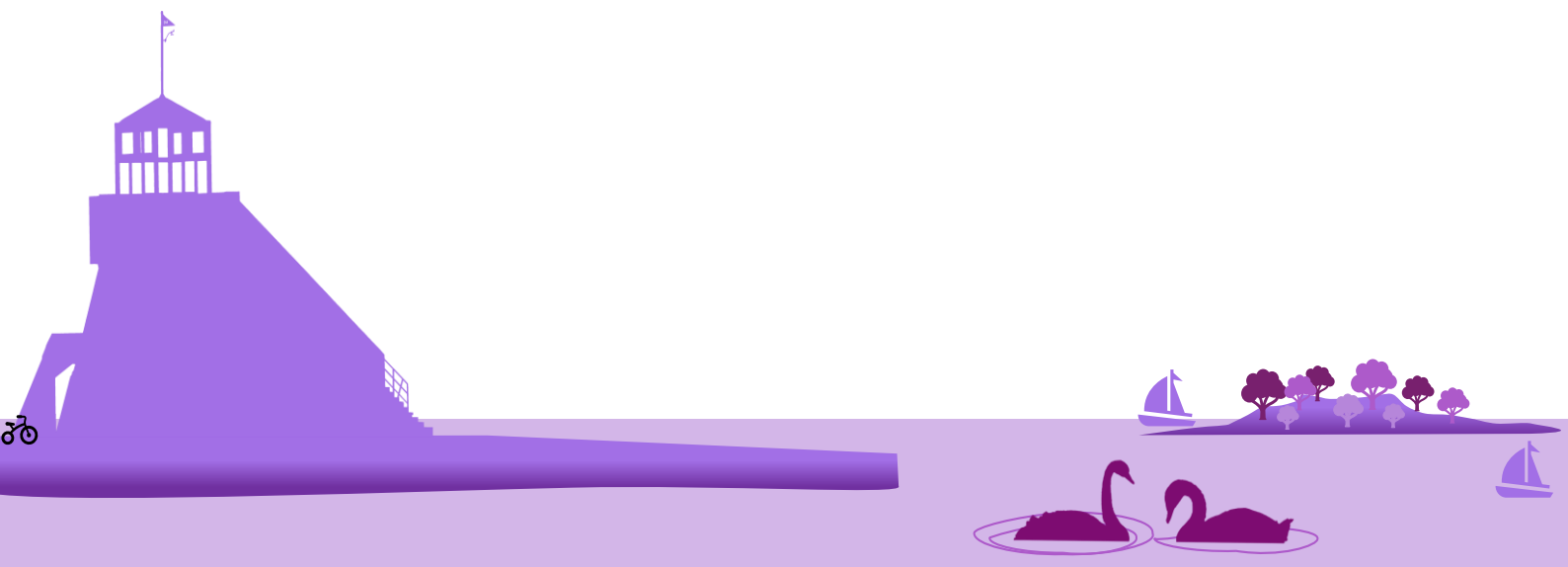
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Supramolecular hydrogels have been studied for several years for their various applications opportunities, thanks to their versatility and mechanical properties. We focus here on nucleopeptide- based multi-component hydrogels with enhanced properties thanks to possible new intermolecular interactions such as Watson-Crick pairing. Adjusting the hydrogel mechanical properties to new applications relies on a deeper understanding of the atomic-scale structure and dynamics. In this respect, Nuclear Magnetic Resonance appears to be the dedicated technique to gather information at the atomic scale of amorphous and neither solid nor liquid samples. We develop here an original methodology combining solid-state, liquid-state, and low field NMR techniques for characterizing both non-isotropic and isotropic phases of supramolecular hydrogels.

Solid-state NMR CP-based experiments investigate the gelator network. ^{13}C -detected determination of ^1H rotating frame relaxation times ($T1\rho(^1\text{H})$) enables to probe dynamics on the microsecond to millisecond timescale. By taking advantage of a deuterated solvent, we assess the efficiency of the relaxation process for different moieties of the nucleopeptide-based molecules at two temperatures, which is indeed related to the nucleopeptide network organisation and intermolecular contacts that maintains the gel structure.

High-Resolution NMR measurements are used for investigating the gelation kinetics. The study is complemented by Fast-Field Cycling Relaxometry at low field in order to evaluate the dynamical behaviour of the hydrogel isotropic phase. Dispersion curve analysis highlights the presence of different water populations regarding their correlation times, evolution of which can be followed during the gelation process.

The results obtained are supported by Molecular Dynamics simulations, which evidence interactions driving the nucleation steps leading to the gel formation.



Beware of the structure: Phosphorus Dynamics in Post-Synthetically Modified NU-1000 MOF in the presence of water – Insights from Advanced NMR Spectroscopy

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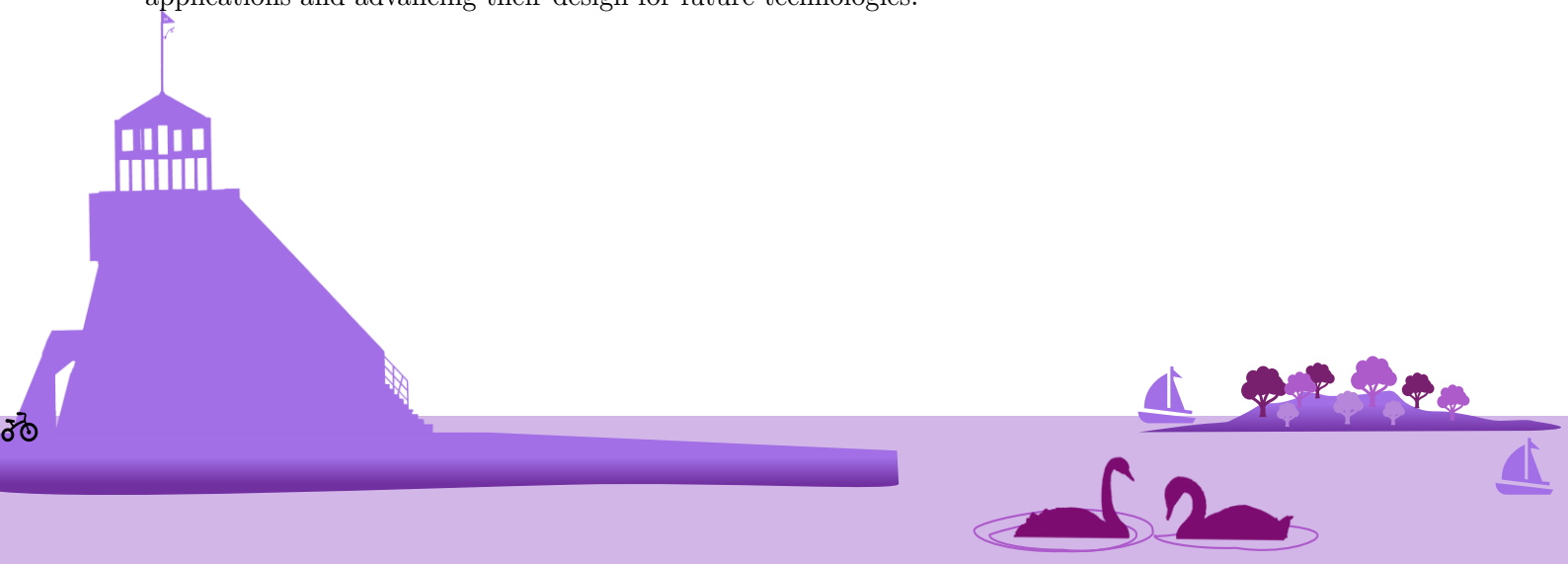
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Understanding the structure and chemistry of materials at the atomic scale is essential to elucidate and rationalize their properties as a key step to boost their application in future technologies. Metal-organic frameworks (MOFs), a versatile class of porous coordination polymers, consist of metal ions or clusters linked by organic molecules. Their highly tunable characteristics, including surface area, pore structure, and chemical composition, make MOFs promising candidates for various applications such as gas storage, catalysis, and sensing. The functionality of MOFs can be further expanded through post-synthetic modification, such as metal node functionalization via solvent-assisted ligand incorporation (SALI). This study investigates the post-synthetic modification of the Zr-based metal node in NU-1000 MOF through reaction with phenylphosphonic acid (PhPA).

To probe the dynamic behavior of this modified material in response to hydration, samples were exposed to three distinct storage conditions over an eight-week period. Solid-state ^{31}P -MAS NMR and ^{31}P - ^1H two-dimensional dipolar heteronuclear correlation (^{31}P - ^1H HETCOR) experiments were employed to monitor structural changes. The HETCOR experiments provided crucial insights into the local phosphorus environment, enabling detailed analysis of the structural evolution under varying hydration conditions. The technique allowed distinguishing between distinct phosphorus coordination modes and regions within the MOF where water and hydrogen bonding were either present or absent, depending on the PhPA loading.

Our findings demonstrate the efficacy of 2D dipolar HETCOR NMR techniques in elucidating structural transformations and coordination environments within MOFs. The approach provides crucial insights into water-induced modulation of material properties over time, contributing to optimizing MOF-based applications and advancing their design for future technologies.



^1H – ^{87}Rb Double Resonance NMR to Detect the Incorporation of Formamidinium into Rb-based Non-perovskite Phases

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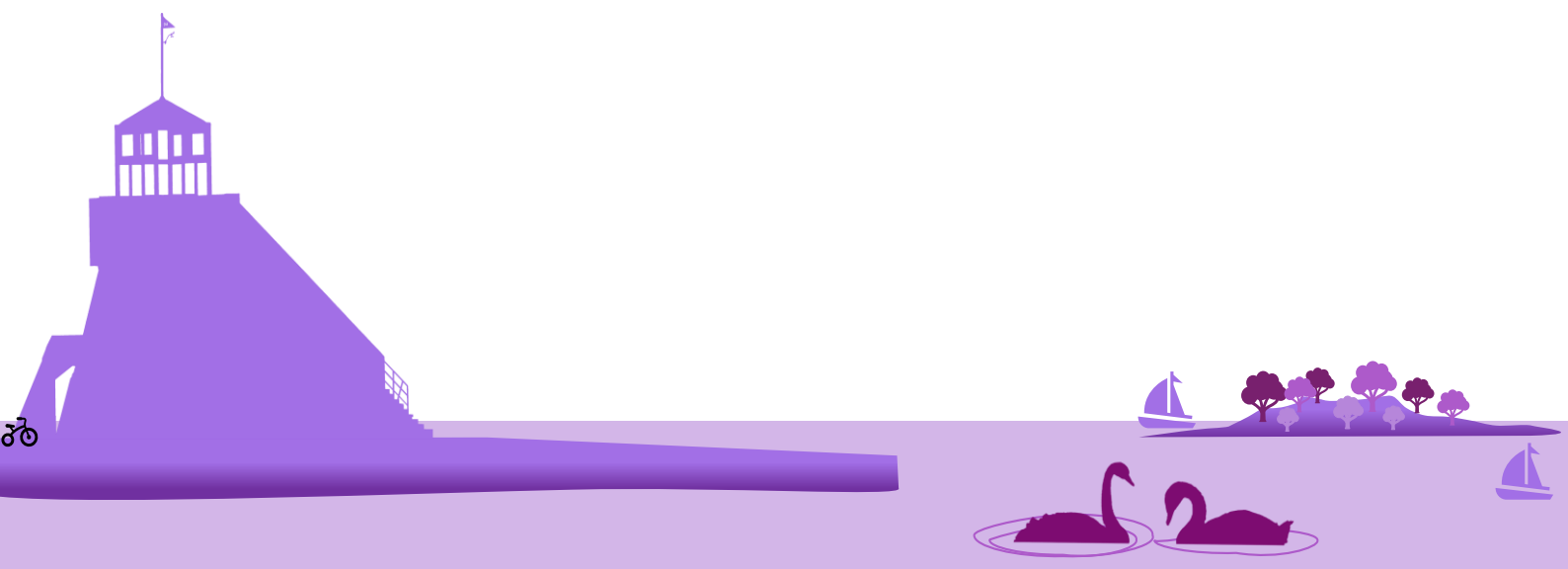
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Hybrid organic–inorganic perovskite materials, such as formamidinium lead iodide (FAPbI₃), are promising candidates for next-generation photovoltaic materials. However, the utilization of FAPbI₃ perovskite in commercial solar cells is hindered due to its the spontaneous phase transition (from the photoactive perovskite phase to a photoinactive non-perovskite phase). This phase transformation issue can be solved by including alkali metal cations such as Cs⁺ and Rb⁺ in the perovskite synthesis. It has been demonstrated by solid-state NMR spectroscopy that the Rb⁺ cation cannot incorporate into the hybrid perovskite lattice in single and double halide systems but instead forms a non-perovskite Rb-based phase. However, the mechanism by which Rb causes improved stability is still unclear.

Here, ^1H – ^{87}Rb double resonance experiments are carried out to show that instead of Rb⁺ cations being incorporated in the perovskite lattice, FA⁺ cations incorporate into the Rb-based non-perovskite phases (FA_xRb_{1-x}Pb₂Br₅ and δ -FA_xRb_{1-x}PbI₃) for both bromide and iodide perovskites. This is shown by ^1H and ^{87}Rb chemical shifts changes, ^1H – ^{87}Rb heteronuclear correlation (HETCOR) experiments, and complete dephasing in the $^{87}\text{Rb}\{^1\text{H}\}$ REDOR spectra. Finally, REDOR dephasing curves are simulated to determine the amount of FA⁺ incorporated into the Rb-based non-perovskite phase. Our findings show that up to ~60% FA⁺ can incorporate for the bromide system and ~15% for the iodide system. We propose that the improved stability imparted by Rb salts in the synthesis of FA-based perovskites can be attributed to the segregation of excess FA⁺ cations.



Beyond boundaries: ultrafast MAS at 160 kHz and high-resolution proton-detected NMR

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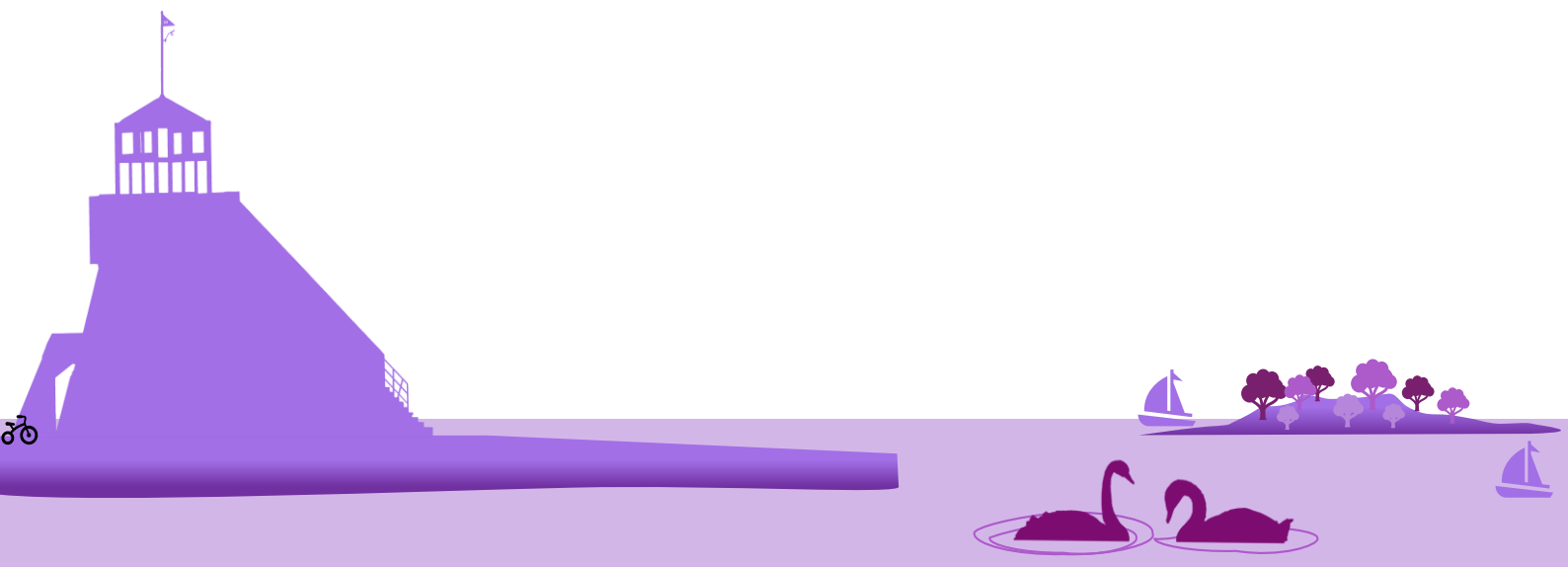
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Over the past two decades, solid-state NMR spectroscopy has undergone transformative advancements driven by increased magnetic field strengths and the advent of ultra-fast magic-angle spinning (MAS). While traditional approaches have relied primarily on ^{13}C and ^{15}N detection, recent developments in ultra-fast MAS (>100 kHz) have revolutionized the field by enabling highly sensitive proton-detected experiments. These advances dramatically reduce strong 1H - 1H dipolar couplings, leading to unprecedented improvements in spectral resolution and sensitivity.

In this presentation, we explore the frontiers of proton-detected NMR spectroscopy under ultra-fast MAS conditions (up to 160 kHz) using the state-of-the-art Bruker 0.4 mm MAS probe. Our investigations span a range of biomolecular systems, from benchmark microcrystalline proteins to integral membrane proteins embedded in native-like lipid bilayers. We demonstrate significant improvements in spectral resolution and coherence lifetimes, enabling rapid protein fingerprinting, efficient resonance assignments, and high-precision structural characterization.

Additionally, we show that the combination of strategic proton dilution through selective deuteration and spin-state selection techniques, coupled with ultra-fast MAS, allows us to achieve the narrowest proton linewidths ever reported in biomolecular solids. By averaging out coherent contributions to relaxation, we further unlock highly sensitive probes of molecular motions. Cutting-edge ^1H , ^{15}N , and ^{13}C relaxation measurements provide atomic-level insights into backbone and side-chain dynamics across multiple timescales, deepening our understanding of biomolecular function.

These results not only enhance our ability to resolve the structural and dynamic complexity of large biomolecular systems but also open new avenues for investigating ligand binding, intermolecular interactions, and hydration or lipid contacts.



Investigating structural parameters via ^{199}Hg NMR spectroscopy

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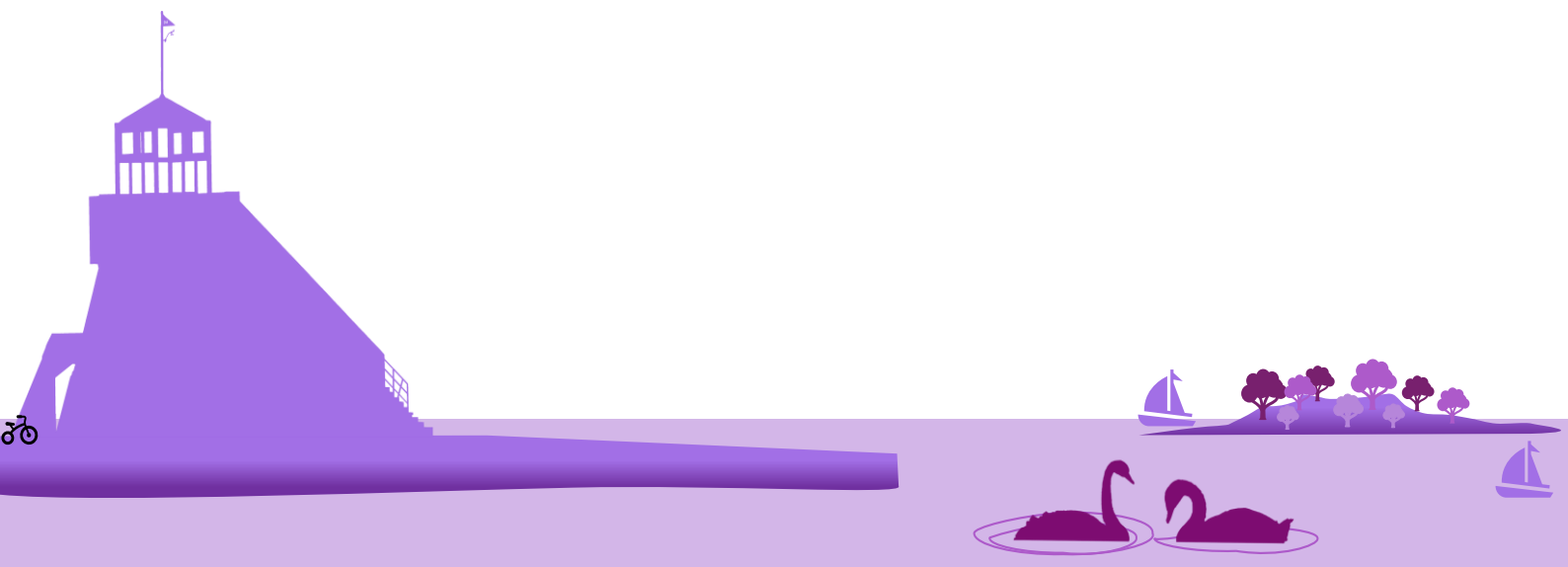
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The key role of structural analysis is to gain insight into the structure of a compound. This allows the structure-reactivity relationship to be studied and the structure of the compounds to be optimised for applications. The gold standard of structural analysis has long been single-crystal X-ray diffraction. However, if the compound does not form single crystals, we have to look for alternative techniques.

In the studies of metal complexes, one of the crucial structural parameters is the coordination environment. In this work, we studied mercury(II) complexes, therefore solid-state ^{199}Hg NMR spectroscopy was a natural choice. The present work explores the potential of ^{199}Hg NMR to provide structural insights into the coordination geometry of $\text{Hg}(\text{II})$ complexes.

Despite its promise, the application of solid-state ^{199}Hg NMR spectroscopy is often challenging. The wide chemical shift range of ^{199}Hg NMR, with peaks often spanning thousands of ppm, poses a significant difficulty in spectra acquisition. In addition, DFT calculations to evaluate the agreement between the experimental spectra and the proposed structural model require a full spin-orbit relativistic treatment, which can be time-consuming.

This research demonstrates the value of solid-state ^{199}Hg NMR as a complementary tool in structural characterization and offers ways to overcome the problems associated with its application. By studying the literature data, we were able to find useful relationships between the experimental NMR parameters and the structural characteristics of the studied compounds. By addressing the challenges of ^{199}Hg NMR spectroscopy, this study lays the groundwork for its broader adoption in the analysis of mercury coordination environments.



The role of formate mobility for adaptive hydrogenation catalysts probed by solid-state NMR spectroscopy

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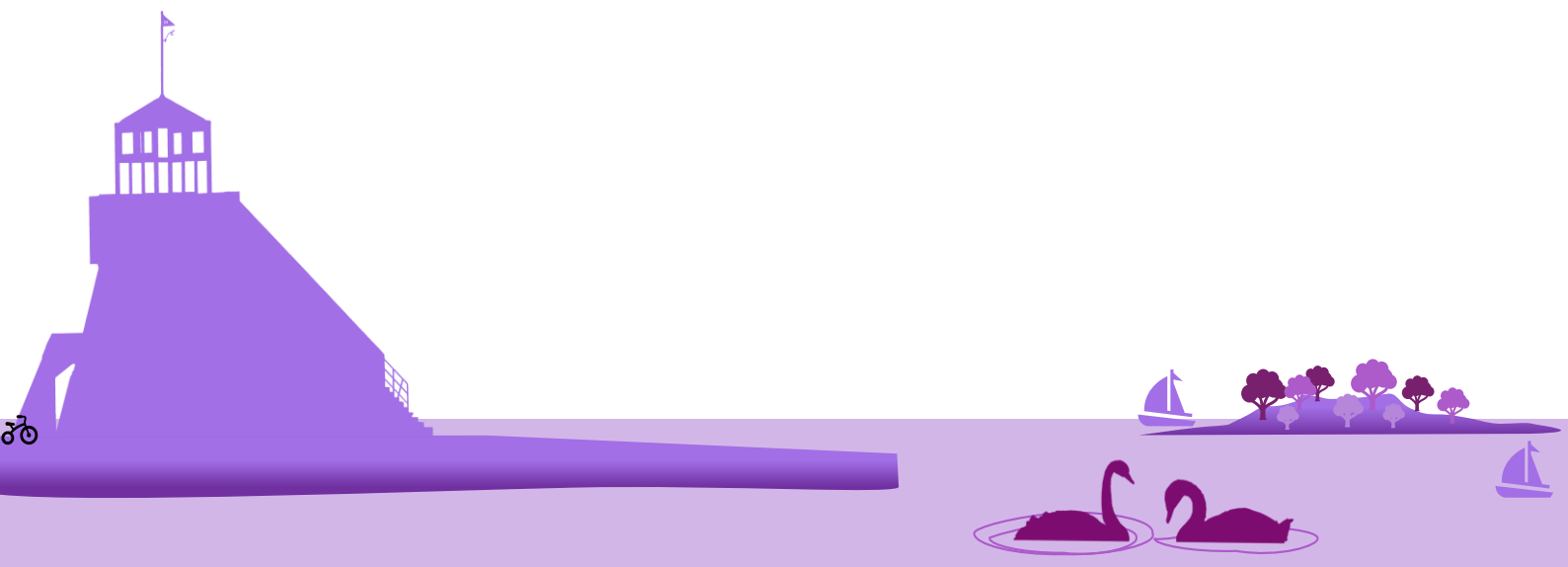
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Ruthenium nanoparticles (Ru NPs) immobilized on supported ionic liquid phases (SILPs) show adaptive catalytic properties in hydrogenation reactions of ketones in response to adding CO₂ to the feed gas. Using SILPs as silica surface modifiers increases the equilibrium concentration of formate species at the catalytically-active centres, which affects the selectivity of hydrogenation reactions [1]. In this study, we apply solid-state Nuclear Magnetic Resonance (NMR) spectroscopy to investigate molecular interactions between formate and the SILPs to unravel the principles responsible for the increased formate equilibrium concentrations on Ru@SILP catalysts.

We investigate the mobility of formate on SILP surfaces using relaxation properties and polarization efficiencies as sensitive probes. Faster ¹H transverse relaxation times and changes of ¹H-¹³C polarization transfer efficiencies in Cross-Polarization (CP) and Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) experiments point to reduced molecular mobility of formate in case of SILPs. Deshielding of formate ¹H and ¹³C chemical-shift values in this case points to an increase in local pH, which underpins the role of the IL head groups as Brønsted bases causing deprotonation of formic acid. The resulting molecular interactions between the IL and formate are responsible for improved adsorption of formate on the surface, leading thus to higher equilibrium concentrations during the hydrogenation reaction.

In addition, we carry out ³¹P-³¹P and ³¹P-²⁹Si homo- and heteronuclear distance measurements to probe conformations and ordering phenomena of the ILs on the surface. With the aid of Molecular Dynamics (MD) simulations, we are in the process of deriving a structural model using NMR-based distance restraints.

[1] Zhang, Y.; Levin, N.; Kang, L.; Müller, F.; Zobel, M.; DeBeer, S.; Leitner, W.; Bordet, A., J. Am. Chem. Soc., 2024, 146, 30057-30067.



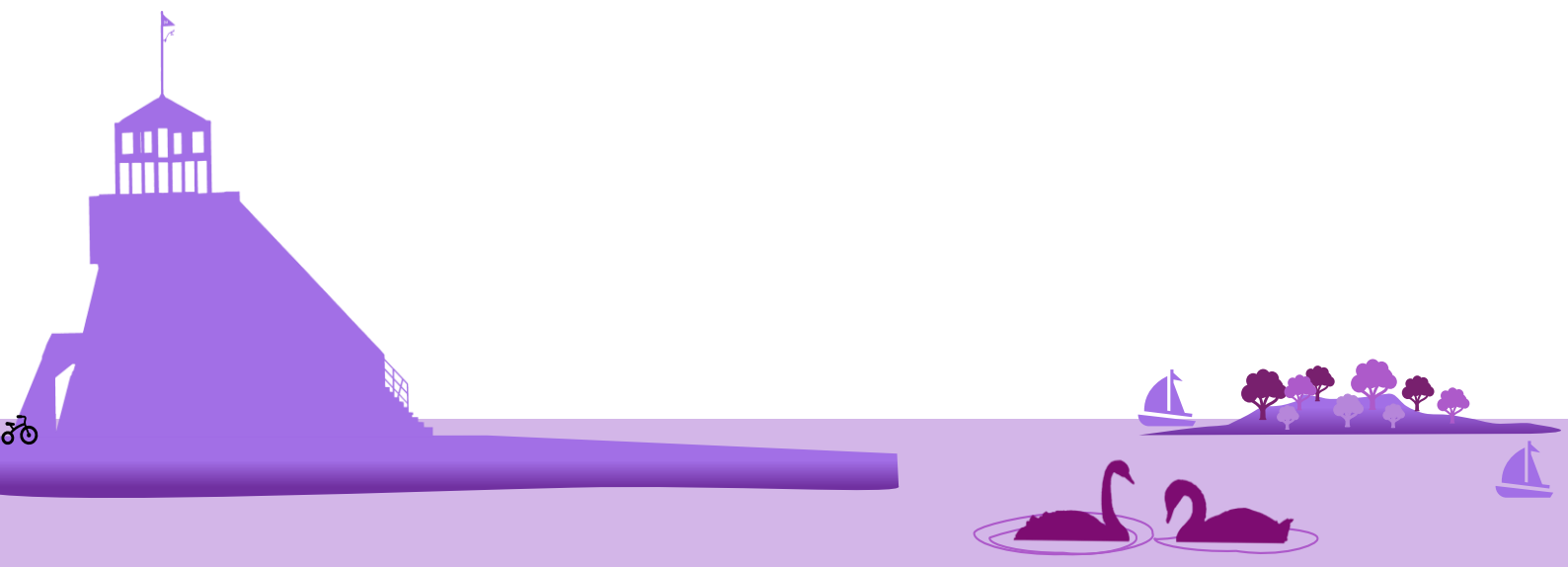
High-resolution in-situ photo-irradiation MAS NMR

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Light plays a pivotal role in many solid-state materials technologies such as photovoltaic substrates, heterogeneous photocatalysts, UV-initiated polymers, and photo-degradation resistant materials. Meanwhile, solid-state nuclear magnetic resonance (NMR) is a key tool for characterizing atomic-scale structure and dynamics in solid materials, but the requisite magic angle spinning (MAS) of the sample-containing rotors limits the measurement of these systems in their irradiated states. Here we report on a bespoke methodology for performing solid-state NMR of a sample irradiated with visible/ultraviolet (vis/UV) light, while undergoing MAS at frequencies up to 15 kHz. A fiber optic insert guides vis/UV light to the spinning NMR rotor, where a glass end cap and dispersion rod acts to illuminate the sample inside the rotor. Hence, low-cost and easy-to-produce parts can be used to adapt existing commercial MAS probes and rotors. As a test case, the methodology was used to follow the photopolymerization reaction of n-butyl acrylate, providing well-resolved ^1H and ^{13}C NMR spectra of the semi-solid polymer gel throughout its formation. Differentiation of the monomer and polymer resonances provides reaction curves, allowing the determination of polymerization rates for different UV radiant powers. Further applications of the in-situ photo-irradiation MAS NMR setup for the study of photo-active solids are explored.



High-Field Solid-State NMR of Pharmaceuticals

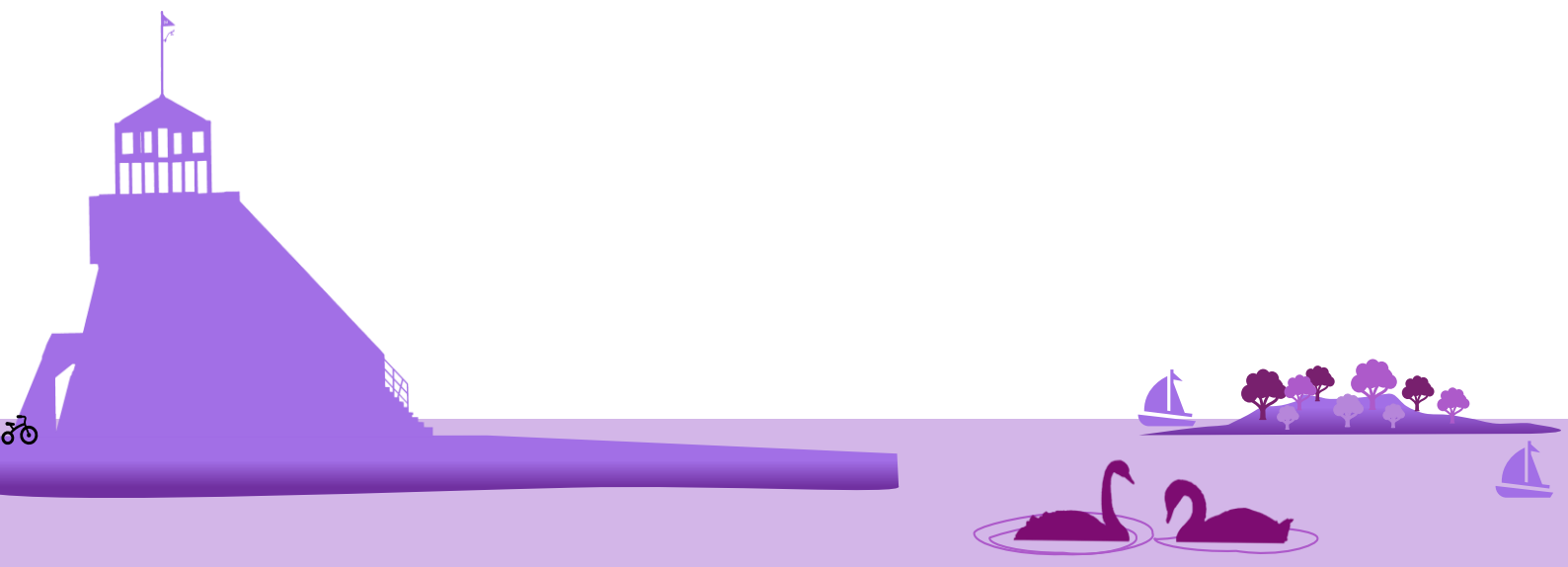
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An NMR crystallography approach that combines magic-angle spinning (MAS) NMR experiments with DFT calculation is applied to characterize two active pharmaceutical ingredients, Lorlatinib [1] and Ritlectinib [2]. Specifically, ^{14}N - ^1H heteronuclear multiple-quantum coherence (HMQC) [3] and ^1H - ^1H double-quantum (DQ) single-quantum (SQ) correlation experiments are presented at a ^1H Larmor frequency of up to 1 GHz and a MAS frequency of up to 60 kHz. A full assignment of the ^1H and ^{13}C chemical shifts is achieved using also ^1H - ^{13}C cross polarization (CP) HETCOR spectra. A particular focus is on probing key hydrogen bonding interactions that drive the adopted molecular packing in a specific polymorph. GIPAW calculations of chemical shifts for ^1H , ^{13}C , $^{14}/^{15}\text{N}$ and ^{19}F as well as of electric field gradients for ^{14}N complement the MAS NMR experiments.

1. Rehman et al. J. Pharm. Sci., 2023, 112, 1915.
2. Rehman et al. Faraday Disc., 2025, 255, 222.
3. Tatman et al. J. Magn. Reson., 2023, 352, 107459.



Kidney stones – Solid-state NMR spectroscopy and imaging

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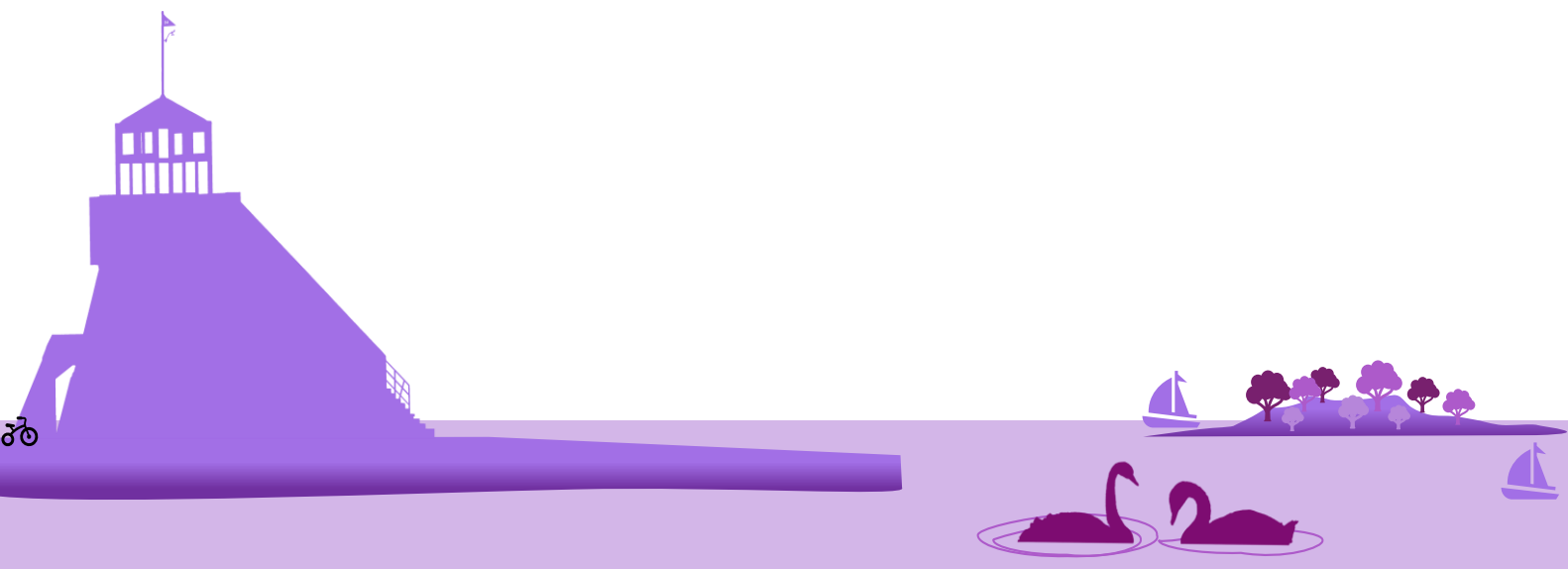
Kidney stones (KS) , also known as nephrolithiasis, are a public health issue, affecting a wide range of ages (with lithiasis symptoms appearing for childrens under the age of 2 years old).

In France, annual costs approaches 800 million euros for the treatment of the renal function. Given the complexity in terms of morphology and composition, the community of clinicians, including those at Tenon hospital (Paris), are particularly interested in any original method of characterization that could led them, as a first result, to understand the laws dictating the growth of kidney stones, then open new prospects for diagnosing and preventing serious calcifications.

In this contribution, we propose the very first 3D MAS MRI (^1H , ^{31}P) images related to KS. The KS samples were kindly provided by Dr M. Daudon, nephrologist at the Tenon hospital in Paris. One sample contained mostly organic mobile components such as lipids and triglycerides and exhibit sharp resonances which are easily imaged at moderate MAS rotation frequency (10 kHz).

On the other hand, a KS made mainly of rigid struvite (NH_4MgPO_4) was successfully imaged by ^1H - ^{31}P CP MAS 3D MRI. In other words, MAS MRI offers a large number of contrast types, probably exceeding those used traditionnaly in standard MRI. The attained spatial resolutions were $40 \times 75 \times 75 \mu\text{m}$ and $50 \times 150 \times 150 \mu\text{m}$ for ^1H and ^{31}P MAS MRI, respectively. Such resolutions are lower than those encountered in micro-CT but MAS MRI has unique potentialities in terms of chemical discrimination and local dynamics (far above micro-CT current capabilities).

The proposed techniques could ultimately provide, new and reproducible, analytical and diagnostic procedures, accessible for the community of clinicians, capable of characterizing kidney stones with precesion, in order to assist tradition methods and assess the causes of nephrolithiasis.



Workflow for Dynamical SS-NMR Simulation of ^7Li Spin Alignment Echo in Energy Materials

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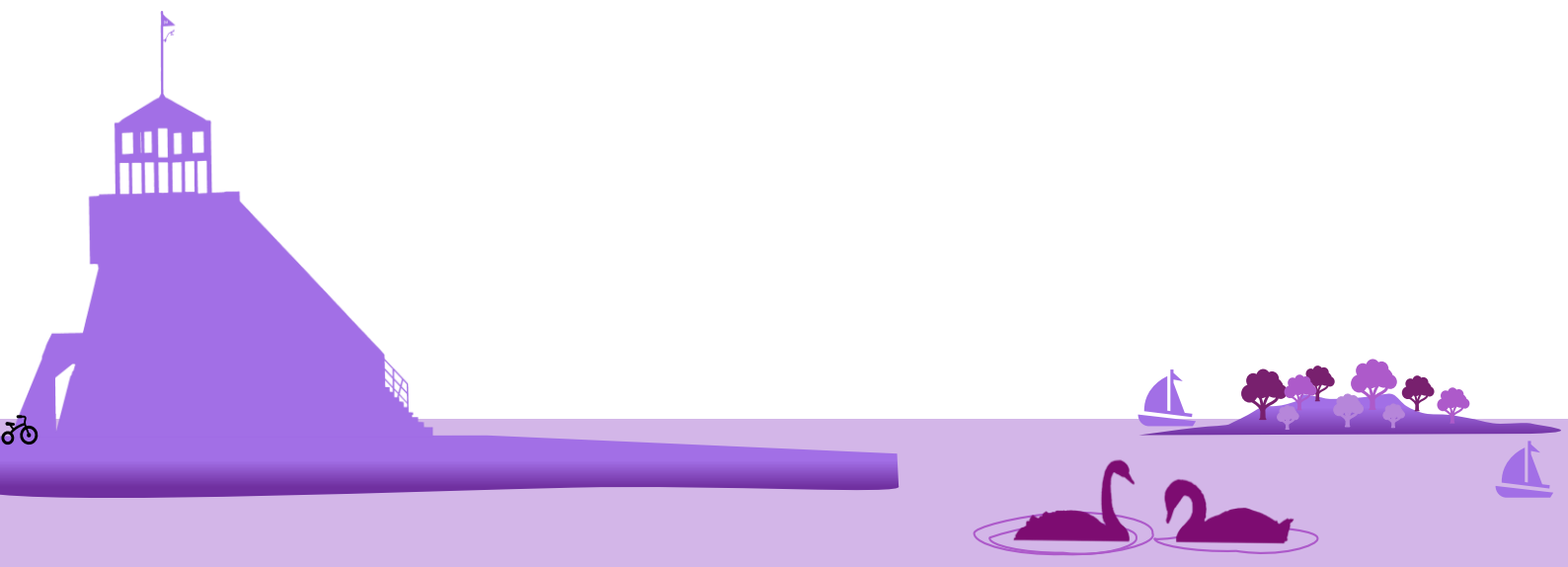
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Solid-state electrolytes are crucial in the development of safe, high-energy lithium-ion (Li) batteries, yet probing Li-ion dynamics in these materials remains challenging. Owing to its non-destructive nature and sensitivity to local atomic environments, solid-state nuclear magnetic resonance (SS-NMR) spectroscopy has emerged as an invaluable tool for characterizing diverse Li-ion environments and investigating ion mobility.

In this work, we present a multiscale, machine-learning (ML) approach for the computation of dynamical SS-NMR observables in the fast ionic conductor $\text{Li}_{10}\text{GeP}_2\text{S}_{12}$ (LGPS). The Li ionic motion is reproduced by molecular dynamics (MD) simulations using a new class of ultra-fast potentials (UFPs), which enable us to approach experimentally relevant microsecond timescales at room temperature. The full electric field gradient (EFG) tensors along the ^7Li trajectories are then computed with a tensorial ML model providing the basis for simulating, in silico, the ^7Li spin-alignment echo (SAE) experiment by computing the ^7Li quadrupolar frequency autocorrelation function (ACF).

Finally, to extract a continuous distribution of relaxation time constants directly from the decaying ACF—without presupposing any particular functional form—we implement an inverse Laplace transform algorithm stabilized by Tikhonov regularization. We therefore obtain a density function of relaxation rates that represents the underlying diffusion processes.

By integrating large-scale MD with UFPs, ML-based prediction of NMR parameters, and rigorous signal post-processing, our comprehensive workflow provides an end-to-end simulation platform for computational SS-NMR. We demonstrate, for LGPS, how to access observable ionic diffusion correlation times and mechanisms from an atomistic description of solid-state materials.



Magnetic orientation of microcrystals

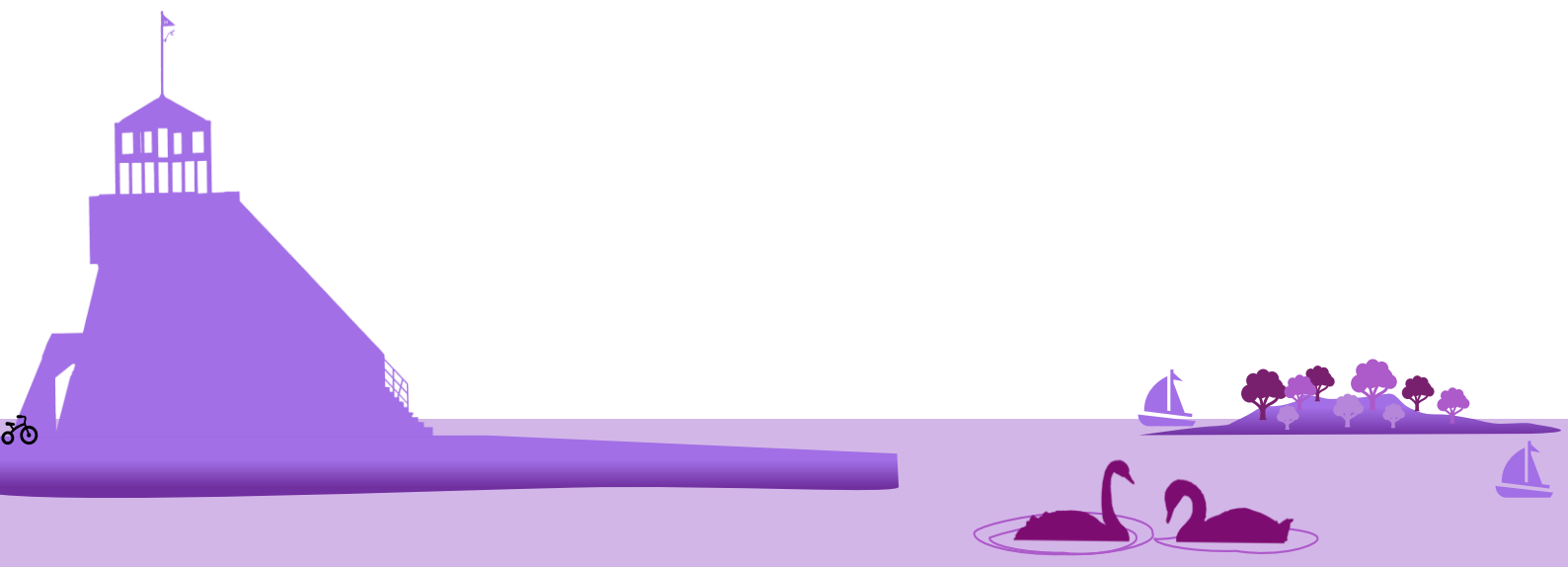
Kusumi, Ryosuke (1,2); Yasui, Hayate (1); Kadoma, Hiroshi (1); Wada, Masahisa (1); Kamide, Tomoya (1); Noda, Yasuto (1); Takeda, Kazuyuki (1)

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We report solid-state NMR of magnetically oriented microcrystals and its applications to ^{14}N NMR as well as full characterization of a ^{13}C chemical shift tensor including orientation of the principal axis system. With a home-built probe capable of modulating the rotation of the sample around the axis normal to the magnetic field, magnetically oriented microcrystal suspension (MOMS) of L-alanine is made. ^{14}N NMR spectra acquired with various timings during intermittent rotation lead to a rotation pattern of the MOMS similar to that of a single crystal. The effect of orientational distribution of the microcrystals to broadening of the resonance line is discussed. We also demonstrate determination of ^{13}C chemical shift tensor in MOMS with modulated rotation and temporal tilting of the sample during the period of NMR signal acquisition.



Homo- and heteronuclear nonsecular resonances

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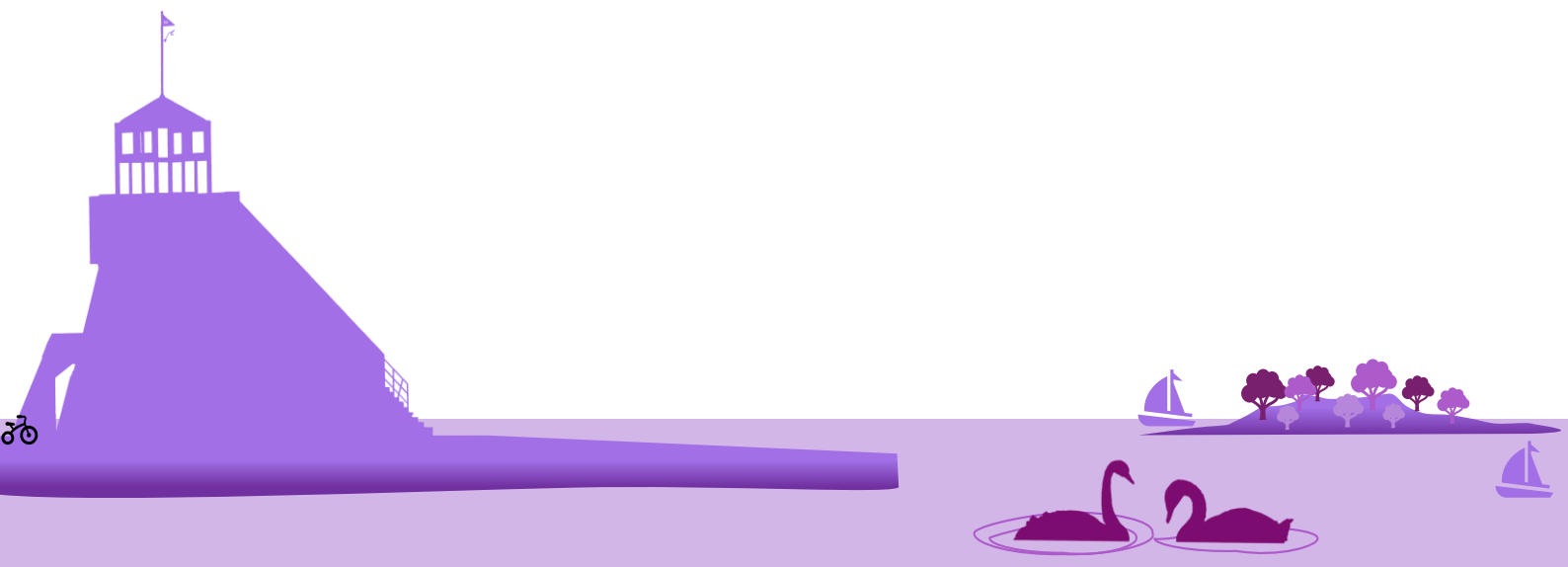
Nonsecular resonances (NSRs) dispense with two fundamental paradigms of high-field magnetic resonance of spins-1/2: (i) the resonance condition, that spins can only be manipulated by time-dependent magnetic fields at or near Larmor frequency Ω_0 , and (ii) the secular truncation, that dipolar spin-spin-interactions are truncated to the secular terms that commute with the Zeeman Hamiltonian.

NSRs do not arise from multi-quantum transitions, but rather are predicted for conditions far off Larmor frequency [1] under which a spin system's dynamics can be described in terms of closed trajectories on the Bloch sphere as opposed to open trajectories representing the generic case. In close analogy to resonant Kolmogorov-Arnold-Moser (KAM) tori that destroy integrable motion, the closed trajectories in our case break the secular approximation for the time-averaged interaction Hamiltonian. As a consequence NSRs lead to effects quite counterintuitive for the experienced NMR-practitioner.

The five homonuclear NSRs occur between $1/3 \Omega_0$ and $2 \Omega_0$ and their irradiation leads to nuclear depolarization, for which we show the first experimental evidence and discuss potential applications. [2] The zoo of heteronuclear NSRs also covers a huge range of frequencies away from the Larmor frequencies. While some heteronuclear NSRs are dissipative, others preserve the total spin polarization.[1] The latter ones can drive incoherent polarization transfer via the irradiation at only one frequency. This frequency is far away from the Larmor frequency of either spin species involved.

[1] C. M. Kropf and B. V. Fine, Nonsecular resonances for the coupling between nuclear spins in solids, Physical Review B 86, 094401 (2012)

[2] Jurkutat et al., Nuclear magnetic resonance far off the Larmor frequency - Nonsecular resonances, under review at Physical Review B



Studying ZnO/ZnS heterostructures by solid-state NMR to unravel mechanistic aspects of ZnO sulfidation process

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Semiconducting heterostructures are regarded as promising candidates for contemporary applications, such as green energy production,[1] pollution control,[2] and sensing.[3] Nevertheless, the performance of these nanomaterials is largely contingent on their preparation methods and hence, their rational optimization requires a detailed understanding of the mechanisms by which they are formed.[4]

One of these heterostructures, ZnO/ZnS (made up of ZnO core and ZnS shell) was the subject of this in-depth study.[5] These nanosized heterostructures with varying O/S stoichiometries were synthesized from pristine ZnO nanoparticles through sulfidation reaction via anion exchange under mild reaction conditions.

The preferential conformation of the surfactants on ZnO and ZnS zones of these heterostructures was determined by ^1H - ^{13}C CP-MAS NMR experiments. Additionally, the local environment of O-atoms in ZnO phase was probed using ^{17}O NMR. Selective labelling studies with ^{17}O -enriched water and the quantitative ^{17}O NMR data for different sulfidation levels enabled the differentiation between surface and core O-atoms. Specifically, it was possible to characterize and quantify the O-atoms at the interfaces between the ZnO and ZnS zones based on the level of sulfidation.

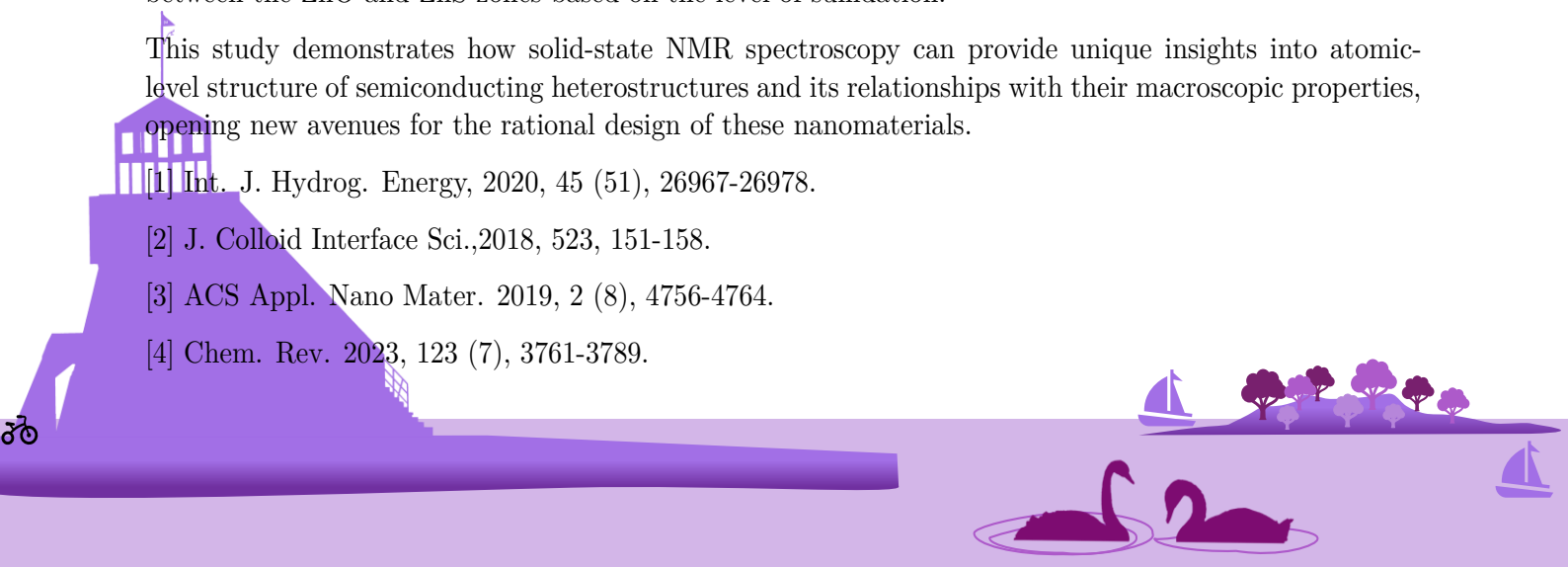
This study demonstrates how solid-state NMR spectroscopy can provide unique insights into atomic-level structure of semiconducting heterostructures and its relationships with their macroscopic properties, opening new avenues for the rational design of these nanomaterials.

[1] Int. J. Hydrog. Energy, 2020, 45 (51), 26967-26978.

[2] J. Colloid Interface Sci., 2018, 523, 151-158.

[3] ACS Appl. Nano Mater. 2019, 2 (8), 4756-4764.

[4] Chem. Rev. 2023, 123 (7), 3761-3789.



Polarization transfer efficiency under spinning frequencies up to 150 kHz - an experimental approach

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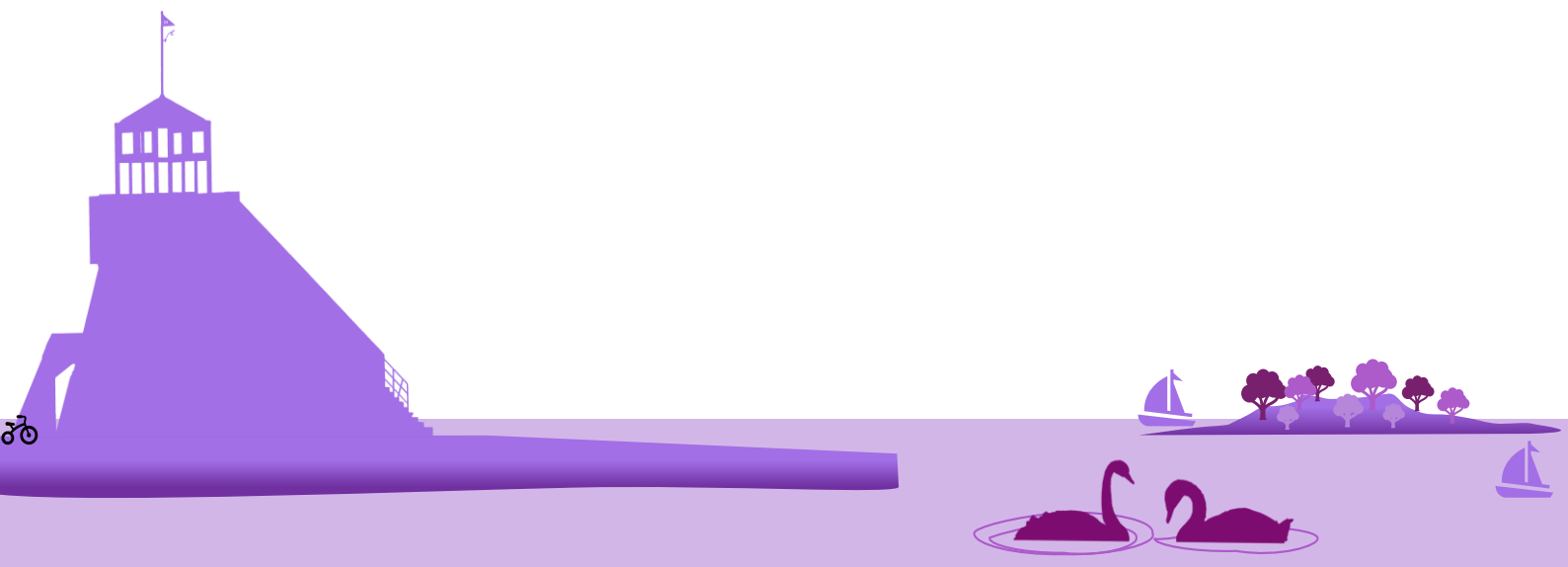
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Despite the necessity and large application of the polarization transfer mechanism, it is quite difficult to obtain a generally uniform enhancement of the NMR signals. This is more evident, especially in the case of the experiments where double cross-polarization (CP), or even CP, is applied under higher spinning frequencies (>60 kHz) and high magnetic fields (>28.2 T). A decisive role in the polarization transfer efficiency could be: (i) the strength of the dipolar coupling, (ii) the radio-frequency field inhomogeneity, large chemical-shifts dispersion, and (iii) molecular motion. However, there is now theoretical or experimental evidence that compares all these facts and explains the contribution of each of them under ultra-fast MAS (e.g., 150 kHz).

In this work, we experimentally investigate how RF field inhomogeneity affects polarization transfer efficiency using a standard CP sequence at spinning speeds up to 150 kHz. Using ^{13}C - ^{15}N labeled glycine as a rigid test molecule, rotors were packed with 20% glycine and 80% KBr in two ways: (i) with glycine in the center and (ii) at the bottom of the rotor. RF inhomogeneity reduced the NMR signal by up to 60% at 20 kHz MAS and up to 90% at 150 kHz MAS.

Secondly, the possibility of polarization transfer through J-couplings under fast MAS was explored by performing INEPT experiments under various conditions. It was demonstrated that polarization transfer can be obtained through J-coupling at 150 kHz MAS (but no NMR signal was obtained through this method for MAS < 100 kHz).

Finally, we also investigated the potential differences in the polarization transfer efficiency (using various conditions: different RF or MAS) induced by the employed instrumentation: we used two probe heads, one 0.5 mm homemade probe, and one 1.3 mm Bruker probe. The former has a higher inhomogeneity, while the latter is more stable. Optimum conditions for their operation will be presented.



15N Solid-State NMR Spectroscopy of Iodine(I) Complexes

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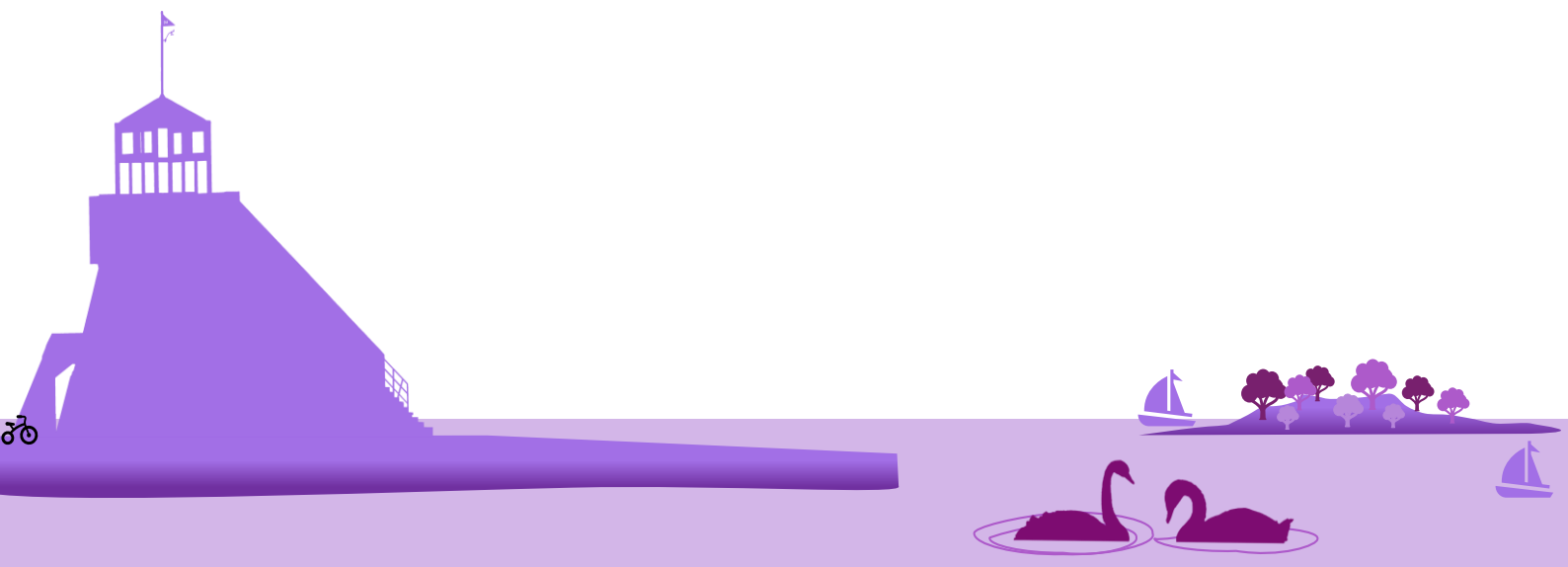
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Halogen(I) complexes are highly reactive in solution, which has limited the availability of their NMR data. [1] The progression from the free ligand to its Ag(I) complex and finally to its halogen(I) complex involves considerable electronic changes, which are easily observed by NMR. The use of ^{15}N NMR spectroscopy in particular, is advantageous in studying these reactions due to the significant changes in the chemical shifts upon interaction with silver and halogens. [2]

We have applied solid-state ^{15}N CPMAS NMR spectroscopy to a series of Barluenga-type iodine(I) [L-X-L]PF₆ complexes as their hexafluorophosphate salts together with their non-liquid ligands (L) and precursor silver(I) complexes. [3] The results were compared to the corresponding solution-state NMR results as well as the single crystal X-ray structures. A significant consistency in the chemical shifts measured in the solid state and those recorded in DCM-d₂ were shown. When compared the solution ^{15}N NMR chemical shifts for [Ag(isoq)₂]PF₆ and [I(isoq)₂]PF₆ (isoq = isoquinoline) measured in MeCN-d₃ [4] to the ones measured in DCM-d₂, a significant difference was observed in the case of the silver complex. This refers to the coordination of MeCN-d₃ to the silver(I) center. The solid-state shift coincides with the shift measured in DCM-d₂, clearly proving that MeCN-d₃ interacts with the silver(I) center.

The solid-state spectra showed practically the same chemical shifts as the solution spectra measured in DCM-d₂, indicating that the technique can be successfully applied to halogen(I) chemistry. More importantly, solid-state NMR excludes the effects originating from NMR solvent's coordination to the active sites, leading to a more reliable view of the halogen(I) complex formation.

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A detailed study of ^{19}F line widths and coherence life-times of fluorinated aliphatic amino acids in solid-state NMR at various MAS rates, ^1H decoupling schemes and magnetic field strengths

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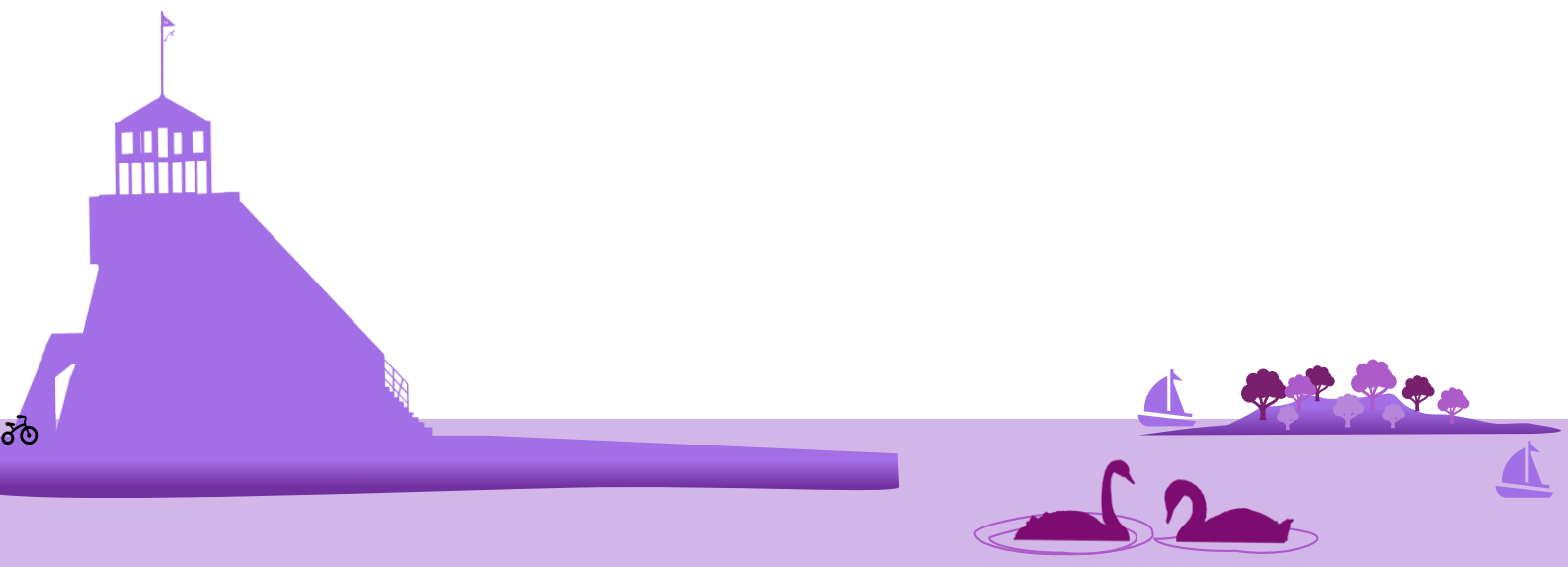
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^{19}F is a highly attractive nucleus for biological solid-state NMR (ssNMR) spectroscopy. Based on the statistical distribution of amino acid sequences in proteins, alanine and valine are the second and fourth most abundant amino acids. Therefore, fluorination of Ala and Val would provide valuable and unique information about protein structure, dynamics, and interactions. Although new ^{19}F ssNMR studies are emerging, detailed research into ^{19}F line widths and relaxation effects at different MAS rates, ^1H decoupling schemes and magnetic field strength are still lacking. Here, we present an in-depth study of ^{19}F detected ssNMR spectra of model fluorinated Ala (with $-\text{CH}_2\text{F}$ labeling) and Val (with $-\text{CF}_3$ labeling) compounds.

The ^{19}F line width analysis revealed that the $-\text{CF}_3$ exhibited only a minor effect from ^1H decoupling and a dependence of MAS rates, while $-\text{CH}_2\text{F}$ group had a much stronger ^{19}F line width reduction effect on these two parameters. This can be attributed to a stronger ^1H dipole coupling contribution due to the closer spatial proximity of ^1H nuclei in the $-\text{CH}_2\text{F}$ group. Analysis of the ^{19}F T_2' coherence life-time revealed a stronger inhomogeneous contributions to the $-\text{CH}_2\text{F}$ group compared to the $-\text{CF}_3$ group. The quantitative ^{19}F line widths analysis was further supported by numerical simulation of the ^{19}F spectra.

This project is funded by European Commission project “MR Latvia” 101160091, under a HORIZON-WIDERA-2023-ACCESS-02 programm.



Bridging the Funding Gap: An Open-Design 30 GHz EPR Spectrometer for Advanced DEER Applications

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We present an open-source Ka-band (30 -31 GHz) EPR pulse spectrometer that leverages the latest advances in 5G and satellite communication at a fraction of the cost of a comparable commercial instrument, addressing the critical challenges posed by worldwide reductions in scientific research funding.

The reduction in scientific funding is most notable in the closure of grants for instrumentation (such as R'Equip in Switzerland), which when combined with a significant number of 1st-generation commercial pulse spectrometers reaching their end-of-life, potentially risks a loss in EPR capacity as equipment is not renewed. Low-cost open-design spectrometers could be an essential part of the solution.

Over the past decade, high-speed arbitrary waveform generators (AWGs) with advanced sequencers and memory options, along with digitisers, have simultaneously become more affordable and more powerful, enabling simpler, more cost-effective and more capable spectrometers to be developed. For homebuilt spectrometer to become wide spread outside of hardware- focused research groups these systems must be easy and fast to build and repair.

Our new spectrometer has been optimized for automation and DEER spectroscopy but is capable of more. It has been configured with an affordable 30 W solid-state power amplifier (SSPA), which when paired with an optimised waveguide path and a high conversion factor loop gap resonator produces high B1 power (>50 MHz) in the sample. A double-super-heterodyne architecture with an initial up conversion to S-Band (3.5-4.0 GHz) has been used to take advantage of the higher quality filters and mixers.

The spectrometer is controlled by an open-source Python-based modular control software with an easy-to-understand Pythonic pulse programming language. The modular design allows for individual components to be replaced depending on the necessary specification (such as using a 34-46 GHz SSPA) and the future development of the field.



Sliding-sample DNP cryostat for thermal-swing applications

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Hyperpolarization by dissolution Dynamic Nuclear Polarization (dDNP) is an efficient way to increase the inherently low NMR sensitivity of liquid state experiments. However, despite high sensitivity boosts, up to 10'000-fold, the method is limited by a major drawback restricting its use, the sample is inevitably diluted and cannot be repolarized, making dDNP a single shot and destructive method.

To broaden the scope of dDNP to multi-shot experiments, such as multidimensional NMR methods, our team has developed a Benchtop DNP approach [1] that we aim to couple with rapid-melt and flow to a solution-state benchtop NMR device. The system under development aims at translating the frozen and polarized sample from a new 77 K custom cryostat design placed inside our 1 T benchtop polarizer, into a dedicated melting station where the solid-to-liquid state transition is performed without dilution. The sample will then be transferred to the benchtop NMR spectrometer for detection.

Here, we present the design and performance of a new custom 3 cm diameter compact stainless-steel and PEEK DNP cryostat, made of stainless-steel, PEEK and 3D printed resin, in which 10 mm o.d. samples can slide towards the melting station. The cryostat structure is conceptualized to reach internal temperature as low as 77K in only two minutes (double-wall outer vacuum chamber isolation), while being transparent to microwave and radio-frequency irradiations for DNP and NMR experiments. It also enables a leak-tight and secure sample slide toward the melting station. The development of this new concept of cryostat is a first very important milestone towards our final goal of producing replenishable hyperpolarization.

[1] Bocquelet, C. et al., Boosting¹ H and¹³ C NMR Signals by Orders of Magnitude on a Bench. Sci. Adv. 2024, 10 (49), eadq3780. <https://doi.org/10.1126/sciadv.adq3780>.



Development of a combined 329 GHz/500 MHz EPR/NMR/DNP system

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Despite the tremendous progress made in magnetic resonance techniques over the decades, many biological molecular systems remain too complex for Nuclear Magnetic Resonance (NMR) alone. Therefore, hyperpolarization techniques, such as Dynamic Nuclear Polarization (DNP), which enhances NMR sensitivity by transferring polarization from unpaired electrons via Electron Paramagnetic Resonance (EPR), offer a promising solution.

We present our novel high-field EPR/NMR/DNP system, FRASCAN II, which is a unique extension for NMR spectrometers (namely the Bruker Avance Neo 500, operating at 11.75 T), that enables the simultaneous use of EPR, NMR, and DNP within a single system. FRASCAN II consists of a custom-made EPR console holding the necessary control modules and electronics, an EPR bridge containing a quasi-optical setup for microwave generation, propagation, and manipulation, and a combined EPR/NMR/DNP quadruple-resonance probe. The probe is home-built, with electron resonance at 329 GHz, proton resonance at 500 MHz, deuterium resonance at 76 MHz, and an X-channel resonance that can be set up to a frequency of NMR-active nuclei within the range of 99-142 MHz thanks to the modular design of the probe.

Such setup allows us to perform pulsed NMR at 15 W in liquid samples, and thanks to the solid-state microwave source used, the system can perform Continuous Wave and Rapid Scan EPR at 60 mW in solid and liquid samples, all at laboratory temperatures, thus allowing us to perform DNP experiments, all within one system. The system uses standard 3-5 mm NMR tubes and is therefore compatible with Bruker SampleCase automation system for convenient insertion and ejection of the sample.

The presented system enables more versatile and efficient use of commercially available NMR instrumentation found in many NMR facilities worldwide, and its key benefits are its low acquisition cost, ease of installation, and user-friendly operation.



An experimental proof of the applicability of cryogen-free technology for high resolution solid state and liquid state NMR

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Introduction

Cryogen-free systems are widely used in Electron Paramagnetic Resonance and Dynamic Nuclear Polarisation. Besides the low maintenance, the cryogen-free technology presents two other major innovations for NMR. First, the magnet is much more compact. Second, the magnetic field in cryogen-free magnets is always ready to be changed. This opens the possibility to use the same magnet at different fields. To be suitable for high-resolution solid- and liquid-state NMR, high-quality data obtained on such systems has yet to be demonstrated.

Method

We performed various long-time multi-scan 1D and 2D measurements on a 400 MHz MAS NMR system for several solid-state and liquid-state samples. Our focus was on the temporal magnetic field distortion generated by the mechanical nature of the cryogen-free cold head operation.

Results

We showed that these distortions can be made smaller than one part per billion with respect to the main magnetic field. That makes the cryogen-free magnets acceptable for solid-state MAS NMR. For liquid-state measurements, an extra suppression of the cold head distortions is required that can be done by a correction AC applied to the Z0 coil from the RT shim set. We also found an extra field distortion coming from the underground train operation. A post-acquisition data correction based on reference deconvolution helped us to improve the quality of our 1D and 2D spectra up to the standard liquid-state NMR conditions. The high-quality liquid-state 2D spectra were recorded at different magnetic fields.

Conclusion

We demonstrated that cryogen-free magnets can be used for high-resolution solid- and liquid-state NMR. We are currently designing a new magnet that will be immune to internal and external magnetic field distortions. Cryogen-free magnets can replace their liquid-cryogen counterparts for frequencies up to 750 MHz. One magnet can be used at various magnetic fields, up to the maximum possible.



Rapid Melting Strategies for benchtop DNP: A Step Toward Replenishable Hyperpolarization in Liquid-State NMR

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Hyperpolarization techniques provide a solution to the inherently low sensitivity of NMR, enabling signal enhancements exceeding 10,000-fold [1]. Dissolution dynamic nuclear polarization (dDNP) is one of the most established methods, but it suffers from key limitations: it is destructive for sample and only for single-use. Once the hyperpolarized frozen sample is dissolved, it is inevitably diluted, leading to rapid signal decay and preventing multiple scans [2]. However, many NMR experiments require phase cycling and multidimensional acquisitions, which are incompatible with this single-shot approach.

We are developing a new Benchtop DNP approach aiming at enabling replenishable hyperpolarization without dilution. Our approach employs hyperpolarizing materials (HYPOPs) [3] in a compact Benchtop polarizer [4], to be coupled to a benchtop NMR spectrometer for solution-state detection. Our final goal is to implement a closed-loop system allowing the sample to undergo repeated freeze-DNP-melt-flow cycles.

An critical aspect of our project is to determine how DNP enhancements can be preserved through the melting process. In our current setup, the sample can be translated from our 77K DNP cryostat inside our 1T benchtop polarizer, into our heating station. The first version of this rapid melting station presented here consists of a metallic structure guiding a high flux – 500L/min – of high temperature – 630°C – air towards the sample, enabling 250 μ L sample melt within 5 seconds. We are now working at speeding up this melting step below a second by implementing higher flows and temperatures and high-power laser light, and coupling the melting station with DNP and solution-state hyperpolarized NMR.

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Micro-chip NMR systems for new microscale applications

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Microcoil NMR is opening new frontiers in NMR research by boosting sensitivity extremely volume-limited volume [1-4]. However, the complexities of micro-coil fabrication and use limit the uptake of these techniques, restricting their application in key areas such as microscopic biological samples [5-8]. We introduce a next-generation micro-scale NMR platform that leverages scalable integrated microchips, combined with additive manufacturing and other flexible production methods. The system is highly adaptable to diverse sample types, scalable for high throughput, and compatible with standard spectrometers. A portfolio of broadband microcoils target volumes from 500 nL down to 1nL, function up to 600 MHz for use with high sensitivity spectrometers, and down to 10 MHz for X-nuclei detection. We report on the system features and design, highlight a proof-of-concept application in embryology, and discuss the future developments of the system.



Automatic bullet-DNP

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Dissolution-DNP [1] is a versatile signal enhancement method for liquid-state NMR spectroscopy. It is primarily limited by low throughput, requiring human intervention after every experiment. Here we show that bullet-DNP [2-4], a variant of dissolution-DNP in which the hyperpolarized material is transferred as a solid, can be automated such that one bullet after another is prepared, hyperpolarized, dissolved and detected without any human intervention. In addition to the obvious gains from increased throughput (the system operates at a duty cycle of 40 min), experiments conducted in this way exhibit a high repeatability. We use the gains in repeatability to (i) optimize conditions for sample transfer, resulting in ^1H signal enhancements well above 1000 for typical ligand molecules (without the need for ^{13}C labelling) and (ii) to conduct drug screening experiments where we target screening in excess of 1000 compounds within a single day.

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AI-assisted NMR Hardware Design with Optimized B₀ and B₁ fields

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The design of nuclear magnetic resonance (NMR) probes with optimized B₁ and B₀ homogeneity is a complex engineering challenge requiring precise electromagnetic field control. Recent advancements in large language model (LLM) artificial intelligence provide a powerful tool for accelerating probe design by integrating computational simulations, optimization algorithms, and heuristic knowledge extraction. LLMs assist in parameter selection, geometry optimization, and automated refinement of coil configurations to enhance radiofrequency (RF) field uniformity (B₁) and static magnetic field homogeneity (B₀). By leveraging AI-driven iterative design processes and predictive modeling, researchers can efficiently explore innovative probe architectures, reduce computational costs, and improve overall probe performance. This approach paves the way for more efficient and accessible NMR hardware development, advancing applications in spectroscopy and imaging.



Digital twin-enabled radiofrequency and gradient decoupling for parallel NMR spectroscopy

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The implementation of parallel nuclear magnetic resonance detection aims to enhance measurement throughput in support of high throughput screening applications including, for example, drug discovery. Multiple detector geometries compatible with commercial NMR magnets (wide-bore) have limited space, introducing challenges in RF and gradient field cross-talk between the detection sites. We describe a compensation scheme based on digital twinning of the hardware for static and dynamic magnetic field prediction coupled with spin dynamic calculations to predict optimized pulse sequences that minimize cross-talk effects. In this contribution we present results for minimizing both RF and gradient field coupling effects across multiple NMR detectors, enabling access to high performance spectroscopy experiments in a parallel NMR environment.



Sample-accessible multi-resonance X-band EPR triple ring resonator

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Electron paramagnetic resonance (EPR) spectroscopy is a powerful technique for sensing unpaired electrons in radicals and metal complexes. It is performed with a resonating microwave cavity to excite the sample at the Larmor frequency. Micro-resonators downsize the resonating structures for EPR spectroscopy, allowing sensitive detection on mass-limited samples. Planar resonators offer accessibility of samples for rapid characterization and screening against environmental parameters such as UV, gas, and liquid flow, as well as temperature gradients. We report a triple-ring resonating structure exploiting the benefits of sample accessibility and miniaturized structure. It offers three simultaneously operating X-band channels (8 - 11 GHz) with real-time accessible 50 nL sample volume for each channel, with an average spin sensitivity of $1.5 \times 10^8 \text{ Spin/Hz}^{(1/2)}$. By cascading the ring resonator in a concentric topology, we improve the quality factor of each resonance by reflecting and containing the electromagnetic energy by the neighboring rings, which are silent to the resonance. The Q-factor of the center resonance at 9.45 GHz, with the enhancement of two passive reflectors, reaches 80. The tuning and matching are achieved through three translational movements, which function additionally as channel suppression and isolation by decoupling the unwanted ring resonator from the feed. The triple ring resonator provides a convenient and flexible platform for in-situ and in-operando experiments for multiple samples with accessibility. It suits scarce or precious samples with the capability of high-throughput screening.



A Magnetic Coil to Generate Magic Angle Offsets

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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. However, mechanical implementations suffer from the problem to move the stator, endangering the stability and quality of MAS. Another 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 18mm radius and 100 turns, operating at 10A affords a 50mT field, which yields a magic angle offset of 0.14° . The calculated relative homogeneity of the field is 0.02% within the volume of a 0.7mm rotor, while a practical relative homogeneity of 1% would suffice for our purpose. To ensure the stability of the coil, a backed coil with 0.45mm diameter copper wire was chosen, with a ceramics holder. Preliminary calculations showed that the coil pair has a resistance of 3Ω , giving a power of 300W at 10A. With a duty cycle of 33.3ms/s, finite elements simulations showed a coil heat-up of 25°C after 100s. Experimental measurements revealed a temperature increase of ca. 15°C after 1s of operation at 9A, indicating the model to insufficiently describe the coil heating. In any case, cooling with an air flow is required to prevent excess heating of the probe and sample. The rise times for the manufactured coils are below 1 ms, during which eddy currents might be generated in the superconducting magnet, with a longer decay than the rise time. To counteract these eddy current formation, a shielding coil was designed such that the outside field is significantly reduced, therefore also reducing magnitude (and decay time) of the eddy currents.



MAS progress

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Improved turbine design, rotor geometry and better materials have allowed us to achieve spinning speeds over 230 kHz if driven by air and 250 kHz if driven by helium. The ultimate value shows clear dependency on the speed of sound in respective media.



Bullet DNP Setup for Titration Experiments

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Dissolution dynamic nuclear polarization (d-DNP) provides liquid samples with high spin polarization of target molecules. However the polarization step can take tens of minutes to several hours - much longer than the typical liquid state relaxation time. At the same time the hyperpolarized liquid can have volume, concentration and polarization that are sufficient for multiple experiments. Using the polarized liquid to prepare several different samples can increase the throughput of hyperpolarized liquid state NMR spectroscopy without the need to modify the polarizer itself.

We present a setup that uses four synchronized syringe pumps and a stripline flow probe [1] to perform multiple NMR experiments with varying sample composition using a single bolus of hyperpolarized liquid produced by the bullet - DNP method [2]. The stream of the polarized liquid is combined with up to two other liquids streams of arbitrary composition. The result is then fed into a microfluidic mixer [3] and from there to the NMR probe. The mixing ratio of the streams is adjusted through the flow rates of the syringe pumps. A fourth syringe pump aspirates the resulting mixture thus controlling the overall flow rate.

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Teaching an old FFC new tricks - Feedback control and other improvements on a 20th-century FFC-setup

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After decades of service at Nottingham University a home-built fast-field-cycling (FFC) setup was given to our lab in Karlsruhe in 2020. The superconducting magnet system is still unique in its capabilities, combining ramp rates of up to 10T/s for fields from 0 to 2.5 T with a temperature range from 3 to 300 K.

Here we present an updated interface for the remote operation of the superconducting magnet's power supply using a field programmable gate array (FPGA) and show data documenting the performance improvements.

The target field value is set via TTL signals from the NMR spectrometer. Thereby it is now compatible with any spectrometer with two TTL channels and the field can be set and altered within the pulse program. The FPGA controls the magnet's current (and therefore field) via a control voltage (0-10V) on the magnet's power supply. Additionally, the FPGA gets feedback from a Hall sensor inside the superconducting magnet on the actual field and adjusts the control voltage via a PID controller implemented in the FPGA's soft processor. This decreases the time for field stabilization following a ramp, compensates for field drifts as well as random spontaneous offsets. Thereby the absolute error in field of previously 2mT is reduced to well below 1mT. This enables signal averaging with this setup for the first time, allowing for relaxation studies on less abundant, low gamma nuclear spin species.



Helium Recovery and Liquefaction Strategies for NMR Labs

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Helium is a finite resource, and users are becoming cognizant of their consumption due to the ongoing fluctuation in price as well as availability concerns. The Cryomech brand has been innovating in the helium management space for nearly 20 years and have enabled countless users to take ownership of their liquid helium usage. This independence allows you to guarantee the longevity of your lab and to continue research despite volatile helium market trends as of late. NMR labs vary greatly in size, appearance, and configuration, Bluefors' Cryomech product line offers several standard solutions that can cover nearly any labs' needs with each system being customizable to create a suitable match for your facility.



527 GHz Solid-State Source for Electron Paramagnetic Resonance and Dynamic Nuclear Polarization Applications

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Microwave sources are key components for any spectrometer capable of electron paramagnetic resonance (EPR) or dynamic nuclear polarization (DNP). At > 100 GHz, the availability of microwave sources is limited to vacuum tube-based options or solid-state (SS) sources. SS-sources, unlike the vacuum tube-based alternatives, are compatible with complex signal generation via arbitrary waveform generators (AWG), have wider tuning bandwidths, and are relatively affordable. The primary disadvantages of SS-sources are their lower output powers and the phase and amplitude distortions introduced by multiple stages of frequency multiplication necessary to reach 100s of GHz. Recently we demonstrated a 250 GHz SS-source compatible with amplitude modulation (AM) via a new mixer-based architecture. The aim of this work is to extend this mixer-based design capable of AM to higher frequencies and present a SS-source capable of operating at 131 GHz, 263 GHz, and 527 GHz that also closes the power gap between SS-sources and the vacuum tube-based alternatives. To obtain higher microwave power, our 131 GHz amplifiers were redesigned to produce more power per chip. Secondly, we eight-way power combined the amplifiers to achieve record breaking power levels for a SS-source at 131 GHz – 1.2 W. To reach our target frequency of 527 GHz, a mixer-based source architecture was used, where a signal at 131 GHz was mixed with an AWG and amplified via the eight-way amplifier block. The modulated signal was doubled to 263 GHz and then doubled again to reach 527 GHz. Each doubler after the mixer will distort the input signal, primarily in terms of amplitude and phase. Reducing the number of doublers after the introduction of the arbitrary waveform will minimize the distortion to the final signal and enable AM at >100 GHz. Here we present a record setting SS-source at 527 GHz that can generate complex signals with 30 mW of power via a mixer -amplifier-doubler-doubler design.



Benchtop DNP on Varying ^1H Spins Quantities Towards Broad Range Sample Hyperpolarization

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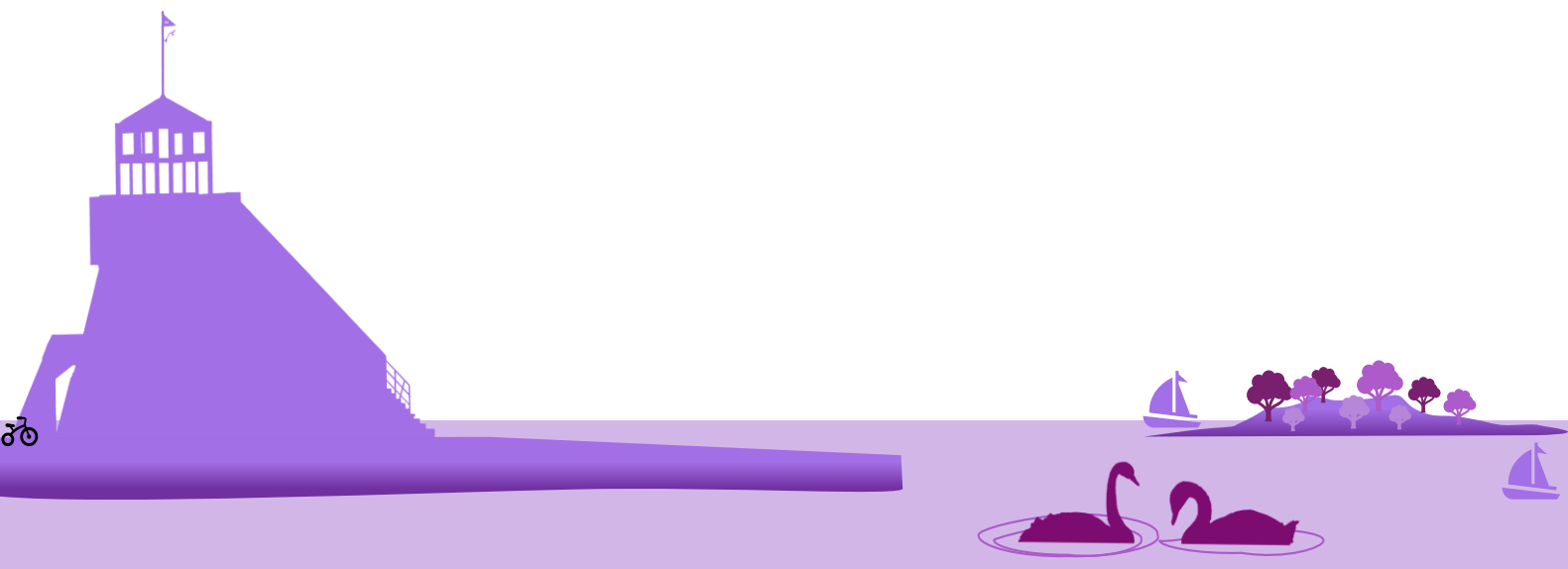
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Hyperpolarization by Dynamic Nuclear Polarization (DNP) at 1 T and 77 K in a benchtop polarizer was recently demonstrated [1], boosting ^1H and ^{13}C sensitivity by two orders of magnitude. The development of such compact instrumentation lies within the HypFlow approach enabling hyperpolarization of native frozen solutions without contamination or dilution. It employs porous hyperpolarizing matrices (HYPSOs) [2] covalently supporting radicals in which the solution is flowed, frozen and hyperpolarized. Initially, the polarizer performances were evaluated using deuterated samples with fixed ^1H and ^{13}C spin quantities. However, assessing DNP efficiency across a broader range of spin concentrations is crucial for native sample analysis. Here, we investigate solid-state DNP in a $\text{H}_2\text{O}:\text{D}_2\text{O}$ and ^{13}C -urea solution impregnated in HYPSOs, varying proton concentrations. We achieved a ^1H DNP enhancement of 50 for a fully protonated sample. The high ^1H polarization was transferred to ^{13}C via $^1\text{H}\rightarrow^{13}\text{C}$ cross-polarization (CP), the efficiency of which was also evaluated. The study also highlights polarization build-up times accelerate with higher proton concentrations, consistent with MAS-DNP observations [3]. Using a frozen solution with varying proton quantities, we applied Prisco et al.'s approach [3] to investigate hyperpolarization spreading across the spin diffusion barrier. Results show that up to ~ 50 M of protons, polarization is limited by ^1H spin diffusion. Beyond this, bulk polarization declines due to rapid spin relaxation with a minimum observed ^1H enhancement of 80. HypFlow hyperpolarization with non-deuterated samples may significantly expand DNP applications where samples need to be analyzed in their natural state. In the future, integrating our benchtop polarizer with sample melting may enable replenishable hyperpolarization, advancing multi-dimensional liquid-state NMR.

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New developments in high-pressure low-field NMR spectroscopy

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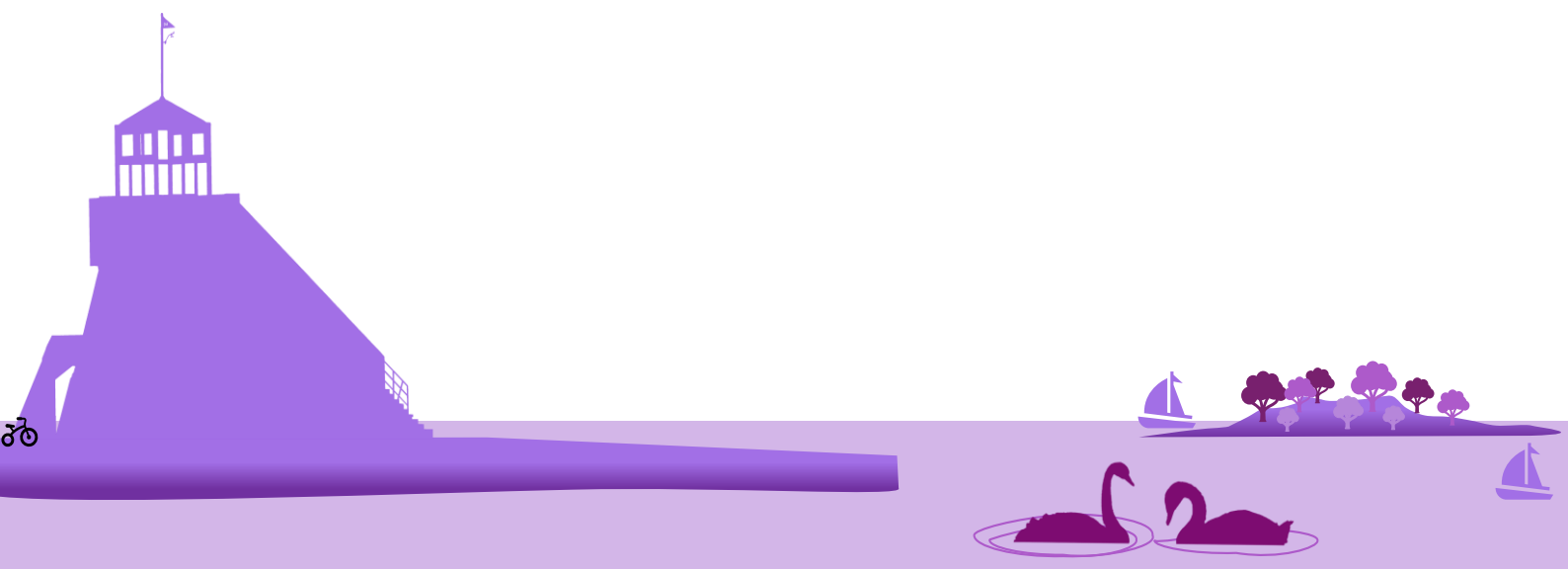
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Benchtop nuclear magnetic resonance (NMR) spectroscopy is finding increasing usage in academic and industrial research. Yet, until now, benchtop NMR spectroscopy was used mainly for the analysis of liquid samples and pressurized samples with pressures up to about 10 bar. High-pressure (HP) NMR at pressures beyond this value would however be very interesting for many applications including the analyses of highly pressurized gases and for parahydrogen-induced hyperpolarisation.

We have recently implemented a low-cost and versatile HP setup for benchtop NMR, which enables spectroscopy measurements at pressures up to 200 bar. Advances in the design of HP tubes, including a novel HP flow-cell, as well as novel procedures for spectra acquisition leading to a dramatic increase of the spectral quality will be presented [1-3]. Various applications will be shown to illustrate the capabilities of benchtop HP NMR: 1) improved composition quantification of complex gaseous mixtures using ^1H HP NMR spectroscopy. Despite of strong signal superposition, the combination of tube design, spectra acquisition procedure, and indirect hard modelling enables the determination of compositions in excellent agreement with the vendor certificates [2]. 2) composition analysis of natural gas mixtures with hydrogen in agreement with the data obtained from the partial pressures approach [3]; and 3) signal amplification by reversible exchange (SABRE) hyperpolarization with up to 200 bar parahydrogen [4]. In standard methanol solutions, a 3-fold signal enhancement was detected for pyrazine by increasing the pressure from 10 bar to 200 bar. Moreover, the use of CO_2 as a quick removable solvent for SABRE experiments was for the first time tested.

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Benchtop NMR analysis of illicit drugs

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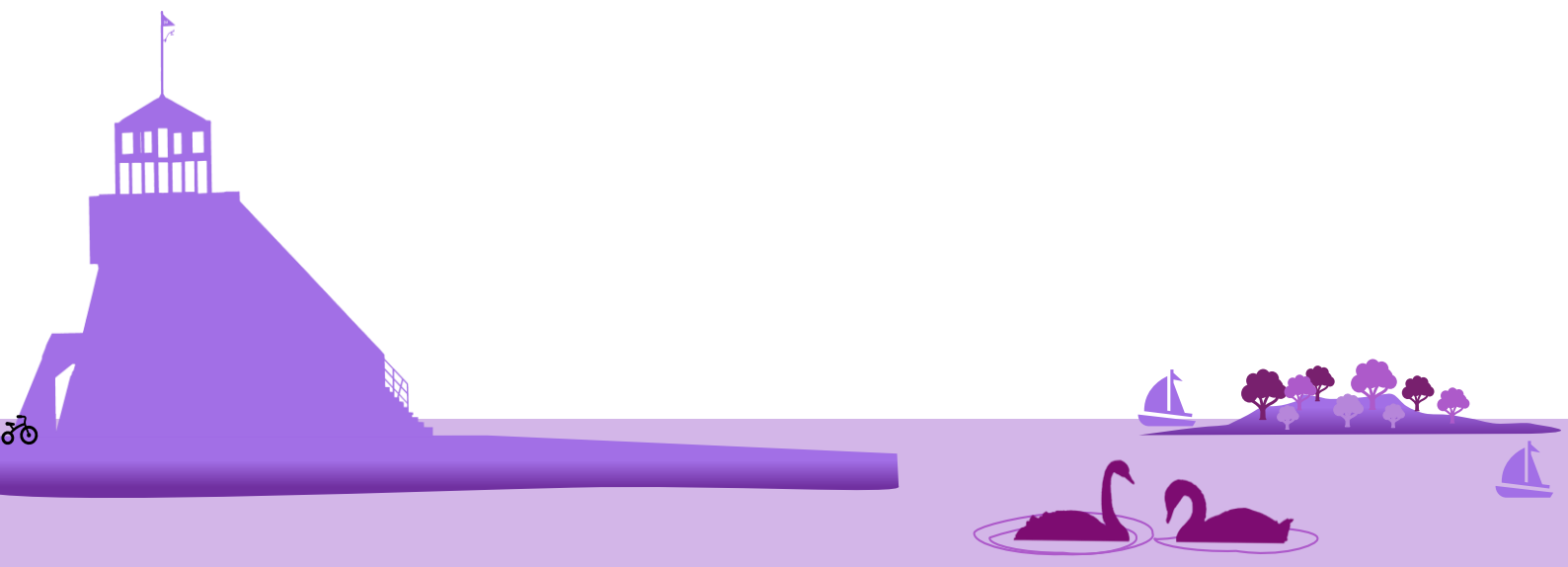
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Quantifying illicit drugs in mixtures is as critical as their identification for forensic and harm-reduction applications. Traditional methods like HPLC-UV offer high precision but are costly, reliant on reference standards, and insufficient for identification. This study evaluates benchtop nuclear magnetic resonance (NMR) spectroscopy, combined with quantum mechanical modelling (Q2NMR), as a cost-effective alternative for illicit drug quantification and identification in complex mixtures.

To develop the quantification method, mixtures containing methamphetamine with purities ranging from 10-90% (w/w) were prepared with cutting agents (methylsulphonylmethane, N-Isopropylbenzylamine, caffeine, and phenethylamine) and/or an impurity (pseudoephedrine). The composition of all species was quantified, with the purity of methamphetamine achieving a root mean squared error (RMSE) of 2.1 mg analyte/100 mg sample. For comparison, the standard analytical technique, HPLC-UV, achieved an RMSE of 1.1 mg/100 mg sample. While HPLC-UV remains the benchmark for accuracy, its high cost, dependency on reference standards, and inability to quantify certain cutting agents limit its routine use in forensics.

Further testing on samples containing methamphetamine, paracetamol, and/or sugar yielded an RMSE of 2.0 mg analyte/100 mg sample. Analysis was then conducted on 47 blind samples, including 15 real-world samples from police casework. For these blind samples, the target analyte was identified from the NMR spectrum using a newly developed pattern matching algorithm for benchtop NMR data. Key analytes were then quantified. Analysis is ongoing, but initial results from 10 samples yielded an RMSE of 1.2 mg analyte/100 mg sample. These results indicate that benchtop NMR offers a rapid, cost-effective alternative to existing forensic chemistry techniques with potential for broader compositional analysis.



Towards quantitative analysis of complex mixtures with benchtop spectroscopy using SABRE hyperpolarisation

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Benchtop NMR spectrometers are a popular alternative to their high field counterparts, because of their low cost and high portability. However, these instruments are relatively insensitive and are generally restricted to the measurement of analytes at concentrations in the order of tens of millimolar. The low sensitivity limitation of benchtop NMR can be addressed by hyperpolarisation methods, such as SABRE – a technique based on the reversible association of analytes to an iridium complex.

In our recent work, we have applied SABRE techniques to measure dilute mixtures at low micromolar concentrations with benchtop NMR. We show that – using simple and cost-effective instrumentation – SABRE benchtop can be used to quantify analytes at micromolar concentrations in mixtures, and that multidimensional NMR experiments can be utilised to gain detailed structural characterisation of these analytes. Using this approach, benchtop NMR can be applied to traditional NMR experiments with sensitivity comparable to modern high-field instruments.

Further progress in this area – particularly in improving spectral resolution – will facilitate the use of benchtop NMR for the quantitative analysis of complex mixtures such as natural extracts and bio-fluids.



Field dependence of long-lived states of ligands and their complexes with protein targets

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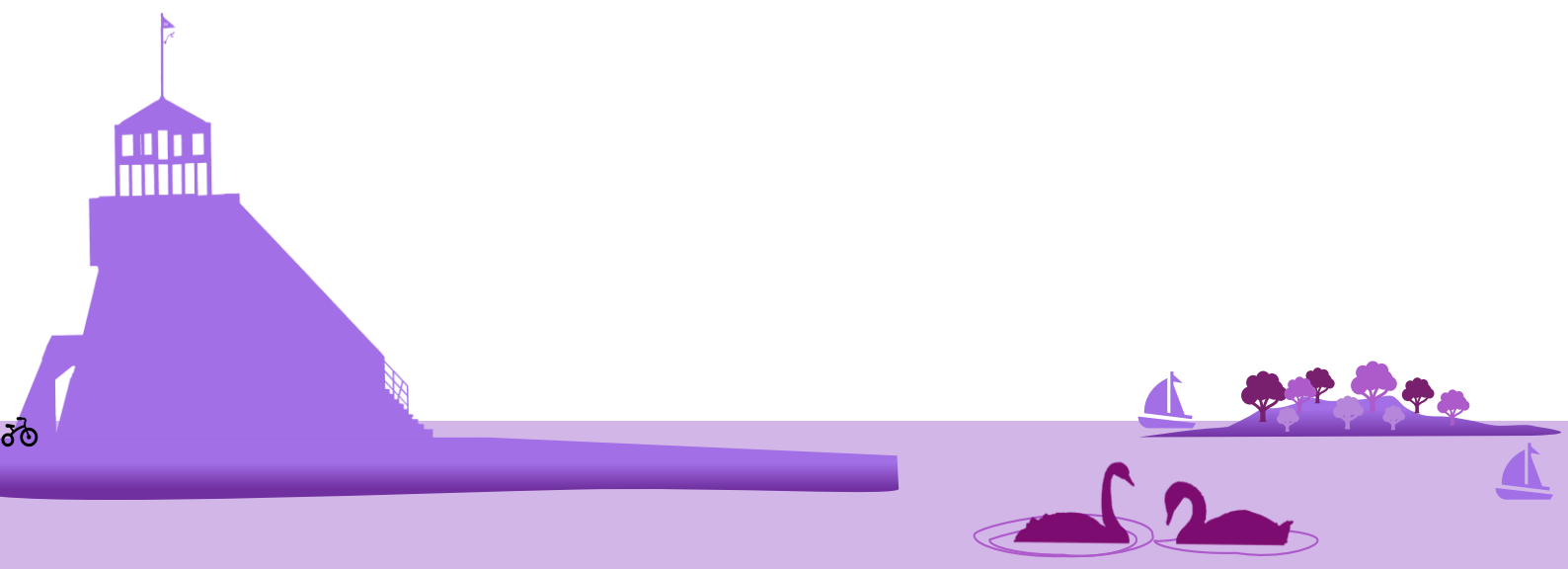
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Long lived states (LLS) are defined as population imbalances that can have longer lifetimes than longitudinal relaxation[1]. Their lifetimes (TLLS) can be used to probe protein-ligand (PL) binding and often offer better contrast than transverse (T2) or longitudinal (T1) relaxation times[2]. Long lived states can be readily excited in a system with two or more proton spins in aliphatic chains with geminal and vicinal couplings. Depending on the spin system, we can choose from a range of pulse sequences: Spin Locked Induced Crossing (SLIC) [1], the sequence often attributed to Sarkar et al.[3] or Selective Excitation of Long Lived States (SELLS) [4] , etc. As we aim to use LLS lifetimes for protein-ligand (PL) binding studies, it is important to make this technique more readily available. Conventional high-field nuclear magnetic resonance (NMR) is costly. Benchtop NMR offers a much cheaper and user-friendly alternative. This study aims to generate LLS on different ligands using various pulse sequences for LLS excitation on a 60 MHz spectrometer. Titration series have been performed using suitable protein-ligand systems. We have studied the field dependence and compared TLLS, T1 and T2 lifetimes and determined binding contrast at both low (1.4 T) and high field (11.7 T). The lack of sensitivity at low field can be overcome by hyperpolarisation. We have successfully created different long-lived states for a range of molecules at low field, which were previously conducted at high field[1]. In addition, we have demonstrated that at low field, the contrast observed upon PL binding is up to 3 times higher when using TLLS instead of T1 lifetimes.

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Extension of the ^1H NMR -based method for crystal water determination to benchtop spectrometers and sparingly water-soluble compounds

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The information about crystal water content of the compound under research is crucial especially in the case of potential drug candidates entering biological tests. Crystal water content affects the total molecular weight of the compound and is needed to avoid drug dosage and formulation errors. Several methods have been used for water content measurements of organic compounds including thermogravimetric analysis (TGA), X-ray diffraction, FT-IR and Raman spectroscopy, differential scanning calorimetry and dynamic vapour sorption [1]. However, most of these methods require accurate weighing of the compound, are time consuming and/or require special knowledge about the method for correct interpretation of the results.

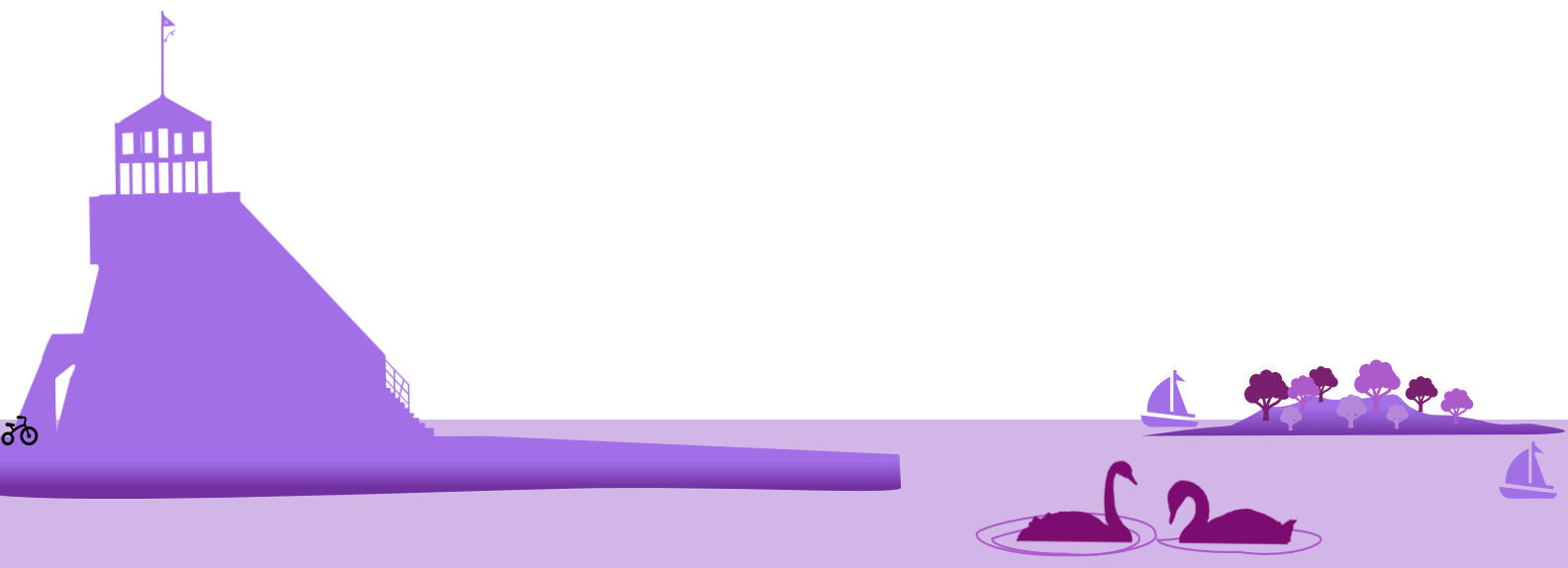
Recently, we have reported a novel quantitative NMR based method for crystal water determination in deuterium oxide [2]. The method was presented to be accurate, fast, simple and non-destructive requiring only minimal sample preparation. The results were comparable with those obtained by TGA and/or X-ray diffraction. High-field NMR spectrometers are often available in modern organic chemistry laboratories, but the very high cost of the equipment can limit the general use of the method. On the other hand, recent development of cheaper benchtop NMR spectrometers has made the NMR methodology more widely accessible. Additionally, despite majority of the drugs are water soluble, there are still compounds (e.g. some bisphosphonates [3]) that are extremely poorly soluble in water, and thus, cannot be analysed with our earlier published method [2].

In this work we extend the previously developed high-field NMR methodology for benchtop NMR instrument and present a solution how crystal water content can be accurately measured from sparingly water-soluble compounds aiming for cheaper and wider use of the method.

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Mixture analysis using SABRE-hyperpolarised benchtop NMR spectroscopy

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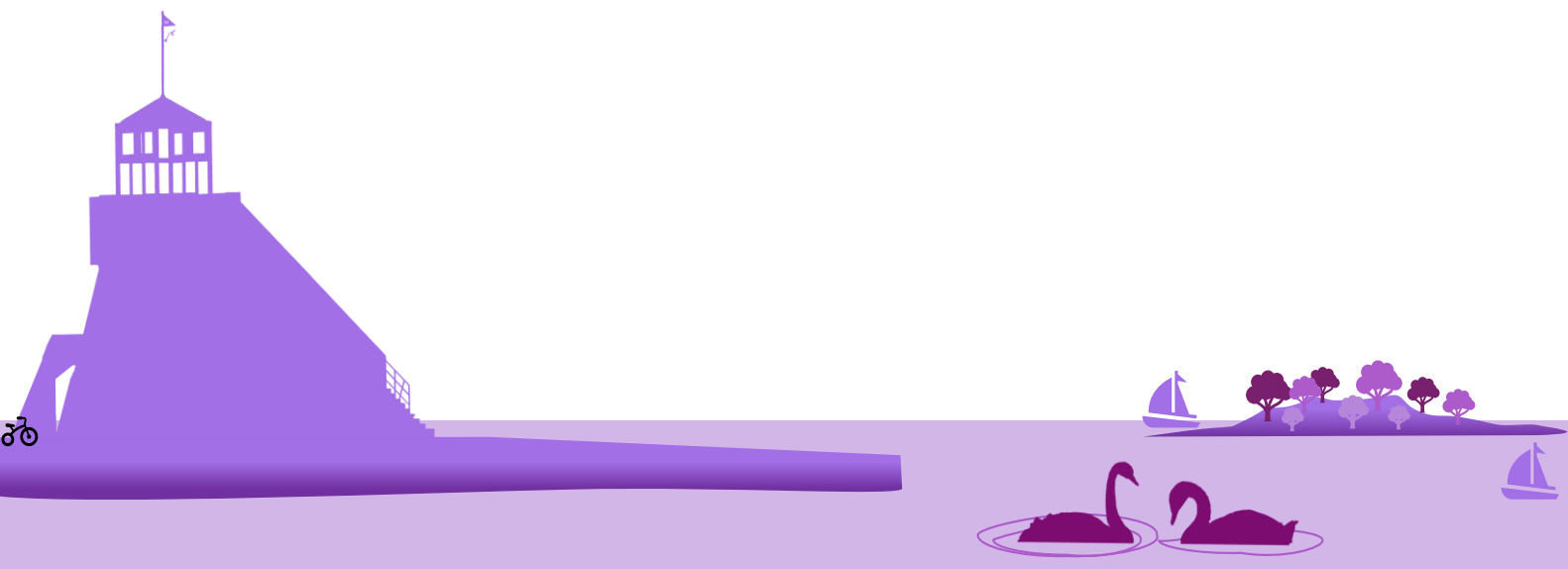
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The lower magnetic field strength of benchtop NMR spectrometers limits sensitivity and reduces signal dispersion, impacting utility. Hyperpolarisation dissociates sensitivity from field strength by creating a large nuclear spin state population imbalance, typically through perturbation of a spin system using a species with intrinsically high polarisation. Of interest for benchtop NMR spectroscopy is parahydrogen induced polarisation (PHIP), which uses the singlet spin isomer of dihydrogen, parahydrogen (pH₂), as the polarisation source. PHIP methods involve a chemical reaction that transforms this singlet state into observable hyperpolarisation on a target substrate, either through its irreversible hydrogenation,[1,2] or in signal amplification by reversible exchange (SABRE), via reversible binding to a catalytic centre.[3] Integration of pH₂ hyperpolarisation methods with benchtop NMR detection requires simple instrumentation to rapidly generate orders of magnitude signal enhancement.[4] However, applications such as mixture analysis are prevented by repeatability and quantitation issues, which generally restrict the use of pH₂ hyperpolarisation to single-shot NMR experiments. In SABRE, these issues arise from manual sample transfer between a low-field polarisation transfer regime and the benchtop NMR spectrometer for detection.

Automation of the SABRE process is shown to improve repeatability, extending compatibility to 2D NMR experiments.[5] Here, we integrate a sample shuttle into our workflow for repeatable SABRE hyperpolarised benchtop NMR spectroscopy. We exemplify its use in multidimensional, pure shift, and ultra-selective observation methods for mixture analysis. The automated field cycled approach is compared to spontaneous ex situ polarisation transfer.

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The study of sedimentation and drying of mining tailing materials by NMR-MOUSE

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Beatification and ore enrichment procedures provide valuable raw materials for different industries. However, these activities can generate massive amounts of waste materials composed of water, fine rocks, and chemical reagents known as tailings. Tailing materials are usually collected and stored in large impediments called tailing storage facilities (TSFs). In the TSFs suspended particles of tailing slurry are settled under gravity forming the dam's beach and as a result, water is separated and reclaimed.

In this work, we aimed to study the sedimentation and drying of tailings utilizing a bench-top, single-sided instrument known as Mobile Universal Surface Explorer (NMR-MOUSE). The NMR-MOUSE can provide valuable information about water content and water dynamics within tailing samples through the monitoring of NMR signal and T2 relaxation times. The NMR results are discussed along with particle size assessment, mineral composition, and elemental analysis to provide a comprehensive insight into the natural and induced sedimentation of the complex tailing materials.

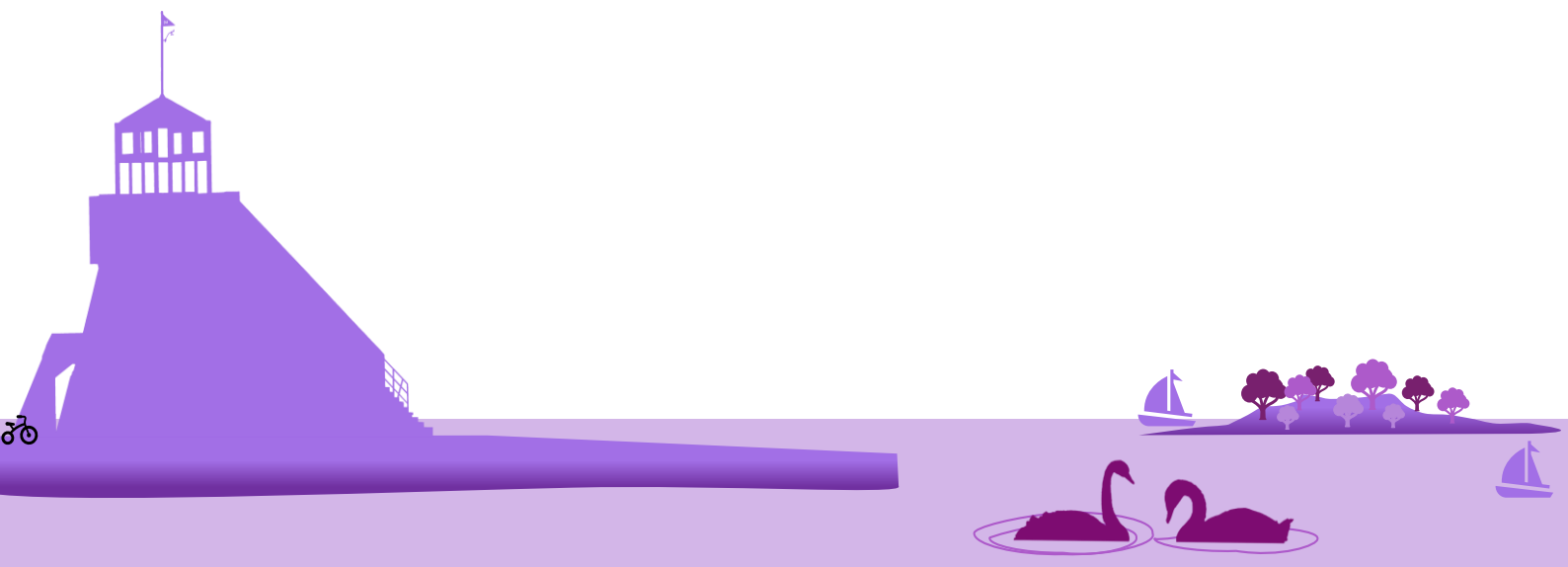
As a result, we observed the potential of the NMR-MOUSE as a non-invasive and real-time method to detect even small changes in water dynamics of tailing materials in different experiments defined as short-term and long-term sedimentation. The findings of this study can provide a better understanding of tailings sedimentation and indicate the ability of the NMR-MOUSE as a diagnostic apparatus for real-time monitoring and optimization of TSF management plans.

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Real-time Monitoring of Small Molecule Reactions Using the X-Pulse Benchtop NMR

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FlowNMR facilitates the real-time monitoring of chemical processes, it provides high-density data acquisition under realistic conditions and allows for the continuous monitoring of reactions by interfacing the NMR system with a flow setup. These setups can provide control over experimental conditions such as temperature and pressure, and in environments sensitive to air, moisture, or light, making it valuable for several processes.

Here we utilise a range of nuclei including ^1H , ^{19}F , ^{13}C , ^7Li , ^{31}P , ^{23}Na , and ^{11}B , we demonstrate reaction monitoring across various types of reactions such as acid-base catalysis, oxidation, rearrangements, and hydrogenations at both high-field (from work carried out previously) and low-field using the Oxford Instruments benchtop NMR, a system which offers broad multi-nuclear capabilities. Each case study will illustrate how FlowNMR can be used to capture quantitative kinetic and mechanistic data as this provides insights into reaction pathways and the efficiency of catalytic processes.



Fast and simple approach towards probing polymer mobility in turbid formulations by TD-NMR

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Distinguishing between particulate and dissolved materials is quite straightforward in clear formulations with small molecules: whenever you see turbidity or a particulate component, it is not completely dissolved. However, things get much more complicated in formulations that are turbid anyway (such as suspensions or emulsions) and/or in which a dissolved polymeric component leads to high viscosity (and thus difficulties for filtration) and some turbidity due to the Tyndall effect. Both issues make simple testing for solubility according to protocols such as OECD guidelines 105 or 120 quite hard.

Proton transverse relaxation time measurements open up a simple way to probe for the presence of non-dissolved proton-containing material inside such a formulation as proton populations with strongly restricted local mobility will manifest themselves with long correlation times and thus short transverse relaxation times. Transverse relaxation curves can be recorded essentially single shot over about 6 orders of magnitude in time with a combination of a solid echo and a CPMG curve and this measurement does not require especially homogeneous magnetic fields so that it also comes quite robust towards suspended non-magnetic particles.

In our contribution, we present examples of various polymer-containing formulations that undergo changes in the dissolution state of the polymer either by concentration or temperature changes and a simple parameter for assessing the degree of mobility restriction in the polymer is presented as well. Also, limitations with respect to polymer concentration and dynamic range of the measurements and possible ways to overcome these will be discussed.



TD-NMR Relaxometry as the effective tool for polymer analysis

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Time-Domain Nuclear Magnetic Resonance (TD-NMR) is a useful technique for studying the structure and dynamics of semicrystalline polymers. Despite the method's versatility, there is still a lack of unified step-by-step methodologies to fully investigate the semicrystalline polymeric materials using capabilities of TD-NMR techniques. In this work, we show how TD-NMR Relaxometry can be used to study polymers below and above glass transition temperatures for extraction of key macroscopic properties like crystallinity and domain size, and microscopic properties such as the abundance of microphases and dynamics.

Experiments were performed using a 1H 18 MHz benchtop TD-NMR spectrometer equipped with a temperature-control unit produced by Resonance Systems GmbH. Solid Echo (SE) and Multiple Quantum (MQ) pulse sequences were used to study relaxation and dipolar interactions.

The obtained melting curves and activation energies were in good agreement with known properties of the samples under study and helped investigate changes in the molecular motions in different materials.

From MQ measurements the build-up curves were produced to obtain the dipolar-coupling distribution to investigate dynamically and structurally distinguishable phases in the polymer matrix. The residual second moments (M_2), which reflect dipolar coupling distributions and relate to crystallinity, and corresponding observed T_2^* were calculated from the NMR peaks. A log-normal distribution was observed in dependence of the DQ-filtered FID signal intensity over the T_2^* values. This type of transverse relaxation time distribution is well known in other structural analysis methods, which supports the validity of the result.

We propose an algorithm for analysis of semicrystalline materials based on the unification of the above-mentioned experiments. It provides complex information about various important parameters on the spatial and dynamical levels along with straightforward interpretation.



Assessing the purity of model glycoconjugate vaccines by low field NMR

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Glycoconjugate vaccines are of growing importance to modern healthcare where they provide an opportunity for high efficacy prophylactic treatment against a growing number of infectious bacterial diseases [1]. Unfortunately, their preparation is highly complex and involves multiple stages of analysis prior to product release [2]. Such analyses must quantify the degree of successful conjugation and the amount of relevant co-expressed and co-purified process impurities. Whilst nuclear magnetic resonance (NMR) spectroscopy can be used for these assessments, the cost of high field systems is significant and hence there is a need to evaluate the performance of low-cost benchtop apparatus.

Here, we set a goal of achieving a satisfactory analysis within 20 min on a series of model glycoconjugates and sought to use hyperpolarization methods based on signal amplification by reversible exchange (SABRE) [3] to enable higher sample throughput. Our analyses demonstrate that a 1 Tesla (T) benchtop NMR can achieve satisfactory dextran-conjugation analysis results without the need for hyperpolarization, although SABRE hyperpolarization offers a route to improvement.

The assessment of the common impurity cell-wall polysaccharide proved more challenging. At high field satisfactory analyses were possible at 10 wt%, 5 wt%, 1 wt% and 0.5 wt% loadings where the resulting signals are distinguishable. However, at 1 T signal overlap precluded simple signal integration and a T1 filter was implemented. This allowed the overlapping signal contributions to be differentiated and made quantification possible for the 10 wt% sample where signal to noise ratios remained high.

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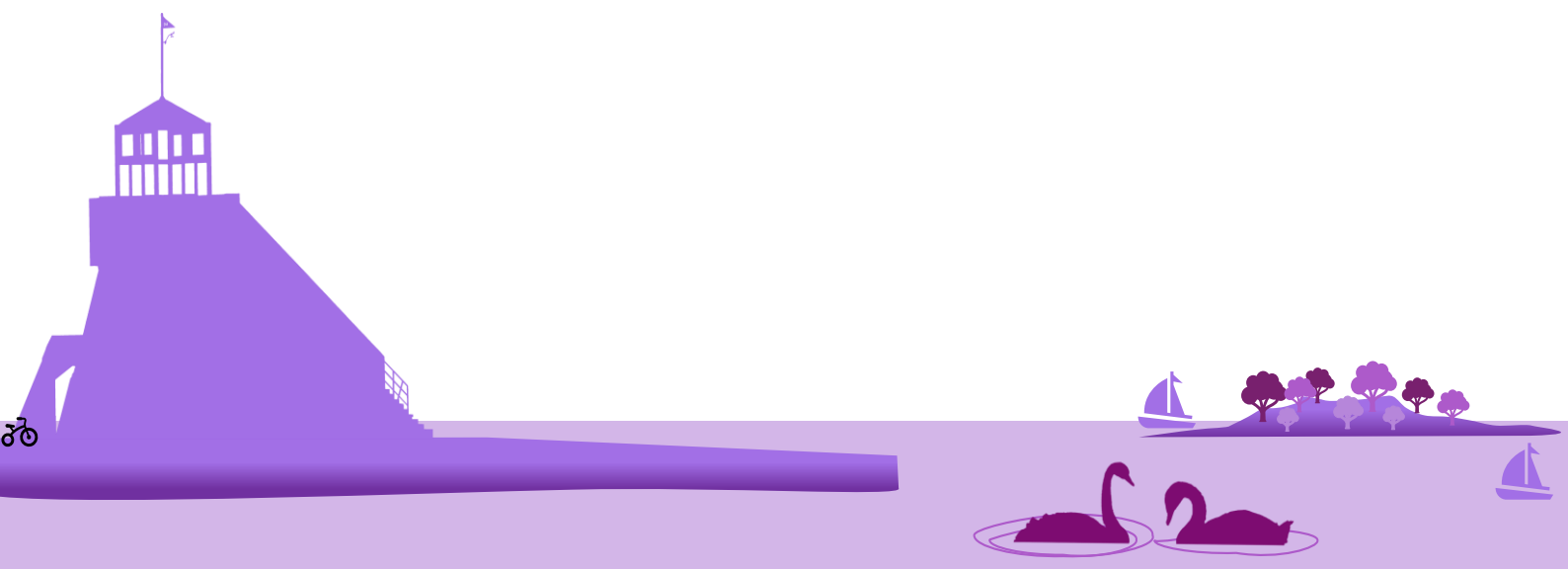


Photo-CIDNP in Motion: A Flow-Cell-based System for Benchtop NMR

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Hyperpolarization methods have emerged over the past decades as one way to overcome the inherent low sensitivity of Nuclear Magnetic Resonance (NMR) spectroscopy. Among various hyperpolarization techniques, photo-CIDNP stands out for its simplicity and accessibility. Photo-CIDNP can be implemented with minimal instrumentation, resulting on significantly lower costs than other hyperpolarization methods. In its simplest form, photo-CIDNP NMR requires only a photosensitizing dye and a light source to generate nuclear spin polarization by a spin-sorting mechanism that involves transient radical pairs. Still, the method suffers from limited throughput due to the need for sample changing.

Here, we present a cost-effective, high-throughput setup for photo-CIDNP in solution NMR using an 80 MHz benchtop NMR spectrometer with a continuous flow setup. A custom-designed flow cell has been modified to include an optical fiber for direct laser illumination. The flow cell can be operated by a peristaltic pump, providing access to continuous reaction monitoring in a closed-loop setup. Alternatively, it can be operated by a high-performance liquid chromatography (HPLC) system, enabling precise and automated sample injection and replacement without manual intervention. All system components are controlled by a single integrated, Python-based software using Transistor-Transistor Logic (TTL) logic. We assess the quality and reproducibility of the combined system and demonstrated its application to a series of photo-CIDNP-active molecules.



Peak Picking and Deconvolution in Fast 2D NMR-Based Metabolomics and Lipidomics of Serum Samples Using the DEEP Picker NMR Tool

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Fast 2D NMR methods are increasingly used for metabolomics and lipidomics studies, providing detailed spectral fingerprints[1] of complex biofluids such as serum. However, accurate extraction of spectral features remains challenging due to peak overlap, baseline distortions, and noise. Additional challenges come from the size of the datasets associated with large sample cohorts, as well as inter-sample spectral variations such as peak misalignment. In such a context, we have evaluated several available tools such as rNMR[2], NMRPipe[3], NMRFAM-SPARKY[4], and DEEP Picker[5]. The latter stands out due to its deep learning-based approach, which improves peak detection accuracy, even in complex spectra with severe peak overlap and noise.

By applying this approach to serum samples, we have developed a processing workflow in order to extract 2D peak volumes from multiple datasets (Ultrafast COSY, NUS TOCSY, and NUS HSQC of metabolic and lipid extracts) with a reasonable throughput and for further statistical analysis. Systematic optimization of key DEEP Picker parameters (scaling factors, noise level, etc.) was performed to improve peak detection sensitivity and reduce false positives. Additionally, line shape fitting was applied to refine peak quantification, ensuring consistent signal integration across 2D NMR metabolomics and lipidomics datasets. The extracted peak integrals are subsequently used for multivariate statistical analysis, including consensus orthogonal partial least squares discriminant analysis (C-OPLS-DA), to identify key metabolic variations.

Our findings demonstrate that DEEP Picker NMR tool, combined with advanced statistical modelling, provides a robust and scalable approach for large-scale fast 2D NMR-based metabolomics and lipidomics studies.

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Translational studies of non-obese type 2 diabetes by NMR reveal insulin resistance onset signatures

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The Goto-Kakizaki (GK) rat is a well-established experimental model for studying spontaneous non-obese type 2 diabetes. However, the mechanisms underlying the onset of the pathology remain unclear. We investigated systemic and skeletal muscle metabolism in just-weaned GK rats by applying liquid (600 MHz, 5 mm) and semisolid state NMR (400 MHz, 4 mm, 12 μ L), respectively. Male and female GK rats were compared to wild-type (WT) Wistar rats, respectively. Serum metabonomics revealed higher glucose levels in male GK than in WT, while no significant differences were observed between female GK and WT rats. Higher concentrations of alanine, lysine, proline, lactate, fatty acyl chains, cholesterol, and glycerophosphocholine in both male and female GK rats were also found. The absolute and normalized wet and dry weights of the extensor digitorum longus (EDL) and soleus muscles were consistently lower in male and female GK rats than in control animals. At this stage of life, the increased skeletal muscle protein catabolism has already led to notable clinical manifestations. However, metabolomics of the EDL muscle revealed reduced alanine, arginine, and creatine levels in both male and female GK rats. However, metabonomics by NMR of the EDL muscle revealed reduced alanine, arginine, and creatine levels in both male and female GK rats. In the EDL muscle of male GK rats, there were also lower levels of glycine, glutamine, lactate, and ATP than in male WT. In the soleus muscle, male GK rats exhibited lower levels of alanine, glycine, lactate, and creatine compared to male WT, while no significant differences were found in female GK rats. These findings demonstrated that GK rats exhibit early systemic and muscular metabolic alterations, leading to increased alanine, glycine, and lactate circulating levels with notable gender differences. These findings underscore metabolic signatures for insulin resistance onset and indicate the importance of considering gender-different features.



Deuterium NMR revealed a reduction in lactate production in hexokinase-2 knockout cells

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Hexokinase enzymes catalyze the first step of glycolysis. In mice, deletion or inhibition of epithelial hexokinase-2 (HK2) protects from acute intestinal inflammation [DOI: 10.1016/j.cmet.2021.11.004]; in humans, HK2 expression in intestinal epithelial cells is elevated during inflammatory bowel disease (IBD) [DOI: 10.1186/s12916-024-03710-7], making it a potential target for inflammation diagnosis and therapy.

We utilized NMR of deuterated glucose-d₂ to probe the cell's compensatory mechanisms during IBD and the impact of HK2 on glycolysis. We used wild-type (WT) Caco-2 and corresponding HK2 knockout (Δ HK2) cells as a model system. Cell-PBS suspension in a Shigemi NMR tube was placed in a 400 MHz NMR spectrometer at 310 K. Subsequently, glucose-d₂ ($\approx$ 8mM) was administrated, and ²H NMR spectroscopy proceeded for an hour. The resulting ²H spectra were integrated (MNova software), and the kinetics were fitted to obtain glucose-to-lactate conversion rate constants (K). All spectra showed glucose consumption and lactate production. After ca. 30 minutes, lactate accumulation inhibited HK activity and thus further lactate production.

WT cells demonstrated a K of 0.057 ± 0.010 and a maximum lactate level of 2.4 ± 0.3 mM. At the same time, Δ HK2 cells had a K of 0.061 ± 0.028 and lactate level of 2.0 ± 0.3 mM. T-test analysis of K showed no significance between the two groups, but lactate levels had p-value slightly below the 0.05 threshold, hinting at a reduction of Δ HK2 cells enzymatic capacity.

The revealed decline in lactate production in Δ HK cells supports a function during IBD and highlights its potential as a disease marker or target for new therapeutic strategies. Deuterium NMR spectroscopy has proved helpful for noninvasive probing of cellular metabolism. We are extending the method by using ¹³C NMR with hyperpolarized and thermally polarized glucose and other labeled tracers. This way, we aim to find the ideal method for potential in vivo diagnosis using ²H or ¹³C MRI.



Metabolic Effects of Mercury and Selenium Exposure in Amazonian Riverside Communities

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Amazonian riverside communities face significant exposure to mercury (Hg) and selenium (Se) due to their fish-based diet. Fish is often contaminated with Hg, while Se-rich soil contributes to unusually high Se levels. Selenium is essential for immune system function, whereas mercury is a neurotoxin with systemic nocive effects. However, the long-term impacts of chronic Se exposure and its interaction with Hg in the human body remain insufficiently understood. This study is part of an ongoing project investigating metabolic alterations in blood associated with varying levels of selenium and mercury using an NMR-based metabolomics approach. Statistical analysis revealed that chronic exposure to high Hg levels affected energy production, oxidative stress, and branched-chain amino acid metabolism, directly influencing glucose, lactate, proline, isoleucine, and valine levels. Furthermore, individuals with elevated blood Hg and Se levels exhibited metabolic profiles more similar to the control group, suggesting a potential protective role of high Se. Our findings underscore the metabolic impact of continuous Hg exposure on the Amazonian riverside population, as well as the mitigating effect of Se against Hg toxicity. Finally, we intend to perform proteomics analysis to provide a complementary and comprehensive understanding of the biochemical mechanisms underlying these effects, aiming to develop protective interventions to mitigate Hg-induced damage.



Hyperpolarized ^{13}C NMR metabolomics of urine samples at natural abundance applied to chronic kidney disease

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NMR metabolomics mostly relies on 1D ^1H experiments, which often results in significant peak overlap and limits the analysis of inherently complex samples. ^{13}C NMR benefits from wider spectral dispersion and narrower signal linewidth but is barely used in metabolomics due to its low sensitivity. In this context, Dissolution Dynamic Nuclear Polarization (d DNP) provides an efficient opportunity to significantly improve the sensitivity of ^{13}C NMR. Thanks to a meticulous optimization of the experimental workflow [1], we recently reported the first hyperpolarized ^{13}C NMR experiments on urine samples at natural abundance in conditions compatible with metabolomics studies [2].

Here, we will describe the first application of this new approach to clinical urine samples [3]. The analysis of human urine samples from patients with different stages of chronic kidney disease (CKD) was performed using ^{13}C d-DNP NMR and benchmarked to conventional ^1H NMR metabolomics at high magnetic field. Multivariate analysis of the d-DNP ^{13}C NMR dataset provided a statistical model able to distinguish patients with CKD from control patients. Moreover, ^{13}C d-DNP NMR highlighted several biomarkers known to be biologically relevant. The results highlighted the complementarity of biomarker coverage between hyperpolarized and conventional NMR metabolomics. In particular, ^{13}C hyperpolarized NMR allowed the annotation of two biomarkers that could not be detected by ^1H NMR because of peak overlap (i.e. guanine and guanidoacetate).

While showing that this emerging hyperpolarized metabolomics approach can provide meaningful information on clinical samples, results also pave the way towards exciting developments in order to make hyperpolarized metabolomics applicable to large sample cohorts and for various biofluids and pathologies.

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Fluorinated Oils Enhance *E. coli* Growth and Morphology

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Fluorinated oils such as FC-43 and FC-770 are increasingly recognized for their unique physical properties—high oxygen solubility, chemical inertness, and immiscibility with water—which make them promising tools in microbial biotechnology. In this study, we explore their effect on *Escherichia coli* K-12 cultures. Compared to standard LB medium, fluorinated oil-supplemented cultures exhibited:

- Accelerated growth kinetics
- Higher colony-forming potential
- Distinct colony morphologies

Growth enhancements were confirmed through spectrophotometry and CFU assays, while microscopy revealed morphological changes not observed in earlier reports. Preliminary NMR analysis suggested shifts in central carbon metabolism. These findings point to increased oxygen transfer as a driver of metabolic reprogramming.

Our results propose fluorinated oils as novel culture enhancers for aerobic microbes, with potential applications in synthetic biology, protein production, and high-throughput screening platforms.



13C Metabolic Flux Analysis Reveals Cellular Rewiring in Stimulated Peripheral Blood Mononuclear Cells

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Inflammatory processes in humans can be triggered by contact with pathogens, injuries or operations, as well as by endogenous factors, which may lead to immunological dysregulations and severe complications. Upon activation by lipopolysaccharide, components of the outer cell wall of gram-negative bacteria, immune cells rapidly adjust their metabolism to meet the short-term increase in energy demand. It is known that in this situation cells switch to aerobic glycolysis to enhance ATP production. Here, we incubate peripheral blood mononuclear cells with 13C-labeled glucose in vitro and study with NMR spectroscopy how glucose, the key metabolite of glycolysis, is metabolized upon stimulation by lipopolysaccharide.

Isolated peripheral blood mononuclear cells were incubated under identical conditions once with 12C glucose and once with 13C glucose for 96 hours. Supernatants and cells were collected separately at different time points. 1D 1H and 2D 1H13C HSQC-NMR spectroscopy was used to monitor differences in extracellular metabolites between unstimulated and stimulated cells. Through comparison of 12C and 13C cultures we were able to identify downstream metabolites of glucose.

In addition to lactate, a well-established product of glycolysis, we identified several other 13C-labeled metabolites in the extracellular media that were uniquely produced under inflammatory conditions, for example alanine.

The unexpected flux from glucose into alanine suggests a diversion of glucose-derived carbon away from classical glycolytic endpoints in activated immune cells. Alanine, synthesized from pyruvate and glutamate via alanine transaminase, may play a previously underappreciated role in the metabolism of blood immune cells. Its secretion points to a yet unknown metabolic adaptation potentially acting as a downstream signaling molecule to combat inflammation.



GNAT for Stats: A Comprehensive Module for NMR based Chemometrics and Metabolomics

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The General NMR Analysis Toolbox (GNAT) has established itself as a versatile free and open-source software suite for processing and analyzing NMR data¹. Here we present a major expansion for comprehensive metabolomics analysis. The module implements a whole NMR metabolomics pipeline in a single piece of software. This expansion includes a new suite of powerful statistical tools designed for tasks like classification, discrimination, and correlation analysis, including PCA, PLS-DA, OPLS-DA, and STOCYSY2,3. A set of preprocessing tools for binning and variable selection (iPLS and biPLS) before metabolomics analysis is also available. Graphical tools for outlier detection allow researchers to identify and address potential data inconsistencies. Model validation and application to unknown samples are straightforward and supported by relevant analytical figures of merit. All analyses done in the toolbox can be exported as reports, in various formats (including .txt, .xmls, and .mat). The standard version of the GNAT is intended to be run within MATLAB, but standalone compiled versions are also available. The new functionalities are demonstrated using a test dataset for the classification of edible oils.

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NMR metabolic profile in kidney disease

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Chronic kidney disease (CKD) affects nearly 100 million Europeans and 800 million individuals global, a growing number, considered to become the 5th largest cause of death worldwide by 2040 [1,2]. CKD patients exhibit an elevated cardiovascular risk, manifesting myocardial infarction and stroke, coronary artery disease, heart failure, arrhythmias, and sudden cardiac death [3]. Since its early stages of development, NMR urinomics proved its power in predicting post-transplant early renal graft disfunctions [4]. With the recent advent of NMR lipidomics [5-8], integrated urinary and blood plasma metabolomics can lead also to advancement in kidney disease monitoring.

This paper describes a cross-sectional study performed on over 70 stable kidney transplanted and over 100 non-transplanted patients, with and without administration of sodium-glucose co-transporter-2 (SGLT2) inhibitors. Clinical and NMR metabolomics data were combined in order to provide a global profile for all metabolites. For the transplanted patients group, a 3-year follow-up is presented and evaluated for relevant associations between renal failure outcomes and baseline metabolites. The obtained data also describes a correlation of metabolomics records with several physiological parameters, such as glomerular filtration rate and handgrip strength.

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Robust microwave cavity control for NV ensemble manipulation

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Nitrogen-vacancy (NV) center ensembles have the potential to improve a wide range of applications, including nuclear magnetic resonance spectroscopy at the microscale and nanoscale, wide-field magnetometry, and hyperpolarization of nuclear spins via the transfer of optically induced NV polarization to nearby nuclear spin clusters. These NV ensembles can be coherently manipulated with microwave cavities, that deliver strong and homogeneous drivings over large volumes. However, the pulse shaping for microwave cavities presents the challenge that the external controls and intracavity field amplitudes are not identical, leading to adverse effects on the accuracy of operations on the NV ensemble. In this paper, we introduce a method based on gradient ascent pulse engineering (GRAPE) to optimize external controls, resulting in robust pulses within the cavity while minimizing the effects of cavity ringings. The effectiveness of the method is demonstrated by designing both 90° and 180° pulses. These optimized controls are then integrated into a PulsePol sequence, where numerical simulations reveal a resilience to detunings five times larger than those tolerated by the sequence constructed using standard controls.

